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Infrared investigation of hard human teeth tissues exposed to various doses of ionizing radiation from the 1986 Chernobyl accident

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Abstract. Infrared spectroscopy (IR) was applied to study changes in solid teeth tissues of persons exposed to low (0.12–0.20 Gy) and high (0.5–1.7 Gy) doses of ionizing radiation during their work in the Chernobyl zone after the accident. Changes in the inorganic and organic matrix of teeth were noted for both high and low radiation doses. The obtained results demonstrated that high doses of radiation lead to imbalance between phosphate–carbonate phases level (because of increasing of CO_3^{2-} content) and accumulation of soluble phosphates in the mineral part of the teeth. These changes have an effect on dental matrix strength. Low doses of radiation do not induce appreciable negative changes in the mineral part of all tooth tissues but lead to changes in organic matrix of teeth (in collagen).

The present results demonstrated that pathological effect of radiation touches upon all dental tissue and obviously all bone systems of irradiated people.

Keywords: Radiation doses and high (0.5–1.7 Gy), radiation doses and low (0.12–0.20 Gy), teeth tissues, infrared spectroscopy, inorganic matrix, organic matrix

1. Introduction

After the catastrophe with Chernobyl reactor IV in 1986, a certain part of the Ukrainian population inhabits a territory contaminated by radioactive nuclides. As a result of the contamination with radioactive nuclides, there is a constant necessity for people to undergo screening. It was observed that people, who took part in the reconstruction works and maintaining in the Chernobyl zone, were suffering from atypical dental problems: pathological dental abrasion, cuneiform defects, enamel erosion and enamel scissuras. The main pathology was additionally worsening while carious changes occurred, although they were not directly related to irradiation.

Monitoring of dental health of more than 1500 people, who were involved in reconstruction works in the area of Chernobyl power station during the first years after catastrophe, have demonstrated an increasing level of unspecific teeth diseases and periodontal problems after 10 years of taking part in this

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work [7]. Moreover, there is also information available in the open literature about an early age development of osteoporosis among the population living on territories contaminated with radioactive nuclides [6,11]. During a specific medical examination of men with an acute radiation syndrome or chronically exposed after the Chernobyl accident [13] decreasing of bone formation and osteopenia with low bone metabolism were detected. Osteopenia is a result of rebuilding of bone tissue metabolism, which concerns especially osteoblasts, as a reaction on metabolic changes in human organism after influence of high doses irradiation. Another symptom, such as high damage (the II/III level) of parodontium was also detected for people with an acute radiation syndrome [10].

The effect of irradiation on the susceptibility of "radiation caries", the structural changes of teeth enamel and dentin of roots was studied by Zhang et al. [12]. They applied SEM (secondary electron microscope) to register visual changes within the teeth structure. Moreover, they tested also the resistance of collagen fiber to the acidic environment. It appeared that high dose irradiation caused fairly extended damage not only in enamel structure and dentin morphology, but also towards collagen – some fibers vanished and dental tissues resistance to acids was fairly reduced.

The task of our investigation was to study and compare processes which take place in solid teeth tissues of persons exposed to a low and high doses of ionizing radiation during their work in the Chernobyl zone. The most efficient techniques to achieve information about molecular structure of biological tissues are IR and Raman spectroscopy [4]. We used the infrared spectroscopy to study samples of dental tissues.

2. Sample preparation and conditions of experiment

Tooth tissues (enamel, cement, dentin, alveolar) of people who absorbed low (0.12–0.20 Gy, low dose group, LDG, 27 patients) and high (0.5–1.7 Gy, high dose group, HDG, 25 patients) doses of ionizing radiation during the first months – one year of work in the Chernobyl reactor IV zone were examined. It must be emphasized that only patients without bone marrow affection were selected. For comparison the same tooth tissues of people who had not been exposed to radiation (control group, CG, 10 people) were also studied. Samples of teeth were first washed with distilled water and dried at 50°C. After that enamel, dentin, cement and alveolar were cut off and powdered with a diamond dental drill. The powder of each tooth tissue was mixed with vaseline oil and put in a cuvette of KBr. All spectra were obtained by one-beam infrared spectrometer IKS-31.

To minimize spectral components due to absorption of CO_2 and H_2O in the air, we used air exhaust pump to provide a spectral measurement at low vacuum (10^{-1} Pa). The infrared intensity transmitted by the cuvette containing the sample (I_s) and that transmitted by empty cuvette (I_o) were measured in the 600–4000 cm^{-1} spectral range with a resolution of 2–4 cm^{-1}. The absorption intensity $I_a(\nu)$, where ν is wavenumber in cm^{-1}, of investigated samples was obtained as $I_a = (1 - I_s/I_o)$, which is approximately proportional to the sample absorption. For a quantitative analysis, the $I_a(\nu)$ spectra were fitted using a standard procedure which describes the $I_a(\nu)$ spectrum as a sum of Lorentzian components [2].

