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Investigation of some Parameters Influencing the Sensitivity of Human Tooth Enamel to Gamma Radiation using Electron Paramagnetic Resonance

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EPR Dosimetry/Tooth Enamel/Sensitivity.

Electron paramagnetic resonance (EPR) has been successfully used as a physical technique for gamma radiation dose reconstruction using calcified tissues. To minimize potential discrepancies between EPR readings in future studies, the effects of cavity response factor, tooth position and donor gender on the estimated gamma radiation dose were studied. It was found that the EPR response per sample mass used for assessment of doses in teeth outside of the 70–100 mg range should be corrected by a factor which is a function of the sample mass. In the EPR measurements, the difference in sensitivity of different tooth positions to γ-radiation was taken into consideration. It was determined that among all the pre-molars and molars tooth positions, the relative standard deviation of sensitivity was 6.5%, with the wisdom teeth and the first molars having the highest and lowest sensitivity to γ-radiation, respectively. The current results reveal no effect of the donor gender on the sensitivity to γ-radiation.

INTRODUCTION

The estimation of individual gamma radiation doses is an important task when investigating the effects of radiation exposure on health, particularly in the radiation-epidemiological regions. Successful dose reconstructions have been performed using electron paramagnetic resonance (EPR) spectrometry. The EPR technique works by reconstructing the radiation dose absorbed by calcified tissues (teeth and bones). The crystalline hydroxyapatite (HA) contained in the teeth and bones produces CO_2^- free radicals upon exposure to ionizing radiation. The high concentration of HA in human tooth enamel makes it the most suitable calcified tissue for EPR retrospective dosimetry. The principals of EPR dosimetry can be found in detail in the literature, for example.[1,2]

The EPR technique has been used to estimate past accumulated dose in different studies. EPR studies of irradiated tooth enamel were first undertaken by Brady et al.[3] and Cevc et al..[4,5] Ikeya et al.[6] used EPR studies to reconstruct the doses of Hiroshima and Nagasaki A-bomb survivors. After the Chernobyl accident, the EPR technique was used extensively to assess the absorbed doses in workers,[7,8] liquidators (clean-up workers)[9] and the general population in the contaminated regions.[10] The applicability of the EPR dosimetric technique was extended to study the background radiation in uncontaminated regions[11–13] and in radiation-contaminated areas like Techa River in Russia.[14] The success of EPR dosimetry using tooth enamel has not been limited to dose reconstruction but has been extended to many fields, for example radiotherapy[15] and geological and archaeological dating.[16]

This success motivated some researchers to study the parameters influencing EPR dosimetry[17–20] and the factors influencing the accuracy of the estimated EPR dose, especially at low dose ranges. One of these studies was a series of comparisons between fifteen of the most respected EPR laboratories in the world, which provided the most important results published in the field of EPR dosimetry using tooth enamel.[21–23] This study identified problems with assessing native doses and dose levels less than 79 mGy where no standard deviations less than ± 50 mGy were obtained. As well, some of these laboratories had problems in the methodology of dose assessment. After excluding the extreme results, only five laboratories measured 176 mGy doses with an uncertainty < 30% while many others had relative deviations up to 86%.[24] Therefore, more studies are required to clarify the sources behind these discrepancies and to improve the accuracy of EPR dosimetry techniques.

The current study seeks to gain insights into some factors which can help to improve the accuracy of assessing the

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radiation dose by the EPR technique. The effect of the cavity response function on the EPR signal intensity was investigated. In addition, the influence of the tooth's position, and donor gender on its sensitivity to γ-radiation was also studied.

MATERIALS AND METHODS

Sample identification

During a three month period, one dentist removed seventy-five teeth for medical reasons from patients living in Cairo, Egypt. For this study, healthy permanent teeth from patients in excellent physical condition were selected. Canines and incisors were excluded. Twenty-eight teeth of the original sample population met these criteria. The twenty-eight selected teeth included a wide range of tooth positions and ages. The tooth positions included all the pre-molar and molar positions and the range of donor ages was between 11 and 54 years for males and between 11 and 59 years for females. The sample teeth had not been exposed to medical or occupational radiation.

