REVEALING EARLY CARIES BY THE PARAMETERS OF ORAL FLUID INFRARED SPECTRA AND CRYSTAL-CHEMICAL COMPOSITION OF DENTAL TISSUES

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The aim of the investigation is to assess the possibility of studying the changes of crystal-chemical composition indices of tissues of the teeth affected by caries (some phosphor-containing metabolites, carbonates, and proteins) according to infrared spectra (IR spectra) of the oral fluid.

Materials and Methods. Patients with various forms of dental caries (n=30) and with an intact dentition (n=30) were studied. Oral fluid was examined using infrared spectroscopy technique. Four parameters were selected as calculation infrared-spectroscopy values, being quotients of the division of the peak heights of the analytic bands of absorption of phosphates, carbonates and proteins of the mixed saliva by each other: P1 — 1070/1017; P2 — 1070/960; P3 — 1070/860; P4 — 1017/860.

Results. According to IR spectrum data it is estimated, that in the oral fluid of patients with various caries forms the content of phosphates decreases and carbonates increases ($p \leqslant 0.05$) in comparison with patients with intact dentitions. Reduction of the protein component of the oral fluid in patients with caries compared to phosphates and carbonates is noted ($p \leqslant 0.05$).

Conclusion. IR spectroscopy of the oral fluid enables early diagnosis of caries and can be used in revealing the disease, objectivizing the diagnosis, planning the extension of stomatological intervention, and evaluation of the efficacy of treatment.

Key words: infrared spectroscopy; early diagnosis of caries.

For early revealing and preventing the development of caries, the commonest disease of mankind [1, 2], a complex of effective diagnostic methods, enabling identification of the lesion at early stages and minimizing its impact on the organism, is necessary [3].

In practical stomatology there is a great number of methods for caries diagnosis, based on visual detection of enamel defects, application of fiber optics with a directed light beam passing at an angle to the tooth surface, X-ray technique for the assessment of the lesion extension [4, 5]. The aim of the modern medicine is to devise diagnostic techniques, which would reduce traumaticity to patients, simplify the examination procedure, and improve the accuracy of diagnosis-making. At present, morphologic and crystal-chemical tooth structure has been studied rather well [6, 7]. It has beens found, that in the enamel, built from the apatite derivatives and a small quantity of organic substances, intensive ion replacements of the constituent mineral components are going at the level of elementary crystal cell [8, 9]. The elements of the apatite crystal lattice are capable of exchanging with ions of the solution surrounding the crystal, and of changing due to the ions present in this solution. This property of apatites makes them highly sensitive to the ion composition of the oral fluid, blood, and intercellular liquid [9, 10]. The process of elements exchange itself runs in several stages with different velocity.

Normal structure and functioning of enamel is maintained by dynamic balance of the processes of demineralization and remineralization, continuously going on between the tooth tissues and oral fluid. When the balance is changed the process of demineralization prevails [10], which is believed, according to modern views, to lie in the basis of the caries development [6–8]. A symptom of early caries is focal demineralization [3, 6, 10]. However, alongside the processes of demineralization in the damaged tissues, active processes of remineralization of various intensity occur in the superficial layer. Knowledge of these processes may help to use new noninvasive methods of caries diagnosis.

Last decades have been characterized by intensive introduction of the latest physical and chemical methods of analysis, primarily spectral ones, into dental

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practice. Methods of atomic absorption spectrometry, flame photometry, spectroscopy in infrared and ultraviolet regions of electromagnetic spectrum are being successfully used. Luminescence and Raman spectroscopy, X-ray spectrum analysis, nuclear magnetic resonance spectrometry and some other techniques are at the stage of practical implementation [4, 11–13].

One of the tasks of medico-biological investigations is establishing empirical interconnection between the characteristic features of the spectra (positions and intensity of absorption bands) of biological objects and pathologic processes occurring in the body or separate organs.

Quite a lot of works are devoted to the study of IR spectra of biological fluids and tissues [11–15]. Spectrophotometric investigation of saliva gland secretion in dental caries was described only in one publication [16], which speaks of the necessity of further studies in this field.

The aim of investigation is to assess the possibility of studying the changes of crystal-chemical composition indices of tissues of the teeth affected by caries (some phosphor-containing metabolites, carbonates, and proteins) according to infrared spectra (IR spectra) of the oral fluid.

Materials and Methods. Sixty people participated in the study: 30 patients with intact dentition (the 1st control group) and 30 patients with various forms of dental caries (the 2nd group of comparison). The diagnosis was established clinically.

The study complies with the Declaration of Helsinki (the Declaration was passed in Helsinki, Finland, June, 1964, and revised in October, 2000, Edinburg, Scotland) and was performed following approval by the Ethic Committee of Nizhny Novgorod State Medical Academy. Written informed consent was obtained from every patient.

