Introducing Micro-hardness Test in Forensic Odontology as an Aid in Solving Crimes: Multidisciplinary Approach

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Abstract

Background: Many studies have used dental radiography, light microscopy and electron microscopy to study the incinerated tooth for a possible estimation of temperature. However, no study so far has focused on measuring the change in hardness of dental hard tissue owing to higher temperatures.

Objective: To introduce a novel method which may indicate the approximate temperature to which dental hard tissues (teeth) might have been exposed.

Methods: We utilized Vickers hardness testing machine on a set of unrestored, non-carious extracted human teeth which were grouped and exposed to particular temperature of 200, 400, 600 and 800 degree Celsius respectively. Vickers Hardness Number (VHN) was obtained by measuring the diagonals formed after indentation at specific weight and time (constant) for all specimens in every group. Optical Photomicrography was used to view the enhanced images of indentation. The value was computed for all specimens in every group.

Results: We observed overall reduction in VHN values with increase in specified temperature. VHN of enamel was limited to 200 and 400 degrees Celsius, whereas VHN for dentin greatly reduced at 600 and 800 degree Celsius by 10 times. Surprisingly, VHN values for enamel were higher for incinerated tooth (at 200 and 400 degrees Celsius) than VHN reported in literature for non-incinerated tooth.

Conclusion: We suggest that micro-hardness test in forensic odontology is feasible and can be added in the list of already existing techniques for temperature estimation however, further experiments are recommended for its reproducibility.

Keywords*: Forensic dentistry, Forensic Science, Hardness test, high temperature, dentin, incinerated tooth, Microscopy, Dentition, Body remains, Enamel*

Introduction

Dental structures are the hardest and well protected structures in the body. These structures resist decomposition and high temperatures and are among the last ones to disintegrate after death.[1] Incinerated human remains often require dental comparison to establish ones' identity. These remains are often fragile and vulnerable to damage teeth and facial bones on minor forces, disrupting anatomical relationships, and impairing the ability to compare with ante mortem records.[2]

A systematic, conservative approach prevents the loss of valuable dental information before a thorough picture of the individual's dental remains has been

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adequately documented. The presenting conditions of fire victims are often explained and illustrated with photographs, with a series of illustrations and text describing the damage seen in the dentition of the fire victims.[3] Studying the effect of heat on teeth and the restorations would help ascertain the temperature reached by the fire, which would be highly crucial in cases where skeletal remains are recovered.[4] This general estimation of burning temperature could provide important information to reconstruct a criminal act.[5]

Various studies have been done to examine physical changes such as change in colour, texture, or morphology that occurs after exposure of teeth to high temperatures in an incremental pattern. Microscopic, radiographic and ultra-structural examination using scanning electron microscope have also been documented. [6 – 8] In the present study we propose a novel method which may provide reliable range of temperature at which a dental hard tissues (teeth) could have been exposed.

Materials and Method

Permission to conduct the study was obtained from concerned authorities of the institute. Since the present study did not involve any human participants, the procedure followed were in accordance with the standards from Declaration of Helsinki and its amendments. Sixteen unrestored, non-carious extracted human teeth stored in 10 % formalin were conveniently collected and categorized into 4 groups [Group A, Group B, Group C and Group D]. Each group consisted of one central/lateral incisor, one canine, one premolar and one molar [no distinction was made with respect to maxillary or mandibular teeth and the sequence of teeth]. All the teeth specimen were then removed of any soft tissue using a hand surface scaler.

The teeth specimen were then vertically mounted on a tray that could withstand high temperature (Figure 1) and each group was subjected to particular temperature of 200 C, 400 C, 600 C and 800 C [C – Degree Celsius] respectively in an electric muffle furnace (figure 2). The increase in temperature was gradual, starting at 18 C, with an increment of 26 C/min, till the desired temperature was obtained. The teeth specimen were then carefully placed inside the muffle furnace for 15 minutes and then carefully removed at the same temperature. This ensured that the same temperature was maintained when the specimen were removed. The teeth specimen were not observed for any morphological changes.

These specimen were then semi/half-embedded in self-cure resin blocks of uniform dimensions. The specimen were embedded in such a way that the labial surface of the crown and root were almost parallel to the upper surface of the resin block. Each block had teeth specimen which were exposed to particular temperature. These blocks were labelled as Block 1, Block 2, Block 3 and Block 4. Due care was taken to minimize the damage during handling of the specimens.

The resin blocks were filed to reduce the thickness if required to permit ease of procedure. The teeth specimen was not filed to prevent any intentional damage. The embedded teeth specimen were then subjected to micro hardness testing using a micro hardness tester (Shimadzu HMV – G20ST) ® [figure 3] with square based diamond indenter of 136^0 with a constant load of 500 gm for 15 seconds. The minimum spacing between indents was 40µm. Each test condition with the same load and time was conducted on every specimen of the block. Indents having well-formed sharp edges were considered for every specimen in all resin blocks. A total of three reading per specimen was included in calculation. This was followed by calculating average Vickers Hardness Number (VHN) for each resin block irrespective of individual specimen. The VHN values for every indent was automatically calculated and displayed on the screen installed on the micro hardness testing machine. The accuracy of the indentation was determined using optical photomicroscope with 100X resolution. [Figure 4] The procedure was limited to coronal part only since, in-vivo the radicular part of tooth is embedded within the alveolar bone.