Sample preparation

To obtain pure enamel without dentine, the crown was cut off from the tooth by means of a circular saw blade after using a drill to remove any tooth fillings and marks of diseases (dark spots). To remove the crown dentine, the crown was washed with acetone for 5 minutes, 0.1 M Titriplex III solution for 15 minutes, and 5 M NaOH solution for 15 hours. These washings were done in an ultra-sonic bath (27 KHz) at increasing temperature from 40 to 60°C due to self heating. Then, the enamel fragments were crushed gently to 65–125 μm grain size and etched with 20% acetic acid under shaking for 5 minutes at room temperature. The grains were washed with isopropanol and dried under vacuum for one day at 40°C.[18]

Sample irradiation

Each enamel grainy sample was placed in a snap top plastic vial (length: 5 cm and diameter: 9 mm) and was irradiated with different doses (0.1, 0.5, 1 and 10 Gy). The phantom box has a rectangular surface shape with 0.5 cm thick walls on all sides. The inner space of the phantom box was 1 cm in the direction of the beam with a surface area of 9×9 cm^2 perpendicular to the beam. The distance from the ^{60}Co source to the phantom was one meter and the radiation field had a size of 12×12 cm^2. The irradiation process was achieved using a γ-^{60}Co-source (Type Eldorado) with an air kerma rate of 37.5 mGy/min at the outer surface of a Plexiglas phantom. The air kerma rate was measured with an accuracy of ± 2.1% (2σ) and converted to an absorbed dose in hydroxyapatite using a factor of 0.99.[25] The non-uniformity of the dose rate in the phantom was measured with alanine dosimeters and was found to be less than ±1% over the field size of 9×9 cm^2. Therefore, the relative standard deviation of absorbed dose is about 1% and to have accurate results for the estimated EPR dose, the uncertainty calculations of the absorbed dose should include this error source. The irradiation process was performed at the GSF-Research Center for Environment and Health, Neuherberg, Germany.

EPR measurements

The EPR spectrum of each enamel sample was taken 36 days after the irradiation process. In order to average out the residual anisotropy of powder samples and variations in the sample positioning in the microwave cavity each sample was recorded repeatedly three times with removal of the sample tube and sample shaking after each run. The enamel samples were placed in pure quartz tubes of inner diameter 3 mm. EPR spectra were recorded at room temperature with an EPR spectrometer (Bruker ECS106) operating in the X-band (~ 9.5 GHz) for microwave cavity (TMH 9402). The sample tube was placed in the magnetic cavity so that the enamel samples were in the centre of the cavity. The following EPR spectrometer parameters were utilized: microwave power: 25.3 mW; modulation frequency: 50 kHz; modulation amplitude: 0.145 mT; conversion time: 82 ms; time constant: 164 ms; magnetic field sweep: 5 mT; sweep time: 84 s; number of scans: 40.[18]

The analyses of the EPR spectra were carried out with the DOSIMETRY software package developed by GSF and IMP institutes. This software is a modification of the Koshta-version[14] and is used at GSF for X-band EPR-dosimetry with tooth enamel. Using the DOSIMETRY software, EPR spectra can be deconvoluted either by linear combinations of Gaussian functions and/or by simulated powder spectra, which need to be imported from external simulation programs. SimFonia software package (Bruker) was used to simulate the powder spectra. The deconvolution of EPR spectra was based on the results of Vanhaelewyn et al..[26,27] For spectrum deconvolution, the irradiated spectrum can be described as a superposition of an axial species with g = 2.0052 and broadening parameter 0.47 mT and two hydroxyapatite CO_2^- signals, one quasi axial with g = 2.0019 and line width 0.31 mT, and the second isotropic with g = 2.005 and line width 0.18 mT. The superposition leads to a little change in the g-value.

EPR spectra of irradiated molars with well known γ-doses were used for calibration purposes. These calibrated molars were extracted for medical reasons from different tooth positions with non historical exposure to medical or occupational radiation. These calibrated molars were used in the form of 65–125 micron grains and irradiated under the same conditions as in this study.

