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Palatine tonsils functional reserve in children by immunological indicators evaluation

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Today, indications for tonsillectomy are based mainly on clinical criteria rather than on the functional state of palatine tonsils (PT). Determination of secretory immunoglobulin A (sIgA) levels in the oropharyngeal secretion before and after antigen load on PT is one of the methods for determining their functional capacity. However, this method has certain disadvantages, in particular not only PT were in the area of influence of both specific and non-specific stimuli, but also other lymphoid structures of the pharynx.

The aim of the study is to improve the assessment of the immunofunctional state of PT.

Materials and methods. The authors investigated by enzyme-linked immunosorbent assay (ELISA) sIgA and IFN-α content in oropharyngeal secretions in children with CT before and after antigen challenge vaccine OM-85 and the physical impact of ultrasound frequency of 880 kHz on the palatine tonsils. All the results were statistically processed using U-test (Mann–Whitney–Wilcoxon test) and Fisher's z-transformation.

Results and Conclusion. The proposed method has significant advantages over the existing one, namely it is more selective, and therefore more accurate in determining the functional state of PT.

Increased levels of sIgA and IFN-α in children's oropharyngeal secretions suggest maintaining the functional reserve of the tonsils; the absence of positive dynamics or negative dynamics of this indicator shows a functional failure of PT and can serve as an indication for surgical treatment of children with CT.

The research was carried out in accordance with the principles of the Declaration of Helsinki. The research protocol was approved by the Local Ethics Committee of all the institutions mentioned in the work. Informed consent of the children's parents was obtained for the research.

No conflict of interests was declared by the authors.

Key words: chronic tonsillitis, saliva, tonsillectomy indications, functional reserve.

Функціональний резерв піднебінних мигдаликів у дітей за результатами оцінки імунологічних показників

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Сьогодні показання до тонзилектомії ґрунтуються переважно на клінічних критеріях, а не на функціональному стані піднебінних мигдаликів. Визначення рівня секреторного імуноглобуліну А (sIgA) у ротоглотковому секреті до та після антигенного навантаження на піднебінні мигдалики є одним із методів визначення їхньої функціональної спроможності. Однак цей спосіб має певні недоліки, зокрема, у зоні впливу як специфічних, так і неспецифічних подразників виявилися не тільки піднебінні мигдалики, але й інші лімфоїдні структури глотки.

Мета — поліпшити оцінку імунофункціонального стану піднебінних мигдаликів.

Матеріали та методи. Досліджено вміст sIgA та інтерферону альфа (IFN-α) у ротоглотковому секреті дітей з хронічним тонзилітом методом імуноферментного аналізу до та після антигенного навантаження вакциною ОМ-85 та фізичним впливом на піднебінні мигдалики ультразвуку частотою 880 кГц. Усі результати статистично оброблено з використанням U-критерію (критерій Манна—Уітні—Вілкоксона) та z-перетворення за Фішером.

Результати та висновки. Запропонований метод має суттєві переваги перед існуючим, він є більш селективним, а отже, більш точним у визначенні функціонального стану піднебінних мигдаликів.

Підвищення рівня sIgA та IFN-α у ротоглотковому секреті дітей свідчить про збереження функціонального резерву піднебінних мигдаликів; відсутність позитивної динаміки або негативна динаміка цього показника свідчить про функціональну недостатність піднебінних мигдаликів і може бути показанням до хірургічного лікування дітей з хронічним тонзилітом.

Дослідження проведено відповідно до принципів Гельсінської декларації. Протокол дослідження ухвалено локальним комітетом з етики всіх установ, згаданих у роботі. На проведення дослідження отримано інформовану згоду батьків дітей.

Автори заявляють про відсутність конфлікту інтересів.

Ключові слова: хронічний тонзиліт, слина, показання до тонзилектомії, функціональний резерв.

Introduction

Today, indications for tonsillectomy are based mainly on clinical criteria rather than on the functional state of palatine tonsils (PT). Determination of secretory immunoglobulin A (sIgA) levels in the oropharyngeal secretion before and after antigen load on PT is one of the methods for determining their functional capacity [6]. This method allows doctors to assess the functional state of PT by levels of sIgA secretion before and after antigenic action in the form of bacterial lysate application to the surface of PT and non-specific irritation by an alternating magnetic field (non-specific stimulus) from the neck [6]. In this way, the assessment of the functional state of PT was performed by changes in sIgA levels in saliva: an increase in the concentration of this im-

munoglobulin by more than 30% from basal value was considered positive, which allowed doctors to appoint conservative therapy of chronic tonsillitis (CT). However, this method has certain disadvantages, in particular not only PT were in the area of influence of both specific and non-specific stimuli, but also other lymphoid structures of the pharynx.