3. Results and discussion

Spectroscopic analysis of teeth tissues was preceded by a standard dental examination of people from the high dose group (HDG) and low dose group (LDG). In the first case, the patients suffered from a high level of pathologocal dental abrasion, cuneiform defects, enamel scissuras and enamel erosion.

Also dystrophic (noncaries) changes of teeth were typical for this group. Additionally it was observed, that enamel and cement were easily split and fragile.

The same pathological changes within a mineral part of teeth were not detected in the group of patients irradiated with a low dose (LDG). The most characteristic dental problems were inflammatory and caries processes in gingiva. From the dental pathological point of view, it is supposed that the interaction between teeth tissue and absorbed radiation goes along different modifications of dental tissues, considering two different profiles of pathological changes. The results of dental medical examination are presented in Fig. 1.

A deeper insight into the histopathology of the dental tissue, on the molecular level, was sought by means of IR spectroscopy. Enamel, dentin and cementum are complex bioinorganic materials which create the hard component of teeth. Enamel is the hardest and the most crystalline part of teeth; it contains about 95–97% of inorganic material. Dentine is surrounded by cementum at the root and a thin protective layer of enamel. Dentin consists of about 70% of inorganic matrix. The structure of the alveolar is very similar to the bone structure.

The mineral part of teeth tissue and bones is represented by apatite material that contains also carbonate components – carbonate hydroxyapatite, with an approximate formula $Ca_{10}(PO_4)_{6-x}(OH)_{2-y}(CO_3)_{x+y}$, where $0 \leqslant x \leqslant 6$ and $0 \leqslant y \leqslant 2$. The $CO_3{}^{2-}$ ion can substitute into two different locations in the hydroxyapatite unit cell, replacing either OH^- or $PO_4{}^{3-}$. The spectral components which correspond to the mineral composition of teeth tissues can easily be identified in the spectral range below 1500 cm^{-1}. A strong band at 600 cm^{-1} belongs to ν_4-PO_4 mode of vibrations. A strong but wide absorption band at 900–1200 cm^{-1} is due to the ν_1- and ν_3-PO_4 vibrations modes. We could not detect absorption bands at 1482 cm^{-1} and 1420 cm^{-1}, which are ascribed to the ν_3-CO_3 mode of vibration, because of the IR absorption properties of vaseline oil. Vaselin oil has the strong absorption bands at 720, 1380 and 1440 cm^{-1}. Nevertheless, the lower intensity absorption band of carbonates at 886 cm^{-1} could be registered for dentine, cementum and alveolar.

The organic matrix of a dental tissue is formed by collagen which controlls the calcification of bone and crystallization of apatite in bones [3]. Collagen molecules consist of 3 polypeptide chains forming

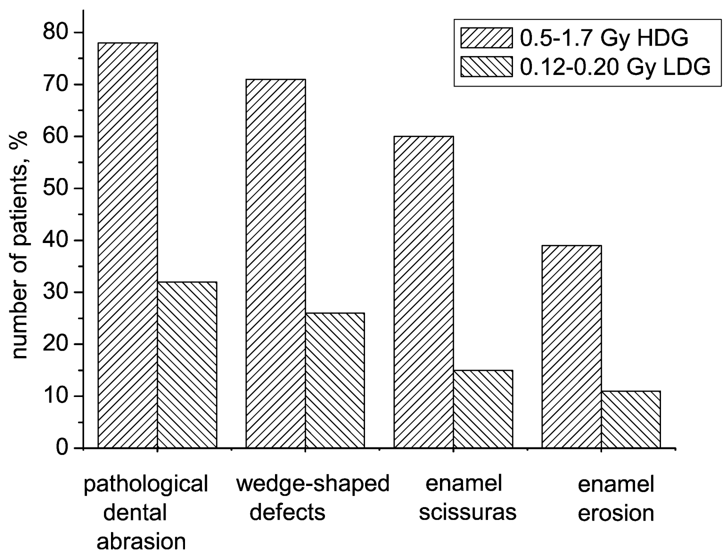

Fig. 1. Frequency of different defects of tooth for person exposed to a high (HDG) and low (LDG) doses of radiation.

turbinal structure. Collagen is rich with glycine, proline and hydroxyproline [8], but the composition of aminoacid of protein depends on the dental tissue type – the ratio of proline:hydroxyproline:glycine is equal to 11:11:55 for enamel and 29:21:62 for dentin [5].

