Dental fluorescence: Potential forensic use

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A B S T R A C T

In cases of identification of bones, skeletal segments or isolated bones, searching for biotypologic diagnostic data to estimate an individual’s age enables comparing these data with those of missing individuals. Enamel, dentin and pulp undergo remarkable changes during an individual’s life. The enamel becomes more mineralized, smoother and thinner, and deteriorates because of physiological and pathological factors. Dental pulp decreases in volume due to the deposition of secondary dentin; thus, the dentin becomes thicker with time. In natural teeth, the fluorescence phenomenon occurs in dentin and enamel and changes in those tissues may alter the expression of the natural tooth color. The aim of this study was to assess the correlation between age and teeth fluorescence for individuals from different age groups. The sample consisted of 66 randomly selected Brazilians of both genders aged 7–63 years old. They were divided into 6 groups: Group 1 – aged 7–12 years, Group 2 – aged 13–20 years, Group 3 – aged 21–30 years, Group 4 – aged 31–40 years, Group 5 – aged 41–50 years and Group 6 – aged between 51 and 63 years. Upper right or left central incisors were used for the study. Restored and aesthetic rehabilitated teeth were excluded from the sample. The measurement of tooth fluorescence was carried out via computer analysis of digital images using the software ScanWhite DMC/Darwin Systems – Brazil. It was observed that dental fluorescence decreases when comparing the age groups 21–30, 31–40, 41–50 and 51–63 years. The results also showed that there is a statistically significant difference between the groups 41–50 years and 21–30 years (p < 0.005) and also among the group 51–63 years and all other groups (p < 0.005). It can be concluded that dental fluorescence is correlated with age and has a similar and stable behavior from 7 to 20 years of age. It reaches its maximum expected value at the age of 26.5 years and thereafter decreases.

1. Introduction

Fluorescence is a phenomenon defined as the absorption of UV light (1–400 nm – invisible light) by objects such as natural teeth, and its spontaneous emission in larger wavelengths (430–450 nm – light visible) [1,2]. The hypothesis of age estimation from changes in dental fluorescence has arisen because it is known that the expression of natural tooth color is dynamic and depends on the interaction of enamel, dentin and pulp with light during the phenomena of refraction and reflection [1]. The enamel, dentin and pulp undergo remarkable changes during an individual’s life. Enamel becomes more mineralized, smoother and thinner. This increase in the mineral content and thinning of enamel makes it more translucent. The physiological and pathological wear of enamel may also lead to exposed areas of dentin, especially in the incisal region; and exposed dentin absorbs stains, leading to an alteration in the expression of the natural tooth color. The pulp decreases in volume due to the deposition of secondary dentin thus the dentin becomes thicker with time. Furthermore, the dentin becomes less permeable as a result of deposition of peritubular dentin, which increases its chromatic saturation and reduces its opacity [3].

Young people usually have anterior teeth with a large pulp volume, and opaque dentin, completely covered by enamel. The enamel is thicker, translucent, shiny and often presents a milky,
white chalk color [4]. Individuals aged 70–80 years of age have significantly reduced enamel thickness and surface texture and a significant increase in translucency. Often, there are large areas of dentin exposure at the incisal edges, which usually suffer from severe extrinsic staining. The pulp virtually disappears, while the dentin becomes thicker and more saturated and decreases in opacity [5]. Thus, if the enamel and dentin are responsible for the dental fluorescence phenomenon and teeth undergo significant changes during life, proper investigation of the phenomenon of fluorescence can provide us a method to estimate an individual’s age.

There are different methods to measure tooth color, including visual assessment with shade guides, spectrophotometry and computer analysis of digital images. The latter method has been successfully used to assess the effects of dental whitening and color changes through longitudinal studies [6–8]. Therefore, the International Commission of Illumination (ICI) established standards that enable the definition of a certain color. It developed the colorimetric model in which a color is located by three values: the luminance (L), expressed as a percentage – 0 for black to 100 for white; two color ranges a* and b*, respectively ranging from green to red, and from blue to yellow; where a* positive tends toward red and the negative toward green, and b* positive tends toward yellow and negative toward blue. Consequently, it is possible to describe a set of visible colors. In CIELAB systems the comparison between two colors (ΔE) can be mathematically calculated. The basis for these calculations are the parameters L* (brightness), a* (red–green), b* (blue–yellow) of the two colors [1,8–13].