Results

The VHN for each group exposed to a particular temperature is shown in table 1. The VHN for Group C and Group D was calculated for dentin. The VHN for un-supported enamel could not be estimated. A decrease in VHN was observed from group A to group B. The VHN for dentin also decreased from group C to group D. The magnified images of indentation for each group (specific temperature) is shown in figure 5. The VHN number could not be obtained for few teeth specimens since the optical microscope could not establish accurate indentations.

Table 1: VHN values obtained across different groups for enamel and dentin

	GROUP A	GROUP B	GROUP C	GROUP D
Mean VHN (Enamel)	448.5 ± 16.8	406.8 ± 41.3	Indentation could not be performed on enamel, rather done on Dentin*	
Mean VHN (Dentin)	Indentation done on Enamel#		42.5 ± 2.1	39.7 ± 1.6
*Indentation not possible since Enamel was chipped off from DEJ at high Temperature				
# Enamel was intact at lower temperatures; Indentation was possible. VHN - Vickers Hardness Number				

Figure 1: Tooth specimens mounted on tray [to withstand high temperatures]

Figure 2: Electric Muffle furnace used in the study to incinerate tooth specimens.

Figure 3: Micro-hardness tester [Shimadzu HMV-G20ST, v1.03]

Figure 4: Optical photomicroscope used in the present study

(a) 200 C
(b) 400 C
(C) 600 C
(d) 800 C

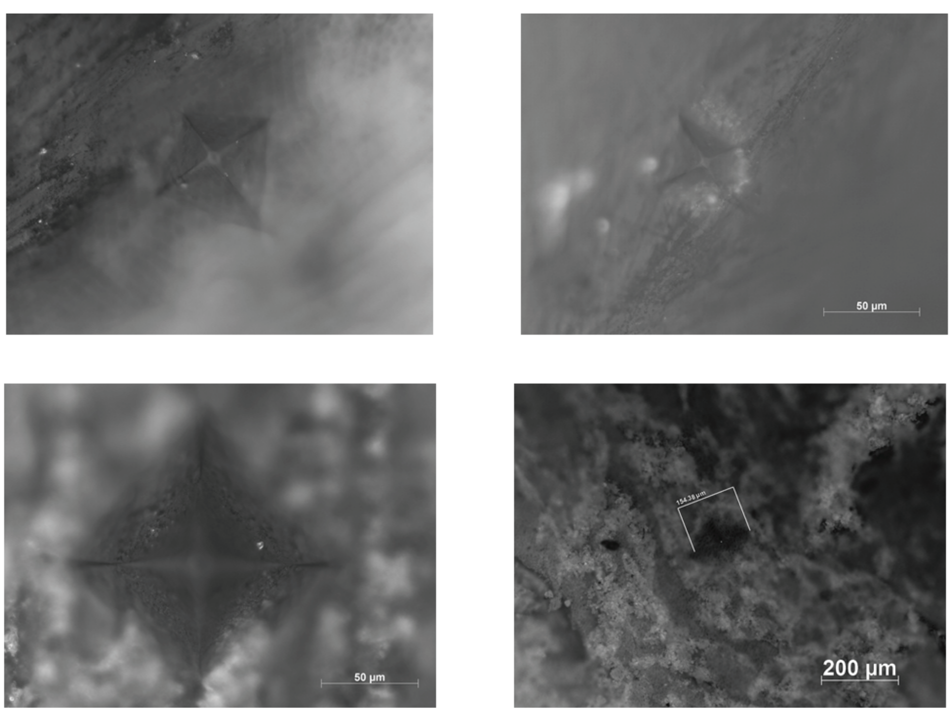

Figure 5: Indentations at different level of temperatures viewed using optical microscope [Indentation diagonals measured as shown in figure 4(d)]

Discussion

There is no standard condition for enamel and/or dentin micro-hardness testing for normal teeth and perhaps neither for incinerated teeth. In the present study, we propose micro-hardness test as a novel method for incinerated dental hard tissues (teeth) for estimation of higher temperatures. Given the paucity of literature on the concept that was experimented in the present study, we took the liberty to select the testing conditions of incinerated dental hard tissue (teeth).

There is literature to support that fragmentation is an important complication when human remains are exposed to higher temperature. This also decreases the hardness of teeth structure and makes it more brittle.[9] We have made an attempt to overcome the above mentioned characteristic using a multidisciplinary approach. To our knowledge, there is no other study reported in literature which have implemented the concept of micro-hardness testing for estimating temperatures in forensic odontology.