Accuracy of EPR measurements

The sources of errors which influence the accuracy and reproducibility of quantitative EPR measurements have been described in detail in many publications.[17,28,29] The error sources can be divided into primary and secondary sources. The first group includes sample-associated factors (preparation, sample physical characteristics, and positioning within the microwave cavity) and instrumental-associated factors (EPR set-up parameters, spectrometer sensitivity, and cavity Q-factor). The second group includes the data processing, EPR calibration, and human- associated factors. All these factors may cause significant systematic and/or non-systematic errors in quantitative EPR measurements. To minimize these errors, the following steps were taken; (1) the front teeth, incisors and canines, were excluded because the previous work proved a non linear correlation between their dose absorbed in enamel and their tooth age, (2) all

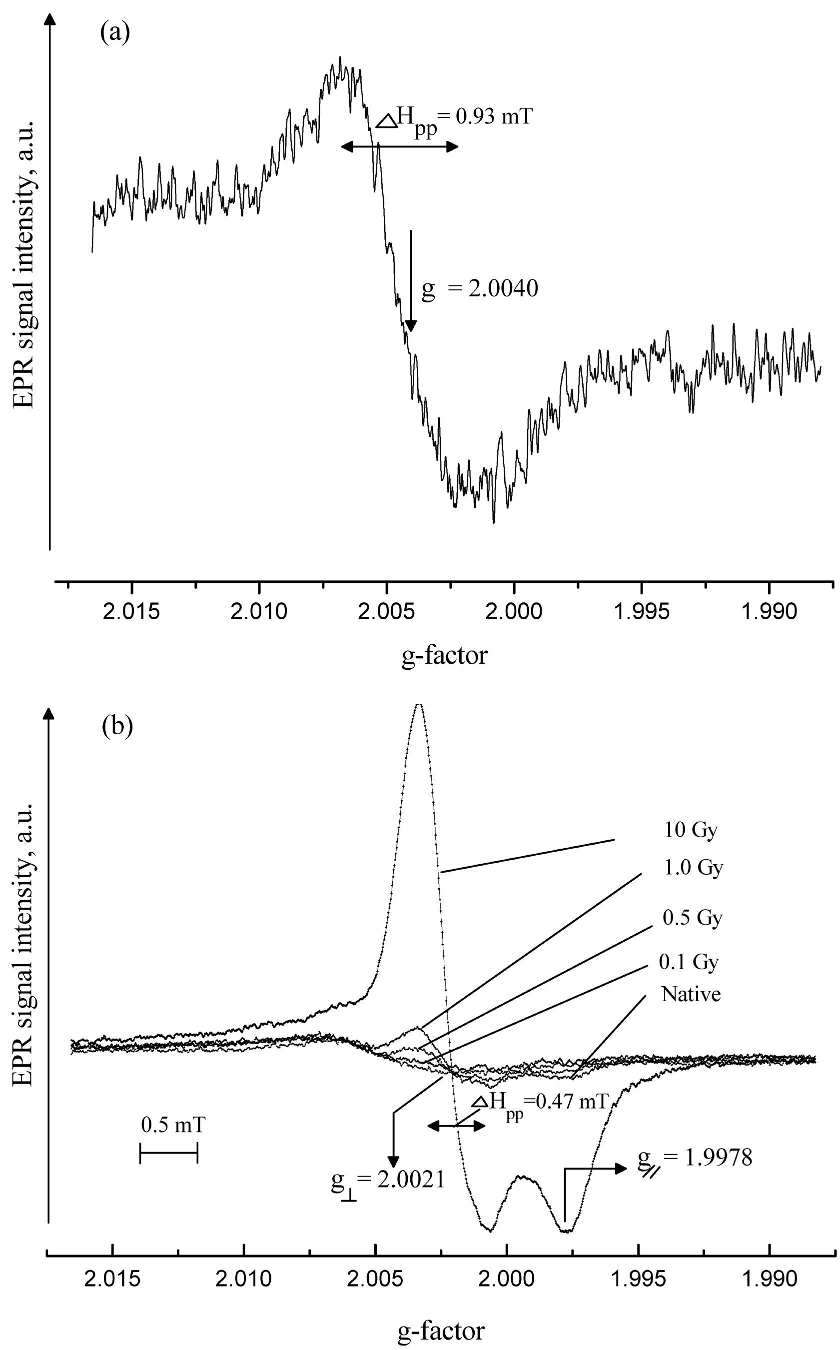

Fig. 1. The EPR spectrum of tooth enamel at (a) the native dose and (b) different ^{60}Co-γ-doses. The spectra display a fixed peak-to-peak line width, ΔH_{pp}, and peak position, g, which are indicated.

samples were prepared and measured under the same conditions, (3) the post-recording spectral manipulations were the same, and (4) the native signal was subtracted from the additive dose signals for each sample.