Studies should be carried out to verify and quantify the change in tooth fluorescence through the lifetime of an individual and establish a new age estimation method, which would be of great value, especially in individuals older than 20 years. The aim of this study was to assess the correlation between age and the change in fluorescence of dental teeth in groups of individuals with different ages The variables assessed in this study were: total fluorescence, L* (brightness), a* (red/green), and b* (yellow/blue).

2. Materials and methods

The research project was appreciated by the Ethics Committee of the University of São Paulo, Brazil, and approved under number 134/2010.

Photographs of the maxillary central incisor tooth (right or left) obtained in an environment illuminated by ultraviolet light (UV), commonly known as “black light” were analyzed. The sample consisted of 66 Brazilians, 25 males and 41 females who were randomly selected in the clinics of graduate courses. The study subjects were aged between 7 and 63 years and were divided into 6 groups: Group 1 – 7–12 years, Group 2 – 13–20 years, Group 3 – 21–30 years, Group 4 – 31–40 years, Group 5 – 41–50 years and Group 6 – 51–63 years. The division of groups was based on a study by Hasegawa et al. [10] who studied the color and translucency of natural teeth in all age groups and established 6 groups where the color and translucency vary throughout life.

The following exclusion criteria were used: presence of restorations or any other rehabilitative or aesthetic procedure (such as tooth whitening), the presence of stains, imperfect amelogenesis, fluorosis and any change on the tooth surfaces where fluorescence was measured, as well as traumatized incisors. There was no cleaning procedure prior to photography, because the central incisor is an easily accessible tooth for hygiene and has a smooth surface. The recorded data were registered in a special form developed for the study.

A box of expanded polystyrene (styrofoam) was manufactured with depth, width and height of 50 cm for each. Two 25 W/127 V Golden® UV lamp bulbs were installed, placed 30 cm apart, with a slot between them through which the photographs were taken. The opposite surface (front) of the box remained open to enable proper positioning of the volunteer. The subject’s head position was standardized with the Frankfurt plane (tragus line to the wing of the nose) parallel to the ground. The subject was positioned at a distance of 65 cm from the camera.

The photographs of the selected maxillary central incisor were obtained in a dark environment with the UV lamp bulb as the only light source. A Sony cybershot DSC (Digital Still Camera) camera (Sony USA – Sony Corporation of America) was used set at Macro f/2.8, ISO 400, speed of aperture 30, at a distance of 65 cm from the object. The camera was fixed by a tripod and no flash was used. The images were coded and the differences in the level of fluorescence from each analyzed condition were determined using a digital image processing with the help of software ScanWhite DMC/ Darwin Systems software from Brazil, which is able of assessing the level of tooth whitening. This software calculates the parameters L*, a*, b* according to the CIE lab.

For the statistical analysis to compare the different age groups regarding the variables considered in this study, the model analysis of variance was applied with a source of variation. In case of rejection of the hypothesis of equal means in all age groups, these bands were compared in pairs considering the LSD test. To assess the relationship between age and each variable of interest fit regression models were used. To evaluate the error of method the model of variance components and estimation of the intraclass correlation coefficient was considered. p values less than 0.05 were considered statistically significant. The null hypothesis of total fluorescence was tested, L*, a* and b* mean was equal in all age groups versus the alternative hypothesis that at least one age group had mean total fluorescence, L*, a* and b* different from the others. Statistically significant difference was observed in at least one age group. Due to the rejection of the hypothesis of an equal mean in all age groups, these age groups were compared in pairs for each variable.

3. Results

From the experiments the data results observed for the total fluorescence are shown in Table 1 and Fig. 1. At Table 1 it can be seen that some age groups has a statistically significant difference when compared with the age groups studied. Fig. 1 shows a descending dental fluorescence from the age group of 21 to 30 years onwards.

The observed data for the L* (brightness) are shown in Table 2 and Fig. 2. Table 2 shows that some age group has a statistically significant different fluorescence when compared to all the age groups studied.