The organic matrix of teeth tissues is represented by amide groups of proteins ($CONH_2$). The amide absorption bands are well detectable at 1660 cm^{-1} (Amide I: C–N, C–C–O, C–N–C vibration), 1550 cm^{-1} (Amide II: C–N and N–H vibration) and at 1240 cm^{-1} (weak band, Amide III: N–H deformation vibration).

Figures 2–4 show IR absorption intensity I_a of enamel, dentin and alveolar, respectively, for people exposed to high and low doses of irradiation. For a comparison, the spectra of unchanged tissues are also presented (control group – CG). All the spectra were normalized to the intensity of the 600 cm^{-1} band (ν_4-PO_4 mode) of each spectrum.

The IR spectra of enamel for all groups consist of strong bands which belong to a phosphate group and very weak bands in the amide region (Fig. 2). A comparison of enamel IR absorption spectra of patients from the control group and people exposed to a low and high doses of irradiation demonstrates only a small increase of the CO_3^{2-} group band at 885 cm^{-1} for the case of high irradiation doses. Obviously the increase of the carbonate content in the enamel is the result of a lack of equilibrium between phosphate and carbonate phases, induced by irradiation. The change in the phosphate–carbonate ratio leads to a decrease of the enamel matrix strength. Spectra of enamel for patients from CG and LDG are very similar, which proves that the influence of low radiation doses on enamel is negligible.

IR absorption spectra of teeth cement are very similar to the spectra of dentin; therefore we show only the spectra of dentin (Fig. 3). They show substantial qualitative changes in the regions of IR activity of both organic and inorganic components after irradiation with high and low doses.

For HDG patients a decreasing content of the mineral part and at the same time an accumulation of the more soluble phosphates (pyrophosphates) was determined. Pyrophosphate groups show of P–O–P bond signals with typical bands at 770 cm^{-1} and 880 cm^{-1}. However, an absorption band of pyrophosphates at 880 cm^{-1} is too close to the one of carbonates at 886 cm^{-1}, and it is difficult to distinguish

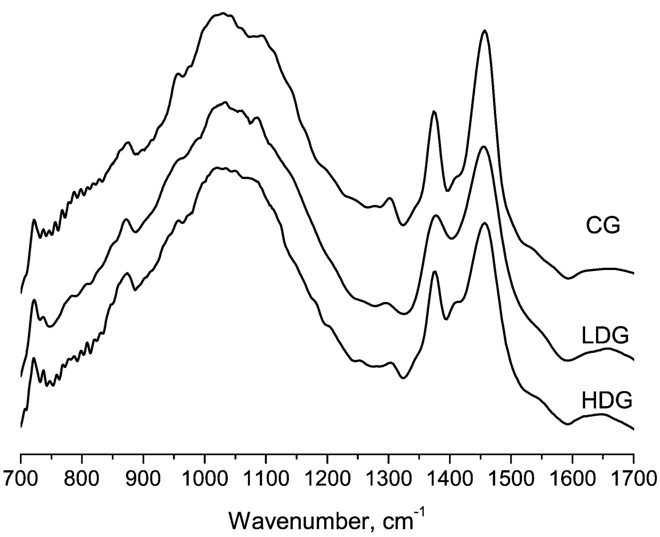

Fig. 2. Typical IR absorption spectra of enamel for patients exposed to a high (HDG) and low (LDG) doses of radiation and people from control group (CG).

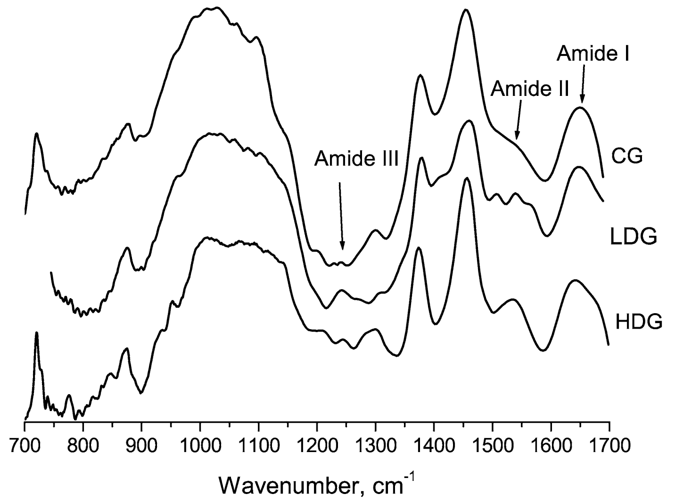

Fig. 3. Typical IR absorption spectra of dentin for patients exposed to a high (HDG) and low (LDG) doses of radiation and people from control group (CG).