RESULTS AND DISCUSSION

EPR signal analysis

The deconvolution of the X-band spectrum in enamel samples reveals two components for CO_2^- radicals. Other different paramagnetic species are generated in tooth enamel under exposure to ionizing radiation, but most of them are unstable and decay in a very short time. More details can be found in many publications, for example.[30,31] The CO_2^- radicals are generated from CO_3^{2-} ions present in the hydroxyapatite crystals (3% by mass CO_3^{2-}).[32] The first component is due to CO_2^- radicals found at the surface of the enamel micro crystallites and comprises an orthorhombic signal with a Lorentzian line shape (g_x = 2.0032; g_y = 1.9972; g_z = 2.0019 and line widths of 0.20, 0.21 and 0.2 mT, respectively). The second component is produced by CO_2^- radicals located in the bulk of the micro crystallites and comprises a quasi-axial signal with a Gaussian line shape (g_x = 2.0027; g_y = 1.9972; g_z = 2.0025 and line widths of 0.46, 0.38 and 0.22 mT, respectively).

The EPR signal of unirradiated samples (native signal) was isotropic with a Gaussian line shape at g = 2.0040 and peak-to-peak width of ΔH_{pp} = 0.93 mT (Fig. 1a). A broad organic signal at g = 2.0087, which is due to organic radicals, is relatively less intense but still present after careful removal of the dentine and roots. The spectra of 10 Gy irradiated samples exhibit a main anisotropic signal peak which is associated with CO_2^- free radicals at $g_{//}$ = 1.9978 and $g_\perp$ = 2.0021 Fig. (1b).

Cavity response function

To investigate the cavity response function, which is dependent upon the geometric positioning of the paramagnetic species within the magnetic cavity, the EPR signal intensity (**SI**) as a function of sample mass (m) was obtained for masses ranging up to 100 mg. The samples were irradiated at 10 Gy to avoid the large uncertainties at low doses. As shown in Fig. 2, for masses between 30 and 70 mg, the value of **SI** increases approximately exponentially with increasing mass. From 70 to 100 mg, the **SI** values show evidence of more stability. By fitting the results with growth exponential function, a constancy in **SI** value was reached using sample mass of 70 mg to 100 mg with regression coefficient r = 0.83. Many findings can be deduced from Fig. 2 as:

(1) **SI** is independent on the sample mass and has a constant value in the range of mass of 70 to 100 mg. The current study could not test the effect for bigger masses on **SI** value where a considerable amount was missing during the experimental procedure. Note that, in general it could not get more than 120 mg from the enamel grains with size of 65–125 µm from healthy molars. However, it is expected that the **SI** will be decreased for sample mass more than 110 mg where the length the sample inside of the cavity will leave the uniform region of the magnetic filed.