From Fig. 2, a decreasing value of L* can be observed from the age group of 21–30 years onwards. The results enable us to infer that the age where the maximum expected value of L* is achieved is at the age of 29.2 years, with a maximum expected fluorescence of 53.91.

Table 1

<table>
<thead>
<tr>
<th>p values for comparisons between the groups of total fluorescence variable.</th>
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</thead>
<tbody>
<tr>
<td>Age groups</td>
<td>13–20</td>
<td>21–30</td>
<td>31–40</td>
<td>41–50</td>
<td>51–63</td>
</tr>
<tr>
<td>Age groups</td>
<td>7–12</td>
<td>0.959</td>
<td>0.023</td>
<td>0.497</td>
<td>0.360</td>
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<td>12–20</td>
<td>0.027</td>
<td>0.490</td>
<td>0.407</td>
<td>&lt;0.001</td>
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<tr>
<td>21–30</td>
<td>0.118</td>
<td>0.005</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31–40</td>
<td>0.146</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>41–50</td>
<td>0.002</td>
<td></td>
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<td></td>
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</tbody>
</table>

p < 0.05 = statistically significant difference.

Bold numbers mean statistically significant difference.
The $p$ values for comparisons between groups of variable $a^*$ show that the age group 21–30 years presents a statistically significant difference when compared with age groups 07–12, 13–20, 41–50 and 51–63; and that the age group of 31–40 years has a statistically significant difference when compared with ages 7–12 and 13–20 years. It is observed an increasing pattern of $a^*$ variable from the 21 to 30 years age group onwards. The results indicate that the age when $a^*$ reaches the minimum expected value is 35.4 years, with the expected minimum of 27.09.

The $p$ values for comparisons between the groups of the variable $b^*$ show that the age group 21–30 years has a statistically significant difference compared with the age groups 7–12, 13–20, 41–50 and 51–63 and that the age groups 31–40 and 51–63 have a statistically significant difference compared with the age groups 7–12 and 13–20 years. It is observed a decreasing behavior of the variable $b^*$ from the age group of 21–30 years onwards. The results enable one to infer that the age where $b^*$ reaches the maximum expected value is the age of 40.9 years, with a maximum value of −68.12.

<table>
<thead>
<tr>
<th>Age groups</th>
<th>13–20</th>
<th>21–30</th>
<th>31–40</th>
<th>41–50</th>
<th>51–63</th>
</tr>
</thead>
<tbody>
<tr>
<td>7–12</td>
<td>0.995</td>
<td>&lt;0.001</td>
<td>0.084</td>
<td>0.994</td>
<td>0.002</td>
</tr>
<tr>
<td>13–20</td>
<td>0.001</td>
<td>0.103</td>
<td>0.999</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>21–30</td>
<td>0.059</td>
<td>0.002</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31–40</td>
<td>0.134</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>41–50</td>
<td>0.006</td>
<td></td>
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<td></td>
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</tbody>
</table>

$p < 0.05$ = statistically significant difference. Bold numbers mean statistically significant difference.

From Fig. 3 was observed that the age where it reaches the maximum expected fluorescence total is the age of 26.5 years, with the expected maximum value of −141.29. There is a very strong similarity within different measurements that used the same unit, and the degree of similarity was 99.17%.

4. Discussion

Enamel, dentin, cementum and pulp undergo changes with age [14–23]. Some increase in volume based on the cellular activity that occurs in dentin, a fact which leads to a different pattern of light reflection in the teeth of elderly when compared with the teeth of young individuals and consequently the correspondent color change occurs [24,25,5,26–28]. Based on these facts studies have arisen correlating color changes with the age factor using digital analysis with color system software [7,10,29–32].

According to this rationale there is the fluorescence, a phenomenon which arises when an object is illuminated with a short wavelength light source (1–400 nm) invisible to the naked eye and light is emitted with a long wavelength 430–450 nm, visible light [33,34]. The structure of the natural tooth fluorescence when exposed to ultraviolet light (UV), which after penetrating the enamel, excites the dentin's photosensitivity [32–34]. Thus, age-related dentinal alterations that change dentinal coloring also affect the phenomenon of fluorescence. It is likely that auto fluorescence of dentin may be used as a reliable indicator of maturation of the human body [32–34]. The present study assessed the variations of fluorescence in different age groups by evaluating digital images using software that was designed for tooth whitening analysis [35].