The study was performed using IR spectroscopy of the oral fluid [10]. The material was taken by collecting of noninduced saliva into the tube in the quantity of 2–3 ml in the morning on an empty stomach. Then the oral fluid was dried for 2 days at room temperature. The specimen was prepared in the form of suspension in vaseline oil. Absorption spectra were recorded by a Specord IR-75 spectraphotometer (Carl Zeiss, Jena Germany) in the range of the wave numbers 1100–800 cm^{-1}. Four parameters were selected as calculation infrared-spectroscopy values, being quotients of the division of the peak heights of analytic bands of absorption of phosphates, carbonates and proteins of the mixed saliva by each other [9, 11, 12]: P1 — 1070/1017; P2 — 1070/960; P3 — 1070/860; P4 — 1017/860.

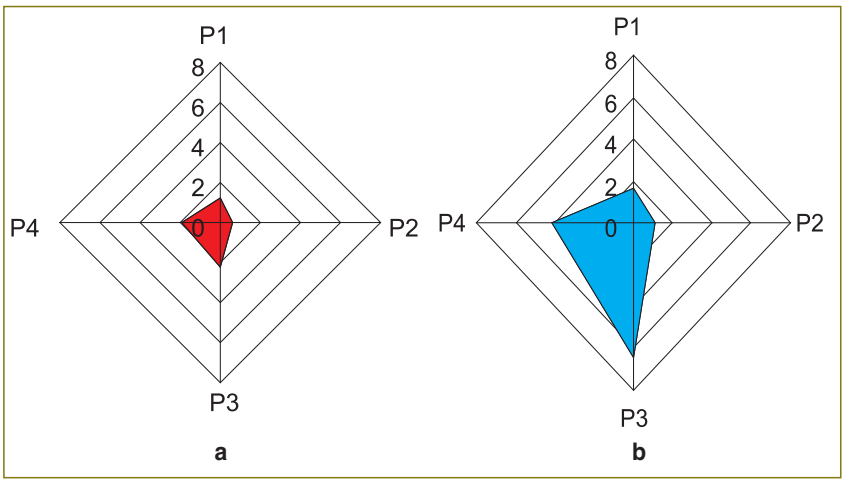
The data obtained were processed by the applied program package Statistica 6.0 and Microsoft Excel using one-dimensional statistics method. The results are presented in the form of M±m, where M is arithmetic mean, m — standard deviation. Confidence of different mean values was determined by Student t-criterion, using Bonferroni correction. Double intragroup and intergroup comparisons of the mean values were also identified according to Wilcoxon and Mann–Whitney criteria. Samples were considered to belong to different total populations at $p \leqslant 0.05$.

Results and Discussion. On the basis of the calculated parameters of oral fluid IR spectra for the control group and the group of comparison differentially diagnostic profiles of the “norm” and “caries” were built (See the Figure).

The Figure clearly demonstrates, how the outlines of the profile are changed in case of caries, its area increases especially in the direction of P3 parameter, the angles of the perimeter sides are altered. And these changes are directly dependent on the severity of the carious process: they are mostly marked in patients with heavy forms of caries. These and other alterations are readily fixed be special computer programs, by means of which objective conclusion on the patient’s condition is drawn.

In the works [9, 11, 12] it is shown that absorption bands of 1070 cm^{-1} corresponds to oscillations of carbonate-ion, 1017 cm^{-1} — oscillations of nonreplaced phosphate-ion, 960 cm^{-1} — oscillations of disordered phosphate-ion, 860 cm^{-1} — oscillations of collagen (an organic component of dental tissue).

In the present investigation increase of parameter values P1 and P2 of saliva IR spectra is noted in the group of patients with caries, i.e. the quotient of the division of the absorption peak height of 1070 cm^{-1} by absorption peak heights of 1017 and 960 cm^{-1} increases, meaning that the content of phosphates decreases in the oral fluid of these patients and the content of carbonates



Differencially diagnostic profiles: *a* — “norm” (control group) and *b* — “caries” (group of comparison)

grows ($p \leqslant 0.05$). These findings are in line with the data on biochemical transformation of hydroxiapatite crystals in the dental tissues in carious process [13] in which the reduction of structural order and crystallicity of hydroxiapatite in dental tissues, as well as the decrease of calcium ion concentration and phosphor content were noted, besides the reverse interrelations between phosphate-ions were also found out.

Increase of values of P3 and P4 parameters of saliva IR spectra in patients from the group of comparison was also found to occur, i.e. reduction of the protein component of the oral fluid was noted in comparison with phosphates and carbonates ($p \leqslant 0.05$), which is confirmed by other works [9, 11], in which, on the basis of calculated characteristics of Raman spectra of dental tissue, the reduction of the organic component compared to the mineral phase was found.

The data obtained show that the occurrence of carious process in the tooth results in the changes of the quantitative composition of the oral fluid components, that is the relationship of phosphates, carbonates and proteins alters. And the character of this alteration corresponds to the degree of the carious process severity. It is interesting to note, that obtaining differentially diagnostic profile of "caries" allows the dentists to diagnose the disease earlier than in visual examination.

Conclusion. IR spectroscopy of the oral fluid enables early diagnosis of caries, and can be used for revealing the disease both at the initial and late stages of its development for objectivizing the diagnosis, planning the extension of stomatological intervention, and evaluation of treatment efficacy.

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