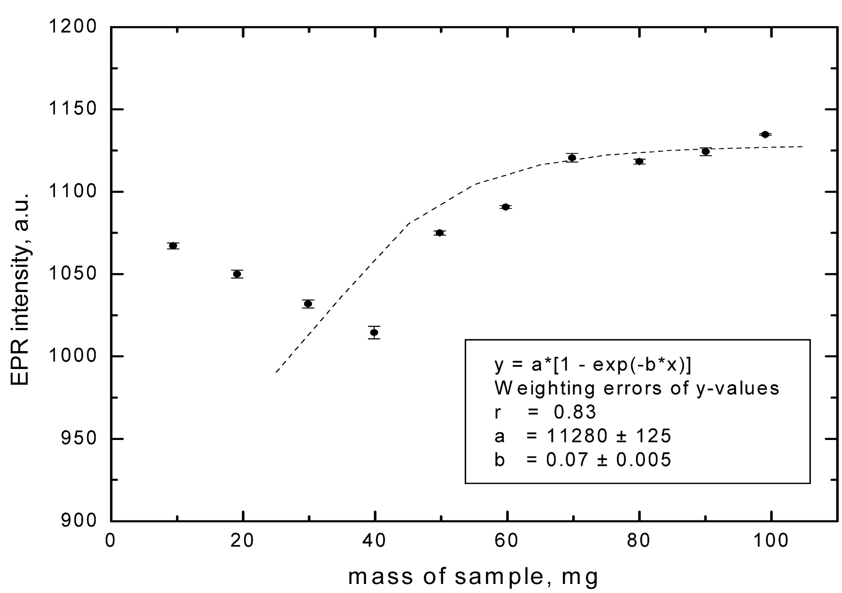

Fig. 2. The dependence of the EPR signal intensity (**SI**) to sample mass (**m**) ratio. After excluding the results at low masses (< 30 mg), the fit curve (dashed lines) exhibits an exponential growth relationship between **SI** and **m**, with a correlation coefficient of 0.825 and an independency on sample mass after 70 mg.

Table 1. Factors at different sample masses for correcting of the non-constant EPR response to sample mass ratio.

Sample mass (mg)	**Correction factor** (RSD in %)	**Sample mass (mg)**	**Correction factor** (RSD in %)
35	1.09 (3.8)	55	1.02 (1.7)
40	1.06 (4.4)	60	1.01 (1.6)
45	1.04 (3.1)	65	1.01 (0.9)
50	1.03 (1.9)	70 ≤ mass ≤ 100	1.00 (1.5)

(2) For sample masses less than 70 mg, **SI** values must be normalized to the steadiness level by multiplying some factor. The values of these correction factors were calculated from the suggested fitting exponential curve and their values are shown in Table 1. Take into consideration that these values are valid for only the used type of EPR spectrometer with certain specifications and it can be changed if the conditions of the experiment are different. These factors are very useful when front teeth or non-healthy teeth are used, in which the enamel produced may be less than 50 mg.

(3) For sample mass less than 30 mg, extreme high values of **SI** had been displayed. The interpretation of this phenomenon can be attributed to one of the two reasons or both. The first one can be pointed to a very low unfavorable signal to noise ratio which causes faults in spectra deconvolution procedures. The second reason can be initiated from the distribution of the generated free radicals in the enamel micro-crystallites. As mentioned in the previous sub-section, γ-radiation generates different types of free radicals, part of them located on the surface and other located on the bulk of the enamel micro-crystallites. In case of suspension of some grains on the surface walls of the used tube, when a small amount of sample is available, the radical intermolecular interactions are so small such that the intensity of the EPR signal is higher than in case when the sample is collected in the bed of the tube. This would result in greater intermolecular interactions and consequently in an EPR signal of low intensity.[33] The net result of these two reasons is increasing in SI values more than its realistic value when low masses are used. To complete the aspect, in the case where all the induced free radicals are distributed uniformly, the net intensity would not differ whether the sample grains were spread over the sample tube or are collected in the ground.

Radiosensitivity and dependence on tooth position

The radiosensitivity **S** of tooth enamel, which is defined as the net ratio of the quantity of the estimated absorbed dose by EPR technique due to the induced paramagnetic centers and the corresponding applied γ-dose, was investigated. The values of **S** for different tooth positions (from the first pre-molar, position-4; to wisdom tooth, position-8) and for different applied gamma doses are listed in Table 2. Each value of **S** in Table 2 was calculated as follows:

1. The measured EPR dose of each sample from three measurements (D_s) is given by:

$$D_s = \frac{1}{3} * \sum_{i=1}^{3} D_i \pm \sigma_s \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where, σ_s is the standard deviation of D_s over three repeatedly measurements for each sample.

2. The native EPR estimated dose ($D_{nat.}$) and its standard deviation ($\sigma_{nat.}$) were also evaluated using equation (1). Therefore, the radiosensitivity (**S**) of any enamel sample can be deduced from equation (1):

$$S = \frac{D_s - D_{nat.}}{D_{ap}} \pm RSD(S) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots..(2)$$

Where, D_{ap} is the applied dose and RSD(S) is the relative standard deviation of **S** due to the different sources of errors and it can be specified as follow:

$$RSD(S) = \left[\left(RSD(D_s)\right)^2 + \left(RSD(D_{nat})\right)^2 + \left(RSD(D_{ap})\right)^2 \right]^{1/2}$$
$$\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots..(3)$$