The phenomenon of fluorescence in the tooth structure caused by UV radiation during the day makes the teeth whiter and brighter and to photograph the blue light beam special lenses and filters must be used. In dark places, where the only light source is an ultraviolet lamp bulb known as “black light,” dental fluorescence becomes more evident, because UV radiation is absorbed by the tooth and the fluorescence returns as a beam of blue light [19,32,35], visible phenomenon (wavelength 430–450 nm) [2,33,36] and that can be photographed with normal lenses.

In this study the only environmental light source was artificial UV light – black light – because of this the images obtained of the central incisors were blue. The teeth selected for this study were the maxillary central incisors due to easy photographic access. Furthermore, there is no difference in fluorescence intensity between the types of teeth from the same individual [36]. The images were analyzed using the ScanWhite DMC/Darwin Systems – Brazil software, and the values of $L^*$ (brightness), $a^*$ (red–green), $b^*$ (blue–yellow) according to the CIELAB system were measured.
When the total fluorescence was analyzed the following behavior was observed: the age group 21–30 had a statistically significant difference when compared to the age groups 7–12 and 13–20 and among these age groups the fluorescence remained constant, however there was a statistically significant difference with the other age groups. The fluorescence of age group 41–50 was significantly different from that of the age group 21–30 years. The age group 51–63 years presented a statistically different difference when compared to other age groups studied. One could say, in a global vision, that the total fluorescence presents a similar and stable behavior from 7 to 20 years, reaches its maximum value at the age of 26.5 years and then decreases when we compare the age groups 21–30, 31–40, 41–50 and 51–63. The data indicate a correlation between age and tooth fluorescence which on average decreases after 26.5 years. According to Matsumoto et al. [36] the fluorescent substance is produced as a result of a physiological aging process, but they affirm that the intensity of fluorescence increases with age. Vanini [32] states that the higher the mineral content, the lower the degree of fluorescence. Two changes in dentin occur with age: continuous growth, known as physiological formation of secondary dentin, which reduces the pulp chamber, and the gradual growth that refers to obliteration of dental tubules [28,30]. Such process characterizes a progressive increase in the mineralization of dentin with aging and explains the results of this study.

A descriptive analysis of the fluorescence of the age groups among which there was statistically significant difference shows that the age group 7–12 years had a minimum total fluorescence of −166, maximum of −133 and a mean of −150.9. And individuals aged from 13 to 20 years a minimum of −161, maximum of −138 and a mean of −151.2. The age group 21–30 years had the following value for total fluorescence: minimum of −161, maximum of −111 and mean of −135. Finally, the age group 51–63 years had the following values of total fluorescence: minimum of −227, maximum of −150 and a mean of −180, when the methodology proposed by this study was used.

A visible color has three dimensions L*, a* and b*. Therefore, it is important to discuss the values found in this study for these three variables, which make up the total fluorescence, in the different age groups. Firstly the variable b*, the color range that goes from blue to yellow. It was observed that there was no statistically significant difference between the age groups 7–12 and 13–20 years (p = 0.933) regarding the value of b*. However, there was a statistically significant difference between those two age groups and the 21–30 years age group (p < 0.001). There was also a statistically significant difference in the value of b* when the group 21–30 years was compared to the group 41–50 years (p = 0.018) and with the group 51–63 years (p = 0.034). The peak of value b* is reached in the 21–30 years group, and it significantly decreases in the other two subsequent groups. The descriptive analysis of variable b* revealed that for the 7–12 and 13–20 years of age group there was a statistically significant difference, compared to the other groups. The 7–12 years group showed the following values of b*: minimum of −79, maximum of −65.8 and mean of −74. The individuals aged 13–20 years had the following values: minimum of −80.3, maximum of −65.9 and mean of −73.9. The age group 21–30 years had the following b* value: minimum of −72.8, maximum of −55.4 and mean of −65.7. The ages 51–63 years had a statistically significant difference when compared with the 7–12, 13–20, 21–30 age groups and the following b* values: minimum of −78.9, maximum of −63.3 and mean of −69.8.