There are many old and new proposals for determining the functional state of PT from simple and complex cytological techniques [1] to multifactorial immuno-biochemical analysis of the oropharyngeal secretion, including determining the ratio of cells of different genesis [6]. The basis of the immune protection of mucous membranes is the formation of humoral immunity on their surface, namely in the oropharynx, eyes, upper and lower respiratory tract, and intestines [1]. It is believed that the main local protection factors associated with the palatine tonsils are sIgA, interferon alfa (IFN-α), and non-specific protection factors such as defensins and elafins [9]. So far, the method of determining the changes in sIgA level in saliva before and after antigen load on PT has been considered the most effective one [8]. This method allows doctors to assess the functional state of PT by levels of sIgA secretion before and after antigenic action in the form of bacterial lysate spray application to the surface of PT and non-specific irritation by an alternating magnetic field (non-specific stimulus) from the neck [3]. The assessment of the functional state of PT was performed by changes in sIgA levels in saliva: an increase in the concentration of this immunoglobulin by more than 30% from basal value was considered positive, which allowed doctors to appoint conservative therapy of CT. This method was tested in hospitals in Kyiv, Vinnitsa (Ukraine) and proved its positive role in determining the treatment strategy for patients with CT [7]. However, this method of determining the immunofunctional state of PT in patients with CT showed some disadvantages, and the main ones were as follows:

1) the area of influence of the magnetic field involved the lymph nodes of the neck and blood vessels;

2) the treatment of the surface of tonsils by spraying with microbial antigens was broad-band and poorly guided and affected all lymphoid elements of the pharynx.

Thus, by stimulating additional lymphatic formations, the existing method makes it impossible to separate the reaction of lymphoid tissue of PT from the reaction of other lymphoid formations in this area, and hence to obtain a reliable assessment of the function of PT. New method based on more targeted introduction of antigens into the tonsil tissue, the use of ultrasound directly in contact with the surface of the tonsils, more prolonged exposure to specific and non-specific load on the tonsils, as well as determination of changes in the levels of sIgA and IFN-α, which is actively produced by the lymphoid and nonlymphoid cells [8,9].

The aim of the study — to improve the assessment of the immunofunctional state of PT.

Materials and methods of the research

The study was performed at Otolaryngological Department of OKHMATDYT hospital in Kyiv (Ukraine); immunological studies were conducted in the laboratory of immunology and pathophysiology of the State Institution «Kolomiychenko Institute of Otolaryngology» of the National Academy of Medical Sciences of Ukraine. The study involved children aged 10 to 16 with the diagnosis of chronic tonsilitis. The number of exacerbation episodes was 2–3 per year. There were 12 girls and 8 boys. The control group consisted of virtually healthy children (10 patients) of the same age. The immunofunctional state of PT was determined in two tests: the old one (M_1) with the use of magnetotherapy device Magniter (Ukraine) and microbial challenge of mucosal vaccine (bacterial lysate spray), as described in the Patent «UA 33382A», and the new one (M_n), which is proposed by the authors. In the new method, the bacterial lysate OM-85 was prepared ex tempore at the rate of 3.5 mg of the drug per 1 ml of sterile sodium chloride solution and applied to PT. After its application, ultrasound was applied at a frequency of 880 kHz (LORA-Don apparatus) through the oral cavity directly on the PT surface for 20 seconds once a day for 7 days in a row. The levels of sIgA (ELISA method, Xema-Medica reagents), INF-α (ELISA method, Cytokine reagents), immunoassay analyser Lab-line (Austria) were determined in the saliva, which was collected and prepared for research according to the guidelines of the Institute of Otolaryngology of the National Academy of Medical Sciences of Ukraine [3].

All the results were statistically processed using U-test (Mann–Whitney–Wilcoxon test), Fisher's z-transformation and WinPEPI computer programs.