Micro-hardness tests are commonly used to study the physical properties of materials, and they are widely used to measure the hardness of teeth.[10, 11] In the present study, we chose Vickers Hardness over Knoops Hardness since, the test indentation is very small in Vickers and hence it is more useful in testing very thin materials, surface of a part or small areas. Gutiérrez-Salazar and Reyes-Gasga also proposed that in tooth hardness studies the Vickers indenter is more useful than the Knoop because a square shape indent is always conserved, and because the indentation produced on a non-flat surface, or by the difference in hardness of enamel and dentin, is easily detected.[12]

In the present study, it was interesting to find that VHN values for enamel were slightly higher at 200 C and 400 C respectively than the VHN values of non-incinerated teeth reported in literature. Lupi-Pegurier et al[13] reported micro-hardness of enamel in the range of 322 to 353 VHN, Chuenarrom et al[14] reported in the range of 316 to 328.4 kg/mm^{-2} with the exception of 418 ± 60 VHN reported by Colly et al. [15] We would also like to highlight that the above VHN values mentioned are irrespective of different loads and time duration. In the present study we set our load at 500gm for 15 seconds and the corresponding mean VHN values obtained were slightly higher. The underlying reasons for this interesting observation needs to be further explored. VHN values for enamel at higher temperatures could not be determined since at temperature above 600 C enamel if present was very brittle and limited to few surfaces and at 800 C enamel got chipped off from DEJ and its remnants were in small pieces which often go unnoticed in fire debris. However, these unsupported enamel were also tested for micro hardness testing but could not sustain the force of indenter at 500 gm. Such unsupported enamel needs to be further tested at lower loads and time.

VHN values for dentin were limited to 600 C and 800 C, since enamel was either present in bits and pieces or completely fragmented thereby exposing the dentin. VHN values could not be obtained at 200 and 400 C. This is because we had no baseline values for dentin prior to incineration, which requires slicing of specimens at the beginning of experiment (which we did not) prior to incineration. Hence VHN values obtained after slicing the specimens post incineration would not have helped us to a large extent. However, this shortcoming needs to be rectified in further experiments for more detailed information. We have compared the values with findings previously reported in literature and found that VHN values for dentin, post incineration were slightly lower than for non-incinerated dentin.[12, 14] This difference in temperature and its associated reduction in VHN values at higher temperatures might prove beneficial in forensic dental sciences.

It is evident from the present study that micro-hardness tests can be applied to estimate the temperature range to which the dental hard tissues (teeth) might have been exposed. These temperature ranges vary according to site involved, the oxidant involved, duration of exposure to fire and burning atmosphere. [16] For example, cremation occurs at temperatures ranging from 871 C to 982 C and combustion of petrol occurs at a temperature of 800 – 1100 C. [17] With increase in temperature we believe the values of micro-hardness tests will also vary. Another added point is that the values are provided as an automated output from the micro-hardness tester which eliminates the possibility of any subjective bias. It also provides a feasible method to store teeth specimens/samples (embedded in resin blocks) for further evaluation if required. The values in the present study provides average VHN for a group of teeth exposed to particular temperatures. We have not made any attempt to provide individual VHN values for incisors, canines, premolars and molars. This was not included due to insufficient time and funds. Further studies are recommended to overcome the shortcomings of the present study.

With not much literature to help us with the concept, we highlight the limitations of this experiment. They are a) Small sample size b) The VHN values were not recorded before the samples were exposed to higher temperatures: Had we recorded the values, it would have helped us to statistically analyze the results for more scientific validity. However, we have referred to VHN values from the literature to compare the findings. c) The VHN was not determined for dentin at lower temperatures: This would have involved slicing the mounted samples. Teeth become brittle at higher temperatures, the slicing was avoided since we weren't sure if the resultant samples would be ideal for micro-hardness testing at load as high as 500 gm which was used in the present study. d) The VHN values are obtained only from the indentations on buccal surface of the samples: There are variations in the thickness of enamel on occlusion, middle third and cervical third near the Cemento Enamel Junction (CEJ) on buccal and lingual/palatal surfaces. Any information of VHN at various anatomical regions of enamel (tooth) will improve its sensitivity. e) The load and time duration was constant in the present study; though there is no set value [load and time] for either dentin and/or enamel, variation in time and load across different areas of incinerated teeth might provide more reliable results.

Conclusion

The present study provides a rough outline to map the changes associated with higher temperatures using micro-hardness test. It can be concluded from the present study that VHN values of enamel slightly increase than the normal at 200 C and 400 C, whereas, VHN values for dentin decrease at higher temperatures of 600 C and 800 C, for a set load and time duration. We again reinforce that further experiments are recommended to obtain more reliable results which may aid in providing estimated temperatures to which dental hard tissue might have been exposed.

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Statement of Human Rights: Mentioned

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