The other variable analyzed in this study, the variable L* (lightness) and the variable a* presented variations that were correlated with age. The brightness had a similar behavior from 7 to 20 years and reaches the expected maximum value around 29.2 years. There was a statistically significant difference between the age groups of 21–30 and 7–12 years of age (p < 0.001) and between 21–30 years and 13–20 years of age (p = 0.001). The brightness decreases from age 30 years until 63 years. A statistically significant difference was observed between the age group 51–63 years and the other age groups. Likewise, between the age groups of 41–50 years and 21–30 years (p = 0.002). The descriptive analysis shows that variable L* in the age group 21–30 years had the following values: minimum of 48.1, maximum of 66.3 and mean of 56.1. And for the 51–63 years age group, the values were: minimum of 36.5, maximum of 53.1, and a mean of 45.3 with a standard deviation of 4.1, when using the methodology proposed by this study.

The variable a* behaved in a similar manner from 7 to 20 years, decreased until the age group of 21–30 years, presenting a statistically significant difference between the age groups 7–12 years and 12–20 years (both p < 0.001). It then decreased from age 30 until age 63, with a statistically significant difference between the age groups 51–63 years and 21–30 years (p = 0.003). 41–50 years and 21–30 years (p = 0.016) and between the age groups 31–40 years and 21–30 years (p = 0.080). This variable in the 21–30 years age group was statistically significantly different when compared to the other groups, except for the age group 31–40, and it was observed that individuals aged 21–30 years presented the following a* values: a minimum of 12 and maximum of 34.4, a mean of 23.6 with a standard deviation of 7.8.

It is necessary to establish a numeric value – minimum and maximum – for dental fluorescence for each age group. There are basically two types of equipment available to characterize the color of an object, the colorimeters and spectrophotometers. The Scanwhite is a colorimeter that is based on the RGB system and evaluates the color of digital photos, which creates another possibility of error, which is the quality of the picture and how it was taken. According to Clavijo et al. [35] one of the main challenges for the development of Scanwhite was developing the routine of standardization of photographs, since the reliability of the results depends on this factor.

However, the photographic method provided by Scanwhite is easy to apply, an aspect that favors its use as a method to estimate age using tooth fluorescence, in the field of forensics as suggested by Melchialdes and Boschi [8] who claim it is important to determine the equipment most compatible with the specific needs of each user. This study presents a table with mean values of the variables (total fluorescence, b*, L* and a*) which have the potential to estimate the age of living individuals (Table 3). It suggests the use of mean values of the variables and proposes future validation in a forensic sample.

### Table 3

<table>
<thead>
<tr>
<th>Age</th>
<th>Total fluorescence</th>
<th>b*</th>
<th>L*</th>
<th>a*</th>
</tr>
</thead>
<tbody>
<tr>
<td>7–12</td>
<td>−150.9 ± 9.6</td>
<td>−74.0 ± 4.4</td>
<td>50.2 ± 2.6</td>
<td>33.1 ± 4.7</td>
</tr>
<tr>
<td>13–20</td>
<td>−151.2 ± 7.2</td>
<td>−73.9 ± 3.8</td>
<td>50.2 ± 2.3</td>
<td>33.2 ± 4.7</td>
</tr>
<tr>
<td>21–30</td>
<td>−135 ± 16.6</td>
<td>−65.7 ± 6.0</td>
<td>56.1 ± 5.1</td>
<td>23.6 ± 7.8</td>
</tr>
<tr>
<td>31–40</td>
<td>−146.5 ± 16.8</td>
<td>−67.9 ± 4.4</td>
<td>52.9 ± 3.9</td>
<td>28.0 ± 6.1</td>
</tr>
<tr>
<td>41–50</td>
<td>−157.3 ± 22.4</td>
<td>−70.8 ± 3.2</td>
<td>50.2 ± 4.7</td>
<td>30.3 ± 3.0</td>
</tr>
<tr>
<td>51–63</td>
<td>−180 ± 19.5</td>
<td>−69.8 ± 4.1</td>
<td>45.3 ± 4.1</td>
<td>31.2 ± 6.4</td>
</tr>
</tbody>
</table>

Bold numbers mean statistically significant difference.
References

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