Optical method for assessing the effectiveness of treatment of staphylococcal infection of tonsils

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Abstract. The results of studies of the efficacy of treatment of staphylococcal infection in palatine tonsils using «Amoxiclav» by a method of Raman spectroscopy. Spectral changes in the treatment of palatine tonsils with the antibiotic «Amoxiclav» are revealed. The coefficients allowing to estimate the effectiveness of treatment of staphylococcal infection with antibiotics «Amoxiclav» are introduced. It is established that the antibiotic «Amoxiclav» is more effective when exposed to a strain of staphylococcus culture (I).

1. Introduction

According to the report on the state of sanitary and epidemiological welfare of the population in the Russian Federation, the leading place in the structure of infectious and parasitic diseases in 2016, as in previous years, is acute infections of the upper respiratory tract of multiple and unspecified localization (ARVI). The proportion of which is 84%, for acute tonsillitis - 3.7% [1].

The most significant bacterial pathogen of acute tonsillitis (angina) is the hemolytic streptococcus of group A. Less commonly, acute tonsillitis is caused by viruses and other streptococci, extremely rarely by mycoplasma and chlamydia. The causative agent is transmitted by airborne droplets. Sources of infection are patients, and in some cases carriers of the disease, who do not have obvious symptoms. Chronic tonsillitis is a consequence of repeated angina or insufficient treatment [2].

Treatment of acute tonsillitis is carried out through the use of various antibacterial drugs, the most common among them was the antibiotic «Amoxiclav», consisting of Amoxicillin and clavulanic acid [3].

The reasons for the development of resistance of microorganisms to antibiotics are: unreasonable prescription of antibacterial agents, mistakes in the choice of an antibacterial drug, errors in the choice of the dosing regimen of an antibacterial drug, errors in the combined use of antibiotics, errors associated with the duration of antibiotic therapy [4].

Therefore, monitoring the effectiveness of antibiotic treatment is an urgent task.

To monitor the occurrence of complications and the effectiveness of antibiotic therapy, a number of laboratory studies are necessarily conducted at the beginning and at the end of treatment of acute tonsillitis. This is a general blood test and a general urine test, an electrocardiogram and a biochemical blood test. In most cases, the treating specialists neglect to conduct a laboratory analysis of pharyngeal tonsillar flora. However, in the treatment of tonsillitis it is important to observe the correct concentration of antibiotic for the course of treatment. Usually it is checked by chemical and microbiological methods [5].
At present, optical methods of investigation have been widely used in medical problems [6-11], such as backscattering [6,7], Raman spectroscopy [8], IR spectroscopy [9,10], and confocal microscopy [11].

Raman spectroscopy can also be used to study saliva, with larynx diseases. Thus, it was established in [8] that a decrease in the intensity at wave numbers 635 cm\(^{-1}\) (tyrosine) and 812 cm\(^{-1}\) (L-serine) and an increase of 1619 cm\(^{-1}\) (tryptophan) indicates a change in the amino acids that make up the saliva, and corresponds to the growth of tumor cells in the nasopharynx.

The article [9] considers the conduction of infrared spectroscopy of saliva in patients with clinically diagnosed chronic tonsillitis by determining the average values of the transmittance of infrared radiation in the ranges: 3085-2832 cm\(^{-1}\), 2120-1880 cm\(^{-1}\), 1600-1535 cm\(^{-1}\), 1543-1425 cm\(^{-1}\), 1430-1210 cm\(^{-1}\), 1127-1057 cm\(^{-1}\). Depending on the mean values obtained over several ranges, either compensated or decompensated chronic tonsillitis is diagnosed. This method is painless fast, and can be performed on an outpatient basis.

The aim of the study is to analyze the effectiveness of treatment with «Amoxiclav» of staphylococcal infection in palatine tonsils using Raman spectroscopy.

2. Materials and methods of research

During experiments investigated the 12 samples containing Staphylococcus strain ATCC№29923 (culture I) and ATCC№35591 (culture II), are in saliva of patients and in saline. Half of the samples were treated with an antibiotic, such as «Amoxiclav», the other half was a control.

Samples of the studies were provided by the professor, MD. Zarubina E.G. (Samara Medical University "Reaviz"). The classification of the test samples is shown in Table 1.

| Label | Description                                      |
|-------|--------------------------------------------------|
| 1.1   | 1 ml. saliva + culture (I)                       |
| 1.2   | 1 ml. saline solution + culture (I)              |
| 1.3   | 1 ml. saliva + culture (I) + antibiotic 3 mg.    |
| 1.4   | 1 ml. saline solution + culture (I) + antibiotic 3 mg. |
| 2.1   | 1 ml. saliva + culture (II)                      |
| 2.2   | 1 ml. saline solution + culture (II)             |
| 2.3   | 1 ml. saliva + culture (II) + antibiotic 3 mg.  |
| 2.4   | 1 ml. saline solution + culture (II) + antibiotic 3 mg. |

Spectral characteristics were studied using an experimental stand including the high-resolution digital spectrometer Andor Shamrock sr-303i with the built-in cooled DV420A-OE camera, the fiber-optic probe for Raman spectroscopy RP8785, combined with the LuxxMaster LML-785.0RB-04 laser module adjustable power up to 500 mW, wavelength 785 nm [12, 13]. Processing of Raman spectra was carried out in the program Wolfram Mathematica 8. The investigated spectrum during processing was cleaned of noise by a smoothing median filter (5 points). At the chosen interval of 400-2200 cm\(^{-1}\), using an iterative algorithm by the method of polynomial approximation of the fluorescent component, an approximating line (a polynomial of the fifth degree) of the autofluorescent component was determined, and then this component was subtracted, obtaining a selected Raman spectrum [14].