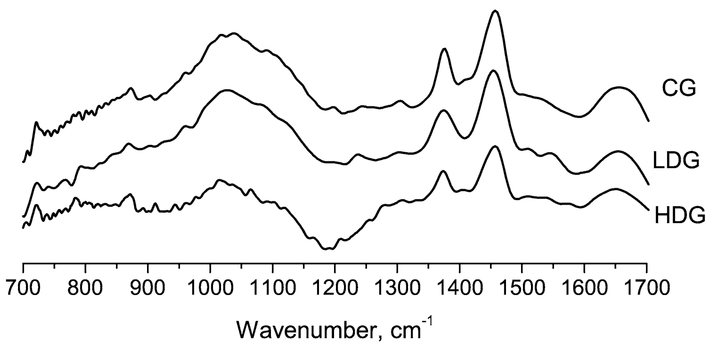

Fig. 4. Typical IR absorption spectra of alveolar for patients exposed to a high (HDG) and low (LDG) doses of radiation and people from control group (CG).

them. Therefore we detected the presence of pyrophosphates in the tooth tissue by an absorption band at 770 cm^{-1}. The appearance of more soluble phosphate forms leads to elimination of these soluble inorganic components from the dental tissues by intercellular liquids and the resorption within the dental inorganic matrix. High doses of radiation have also a strong effect on the organic part of dentine: the ratio between Amide I and Amide II decreases in the dentin tissues. This is clearly visible in Fig. 3, for the absorption intensity at 1660 cm^{-1}, corresponding to vibrational mode of Amide I. High doses of radiation lead also to a decrease of oxyproline and proline levels and to a degradation of the collagen structure.

The instant radiogenic destruction of collagen in dental tissues was demonstrated by Niehoff et al. [9]. According to their hypothesis, there is a destruction of collagen directly in the bone during irradiation. In order to prove that, determination of the bone collagen levels (hydroxylysyl-pyridinoline and lysylpyridinoline) in human urine was performed by Niehoff et al. using high performance liquid chromatography (HPLC). It appeared that the content of these types of collagen in urine increased directly for patients after a course of radiotherapy. This result may reflect either a destruction of collagen or an increased bone resorption after radiotherapy.

Our previous investigations of dried urine spectra for people with acute radiation syndrome (they took part in the reconstruction works in the Chernobyl zone during the first months after the catastrophe) showed an increasing of soluble phosphate elimination from the organism which confirms the existence of osteoporosis process in all bone systems caused by high doses of radiation [1].

For LDG patients qualitative changes of the mineral matrix of cement and dentin are not observed, but we detected changes in the organic part. In Fig. 3, the band corresponding to Amide III (1220 cm^{-1}) becomes stronger in case of the dentin from LDG, which can be ascribed to a higher content for this group. There is also an indication of the changes within signals of Amide II: the band at 1550 cm^{-1} is split into two overlapping weak bands (Fig. 3). This is probably a result of collagen breaking, brought about by radiation.

The pathological changes in the inorganic and organic matrix become especially evident for alveolar (Fig. 4): accumulation of more soluble phosphates (hence, a resorption process takes place in alveolar), imbalance between phosphate–carbonate phases for the HDG patients and changes in the ratio of amide bands for patients from LDG.

4. Conclusion

The present results demonstrate that the pathological effects of radiation touch upon all dental tissue and obviously all bone system of irradiated people. We detected an increase of the CO_3^{2-} content (imbalance between phosphate–carbonate phase levels) in enamel, dentin, cement and alveolar for persons exposed to high doses of radiation. This disorder has an effect on the dental matrix strength. High doses of radiation also lead to mineral composition changes: more soluble phosphate forms (pyrophosphate) are accumulated in the mineral part of the teeth, and afterwards they are washed out by intracellular liquids. As a result of this process, the content of the mineral phase decreases and hard dental tissues are easily split and fragile. On the other hand, low doses of radiation do not induce noticeable changes in the mineral part of all tooth tissues.

Changes of the organic composition of dentin, cement and alveolar were noted for both high and low radiation doses. The alteration of collagen caused by high doses of radiation is evident in the decrease of the Amide I content. The splitting of the Amide II absorption band and the appearance of the Amide III were observed at low doses of radiation. Rebuilding of the collagen matrix can be explained by the changes of amino acids (proline and oxyproline), which are parts of collagen, by radiation.

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