The research was carried out in accordance with the principles of the Declaration of Helsinki. The research protocol was approved by the Local

Table 1

Content of IgA and INF-α in oropharyngeal secretion in children with CT and healthy donors

Groups	Number of observations (n)	sIgA (g/l) M±m	IFN-α (pg/ml) M±m
Control (n=10)	10	0.85±0.2	19.5±2.5
CT (n=20)	20	0.35±0.1*	7.5±2.5*

Note: *P<0.05 in relation to the control group.

Table 2

Content of IgA in saliva of children with CT, who underwent stimulation of the tonsils by various methods

Groups	Number of patients	sIgA (g/l) M ± m	Number of positive reactions
M_1	n=10	0.45 ± 0.15	7
M_n	n=10	0.55 ± 0.1	5

Table 3

Content of IFN-α in saliva of children with CT, who underwent stimulation of the tonsils by various methods

Groups	Number of patients	IFN-α (pg/ml) M ± m	Number of positive reactions
M_1	n=10	10.5 ±0.5	4
M_n	n=10	20.5 ±2.5*	8

Note: *P<0.05 in relation to M1 group.

Ethics Committee of all the institutions mentioned in the work. Informed consent of the children's parents was obtained for the research.

Results of the research

It was found that the levels of IgA and INF-α in oropharyngeal secretion was significantly different in children with CT and those of control group, mainly with a downward trend (Table 1).

The testing according to the method with the use of magnetic field (M_1) and mucosal vaccine and the new method (M_n) showed the following results (Table 2).

As can be seen from Table 2, according to the new method, the increase in sIgA in oropharyngeal secretion was 22%compared to the old method, but the number of cases with a positive reaction of stimulation of sIgA levels was lower by 28.57%.

Table 3 presents data on the activation of IFN-α production and its content in saliva with influence on PT by different methods. In contrast to the level of sIgA, the content of IFN-α in saliva and the number of positive reactions to the test by M_n method are characterized by a significant increase in all cases. This is most likely due to the fact that this cytokine is produced not only by lymphoid cells, but also by the cells of other histogenesis, which makes it impossible to assess the functional reserve of PT on this indicator.

Discussions

The findings indicate that the proposed method of determining the functional state of PT in children with CT is more selective, and therefore more accurate than the old method.

Clinical and immunological example. *Patient M,* aged 12. Diagnosis: chronic tonsillitis with frequent exacerbation (2–3 times per year). A test was performed to evaluate the immunofunctional state of the palatine tonsils by M_1 method and an increase in the level of secretory IgA by 37% was obtained, which indicated that the immunofunctional state of PT was allegedly preserved. After 3 weeks, the immunofunctional state of the patient's PT was tested by M_n method and stimulation of sIgA level <15% was obtained, which may indicate reduced PT function and may be an objective basis for surgical treatment of CT. When using the new method, the stimulation of sIgA levels in saliva decreased by 59.45% in this case.

On the basis of the carried-out research, a new more selective method of evaluating immunofunctional condition of PT was proposed, in which the basic principle of combining non-specific and specific irritation of tonsillar tissue was retained. In the study, OM-85 was used as a new specific stimulus and low-frequency ultrasound (880 kHz) was used as non-specific stimulus, acting directly (targetedly) on PT and excluding other lymphoid

structures of the pharynx from the area of influence. The proposed method has significant advantages over the existing one, namely it is more selective, and therefore more accurate in determining the functional state of PT. The new method of determining the functional state of PT also has advantages over the methods of assessing the clinical state of PT in case of CT. Such methods as the Centor/McIsaac score or the Paradise criteria [8, 9] do not reflect the actual immunofunctional state of PT and the immune system as a whole.

Conclusions

Thus, the new method M_n (Ukrainian patent UA 112528U) [2] 28.57% more accurately determines the functional state of PT, which allows it to be the basis of clinical and immunological scoring of the state of local immunity of patients with CT along with clinical signs. In our opinion, this immunofunctional method is more objective than only Paradise criteria and may be an alternative in determining the indications for tonsillectomy.

The future of the tonsillar problem belongs to the personalized approach to the patient, taking close account of the immunological status, including immunofunctional condition of tonsils of Waldeyer's ring.

Information of research program. The research was carried out within the framework of the research work of the Department of Pediatric Otorhinolaryngology of Shupyk National Healthcare University of Ukraine, Kyiv, Ukraine — «Diagnosis and treatment of diseases of ear, throat and nose in children» (State registration number 0113U002213).

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