3. Results and discussion

Figure 1 shows the averaged Raman spectra of the saliva samples (a) and saline (b).
Figure 1 - Averaged Raman spectra of the investigated samples of saliva (a) and saline (b)
Figure 1 shows the spectral changes at wave numbers: 628 cm\(^{-1}\), 667 cm\(^{-1}\), 735 cm\(^{-1}\), 844 cm\(^{-1}\). Changes in the intensity of the lines at wave numbers of 628 cm\(^{-1}\) and 844 cm\(^{-1}\) correspond to the lines of the introduced antibiotic in the samples under study. The line intensities at 667 cm\(^{-1}\), 735 cm\(^{-1}\) wave numbers correspond to changes in the nitrogenous bases of DNA, such as adenine and guanine, contained in staphylococcal strains. In Fig. 1, spectral features are also noticeable for samples with cultures without antibiotic, which manifest themselves in a change in line intensities at wave numbers of 992 cm\(^{-1}\) and 1635 cm\(^{-1}\), which is associated with a change in glycine and proteins. Changes in the intensity of these lines can be used to assess the effectiveness of treatment of staphylococcal infection with the antibiotic «Amoxiclav».

To evaluate the effectiveness of treatment of staphylococcus in the palatine tonsils with antibiotic «Amoxiclav», coefficients (1-4) were introduced. The denominator in the introduced coefficients was the line intensity at the wave number of 1744 cm\(^{-1}\), corresponding to the acetates.

\[
A = \frac{I_{667}}{I_{1744}} \quad (1)
\]
\[
B = \frac{I_{735}}{I_{1744}} \quad (2)
\]
\[
E = \frac{I_{992}}{I_{1744}} \quad (3)
\]
\[
F = \frac{I_{1635}}{I_{1744}} \quad (4)
\]

\(I_{667}\) – the Raman intensity at a wave number of 667 cm\(^{-1}\), proportional to the content of guanine; 
\(I_{735}\) – the Raman intensity at a wave number of 735 cm\(^{-1}\), proportional to the content of adenine; 
\(I_{992}\) – the Raman intensity at a wave number of 992 cm\(^{-1}\), corresponds to glycines. 
\(I_{1635}\) – the Raman intensity at a wave number of 1635 cm\(^{-1}\), corresponds to proteins. 
\(I_{1744}\) – the Raman intensity at a wave number of 1744 cm\(^{-1}\), proportional to the content of acetates. Based on the introduced coefficients, two-dimensional dependences were constructed (Figure 2).
As can be seen from the figure, the addition of an antibiotic causes a decrease in the coefficients $F$ and $B$ for both saliva samples and saline samples. These coefficients show a decrease in the relative concentration of proteins ($1635 \text{ cm}^{-1}$) and adenine ($735 \text{ cm}^{-1}$), indicating a decrease in the concentration of bacteria in the samples and can be used to monitor the effectiveness of treatment. Furthermore, the antibiotic in saliva samples and spectra, and the figure shows a decrease in the coefficient $A$, indicating the relative concentration of guanine ($667 \text{ cm}^{-1}$). In the spectra of saline solution this is not observed, which is possibly connected with overlapping of this line by other stronger lines. A similar situation is observed for the coefficient $E$ showing the relative concentration of glycine: for saline solution it is well resolved with respect to other lines and its decrease is clearly visible, and for saliva it is lost against the background of other peaks.

Table 2 shows the results of the calculation of the change in the relative concentration of staphylococcal infection of different strains under the influence of the antibiotic «Amoxiclav» with the help of the introduced coefficient $B$.

| Samples                                                                 | Coefficient $B = I_{1735}/I_{1774}$ |
|------------------------------------------------------------------------|-------------------------------------|
| 1.1 – 1 ml. saliva + culture (I)                                        | 100.0%                              |
| 1.3 - 1 ml. saliva + culture (I) + antibiotic 3 mg.                     | 3.5%                                |
| 2.1 – 1 ml. saliva + culture (II)                                       | 100.0%                              |
| 2.3 – 1 ml. saliva + culture (II) + antibiotic 3 mg.                    | 6.9%                                |
| 1.2 – 1 ml. saline solution + culture (I)                               | 100.0%                              |
| 1.4 – 1 ml. saline solution + culture (I) + antibiotic 3 mg.            | 0.02%                               |
| 2.2 – 1 ml. saline solution + culture (II)                               | 100.0%                              |
| 2.4 – 1 ml. saline solution + culture (II) + antibiotic 3 mg.           | 0.4%                                |

It can be seen from Table 2 that when the antibiotic is exposed, the values of the optical coefficient $B$ are reduced to 3.5% - 6.9% (depending on the staphylococcus strain) for saliva and up to 0.02% - 0.4% for saline. Antibiotic «Amoxiclav» is more effective when exposed to the strain of staphylococcus culture (I), from 100% to 3.5% and from 100% to 0.02% compared to exposure to the strain of Staphylococcus culture (II), from 100% to 6, 9% and from 100% to 0.4%.

4. Conclusions
Result of the study:
- Spectral changes were detected in the treatment of palatine tonsils with antibiotic "Amoxiclav", which appear to change lines at wave numbers 667 cm\(^{-1}\), 735 cm\(^{-1}\), 992 cm\(^{-1}\), 1635 cm\(^{-1}\), corresponding to guanine, adenine, glycine and proteins.

- The coefficients allowing to estimate the effectiveness of treatment of staphylococcal infection with antibiotic «Amoxiclav» are introduced. It is established that the antibiotic «Amoxiclav» is more effective when exposed to a strain of staphylococcus culture (I).

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