Where $RSD(D_s)$, $RSD(D_{nat})$ and $RSD(D_{ap})$ are the relative standard deviations of D_s, D_{nat} and D_{ap}, respectively. Equations (3) can also write as:

$$RSD(S) = \left[\left(\frac{\sigma_s}{D_s}\right)^2 + \left(\frac{\sigma_{nat}}{D_s}\right)^2 + \left(\frac{\sigma_{ap}}{D_{ap}}\right)^2 \right]^{1/2} \quad \ldots\ldots\ldots\ldots\ldots (4)$$

3. Consequently, the net value of $\mathbf{S_p}$ for a specific tooth position with an available number of teeth (j = 1, 2, .., N) is given by:

$$S_P = \frac{1}{N} * \sum_{j=1}^{N} S_j \pm \sigma_P \;\ldots\ldots; \qquad \sigma_P = \left[\sum_{j=1}^{N} \left(\sigma_j\right)^2 \right]^{1/2} \ldots\ldots..(5)$$

Where, σ_j and σ_p are the uncertainties for sample (j) and over all the available samples at specific tooth position respectively. The estimated values of **S** for any specific tooth position for each applied dose are tabulated in Table 2. Table 2 includes, also, the mean results of **S** obtained for each tooth position and these results were illustrated in Fig. 3a. The results in Table 2 can be summarized as:

- After excluding the results at 0.1 Gy because of their larger error, for each tooth position, no significant change in **S** values was observed between the irradiated γ-doses from 500 mGy to 10 Gy. The small differences lay within the normal relative standard deviation (5%). The relative standard deviation of the sensitivity of all tooth positions was evaluated to 6.5%.
- The first premolar teeth (position 4) show a higher **S** value among all tooth positions and display more or less uncertainty at 100 mGy. The second premolar teeth show a quite similar behavior but with a lower **S** value and an increase in the uncertainty value at 100 mGy.
- The first molar teeth (position 6) show a lowest **S** value among all tooth positions.
- The wisdom teeth have, approximately, the most constant S-value in whole dose range from 0.1 to 10 Gy.

The above results reveal how the dose-tooth position relationship is an essential parameter for assessing the sensitivity of tooth enamel for γ-radiation. The difference in **S**-value between the different tooth positions can be related to the

Table 2. The radiosensitivity **S** of the different molars and pre-molars for γ-radiation at different doses.

Irradiation dose (Gy)	Radiosensitivity (S)				
	First pre-molar (5)*	**Second pre-molar** (7)	**First molar** (5)	**Second molar** (4)	**Third molar-Wisdom tooth** (7)
0.1	0.97 ± 0.38	1.03 ± 0.56	0.69 ± 1.06	0.61 ± 0.85	0.95 ± 0.64
0.5	1.05 ± 0.10	0.99 ± 0.17	0.88 ± 0.14	0.91 ± 0.14	0.95 ± 0.13
1.0	1.05 ± 0.06	1.01 ± 0.08	0.90 ± 0.08	0.94 ± 0.05	0.97 ± 0.08
10.0	1.05 ± 0.03	1.02 ± 0.03	0.94 ± 0.03	0.95 ± 0.05	0.98 ± 0.03
$S_p^{**} \pm \sigma_p$	**1.05 ± 0.04**	**1.01 ± 0.07**	**0.90 ± 0.06**	**0.93 ± 0.05**	**0.97 ± 0.06**

* The number (N) in brackets represents the number of samples used.
** $\mathbf{S_p}$ is the average of **S** values over all irradiation doses (except that for 0.1 Gy for its high uncertainty) and σ_p is the uncertainty of each specific tooth position.

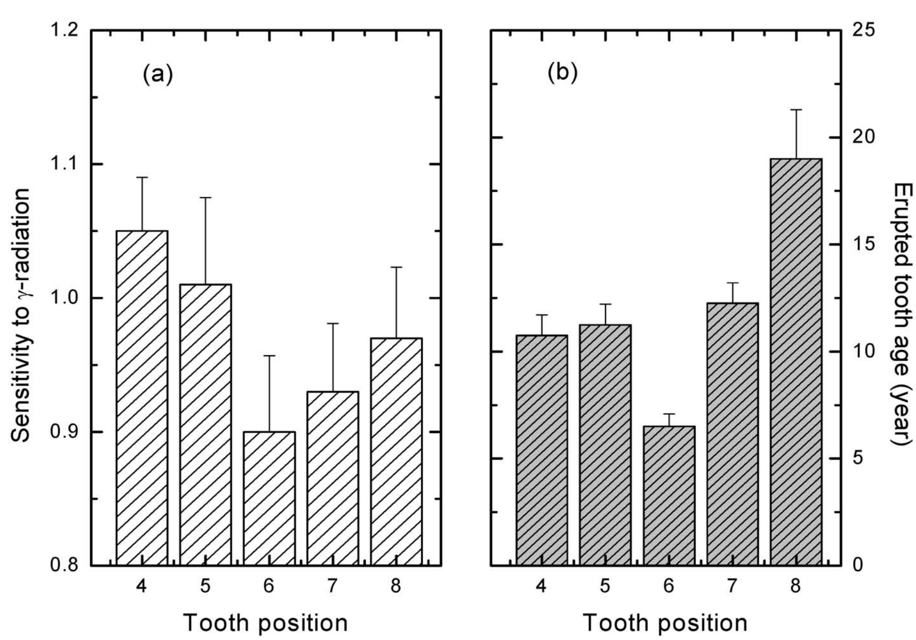

Fig. 3. (a) The dependence of the radiosensitivity **S** of tooth enamel for γ-radiation on both the applied dose and tooth position. The height of each column represents the mean value of **S** over all the applied γ-doses except that of 100 mGy due to its greater uncertainty. (b) The erupted tooth age for the different tooth positions as documented in Ref. 31.

internal chemical composition of each specific tooth and to the stage of the mineralization process of tooth enamel. According to this hypothesis, the wisdom tooth has the most stable **S**-value because its mineralization process has been completed inside the jaw before erupted to the oral cavity. In addition, the ratio between the mean value of **S** of some tooth positions exhibit a similar tendency with their corresponding age of eruption at many situations (see Fig. 3b). Further studies involving larger number of tooth samples from different donors are needed to confirm these findings.

Radiosensitivity and dependence on the gender of tooth donor

The effect of the gender of the tooth donors was investigated as well. The examined tooth samples were chosen, from the first moment, such that equal numbers (14 samples) from both genders were included. The net value of **S** for each gender type was estimated as an average of **S** for all female/male samples, regardless to their positions, by means of equation (4) with $N = 14$. The results reveal a similar value of **S** between male and female teeth for γ-radiation (i.e. 0.97 ± 0.04 and 0.98 ± 0.05, respectively). No obvious effect of the donor gender on the sensitivity to γ-radiation was found.

CONCLUSIONS

In the application of EPR spectroscopy for dose reconstruction some important factors are currently not well investigated and have not been taken into account so far. Consideration of these factors can enhance the accuracy of EPR estimated dose values and should be considered in the protocols of EPR laboratories. The current study investigated some of these parameters: cavity response function, tooth position, tooth age and donor gender.

Theoretically, the signal intensity per unit mass should be constant when the sensitivity of the cavity is constant. However, the practical work was coincident with this theoretical aspect only in the part of a mass range of about of mass > 70 mg, while below this range some factors disturb the signal intensity per mass values. The effect of these disturbing factors is so high at very low masses (mass < 30 mg). The origin of these factors needs more investigations with enough number of tooth samples. Consequently, the present results recommend the scientists working in the field of EPR dosimetry use enamel grain samples of masses in the range of 70–100 mg. In some cases, the enamel produced may be less than 50 mg like in case of not healthy teeth or in case of front positions (incisors and canines). In these conditions, the EPR estimated dose must be modified with some factors depending on the available sample mass.

It was also shown here that the tooth position may play an essential role in the discrepancies between the EPR estimated dose values. The first pre-molar and the first molar teeth exhibited the highest (over estimation) and lowest sensitivity, respectively, with difference of 16%. The sensitivity of the second pre-molar is the most one close to the unity between the other positions; so, it can be taken as a reference. The discrepancy in sensitivity between the different tooth positions might be related to the age of tooth eruption.

Further studies involving a larger number of tooth samples with different donor ages are needed to have a reasonable time range to investigate the period of post-eruptive enamel maturation for different tooth positions ages and also teeth from different positions including the incisors and canines.

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