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AN ADVANCEMENT OF TREATMENT OF NASOPHARYNGEAL SIGNS OF GASTROESOPHAGEAL REFLUX DISEASE IN CHILDREN AGAINST THE BACKGROUND OF VITAMIN D DEFICIENCY

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Abstract

The work aims at substantiating the prescription of vitamin D₃ for treatment and prevention of nasopharyngeal signs of GERD in children through determining the main risk factors of the development of the disease.

Materials and methods. Eighty-eight children aged about 4.6 ± 0.14 years were enrolled in the study. The patients were divided into four groups: group 1 consisted of 22 children with nasopharyngeal signs of gastroesophageal reflux disease (GERD); group 2

comprised 22 children with chronic ENT pathology and without GERD; group 3 was formed of 22 children with GERD and without any ENT pathology; group 4 (control group) included 22 children without an ENT or gastrointestinal pathology. No substantial gender differences were observed in each of the groups. GERD was diagnosed on the basis of symptoms and 24-hour pH monitoring; the test for Vitamin D provision was performed; mucosal immunity characteristics were identified and the contamination of the upper airways mucous membranes was studied. The results are statistically processed using Microsoft Office Excel and Statistica 13 software.

Results. Children with GERD with nasopharyngeal signs have been proved to have acute respiratory viral infections (ARVI) more frequently than children with GERD and without ENT pathologies (the number of aggravations is 8.5 (8.0;10.0) and 3.0 (2.0;3.0) respectively ($p < 0.00001$)).

The contamination of mucus membranes of oropharynx and nose of the children with nasopharyngeal signs of GERD is represented by opportunistic microflora (in 95.5%) with prevailing *Haemophilus influenzae*. More than two kinds of microorganism have been inoculated in 63.6% of children ($p = 0.002$), which indicates considerable impairment of colonization resistance in palatal tonsils. Moreover, the majority of the prolonged acid refluxes has been registered in children with the presence of *Haemophilus influenza* ($7,5 \pm 1,62$ against $3,67 \pm 1,2$, $p = 0,04$). These data confirm that the acid content of the refluxate not only irritates the mucous membrane of the oral cavity but also leads to the impairment of local immunity mechanisms and creates favourable conditions for the colonization and growth of bacteria which results in the development of lesions in the pharyngeal mucous membrane.

Children with GERD accompanied with nasopharyngeal signs are characteristic of vitamin D deficiency which is confirmed by lower levels of 25(OH)D₃. This, in its turn, influences the production of antimicrobial peptides (α -defensins 1-3 and cathelicidins LL 37). Group 1 children present with significantly low levels of antimicrobial peptides (α -defensins 1-3 2474.08 ± 180.4 pg/ml and cathelicidins LL 37 $18.89 \pm 2,84$ pg/ml, $p < 0.05$) against the background of lower 25(OH)D₃ (13.05 ± 0.55 ng/ml, $p = 0.00001$). The determined peculiarities substantiated the introduction of vitamin D₃ into therapy for GERD.

Children of the treatment group were prescribed vitamin D₃ in the dosage of 2000 MU per day for a monthly period, and monotherapy with vitamin D₃ in the dosage of 1000 MU per day for a year, daily from September to April, including the break between May and September. Before the treatment, the yearly incidence of the acute respiratory viral disease (ARVD) in the treatment group children was 9.0 [8.0;10.0], and in the control group children,

it was 8.0 [7.0; 10.0]. After a year since the beginning of the treatment, the incidence of ARVI in the treatment group children was 4,0 [3.0;4.0], which is significantly lower than in the control group children (5.0 [4.0;6.0], $p=0.008$). During the year, aggravation of chronic tonsillitis was registered only in 45.5% of the control group children ($p=0.03$), and GERD symptoms were registered only in 18.2 % of the treatment group patients ($p=0.03$).

Obtained results prove the advisability of including vitamin D₃ in the therapeutic regimen of gastroesophageal reflux disease, and its nasopharyngeal signs in particular, in preschoolers.

Conclusions. It is typical of children with GERD accompanied by nasopharyngeal signs to demonstrate significantly low levels of antimicrobial peptides against the background of even lower 25(OH)D₃ (13.05±0.55 ng/ml, $p=0.00001$). Children with GERD and nasopharyngeal signs tend to have ARVI more frequently than those with GERD but without ENT pathologies (number of aggravations is 8.5 (8.0;10.0) and 3.0 (2.0;3.0) respectively ($p<0.00001$)). The prescription of vitamin D₃ in the complex therapy for GERD with nasopharyngeal signs allows to decrease the incidence of the acute respiratory viral infection (ARVI) over a year ($p=0.008$) and to gain stable regression of the clinical symptomatology ($p=0.03$).

Keywords: gastroesophageal reflux disease; chronic tonsillitis; children; preschool age; pathological gastroesophageal reflux; vitamin D; calcidiol; calcitriol; 25(OH)D₃; 1,25(OH)₂D; vitamin D-binding protein; *Haemophilus influenzae*; mucosal immunity; cathelicidin; α -defensins 1-3.

УДОСКОНАЛЕННЯ ЛІКУВАННЯ НАЗОФАРИНГЕАЛЬНИХ ПРОЯВІВ ГАСТРОЕЗОФАГЕАЛЬНОЇ РЕФЛЮКСНОЇ ХВОРОБИ У ДІТЕЙ НА ТЛІ ДЕФІЦИТУ ВІТАМІНУ D

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Мета роботи - Обґрунтування призначення вітаміну D₃ для лікування та профілактики назофарингеальних проявів ГЕРХ у дітей шляхом визначення основних факторів ризику розвитку захворювання.

Матеріали і методи. В дослідження залучили 88 дітей середній вік яких становив $4,6 \pm 0,14$ року. Пацієнтів розподілили на 4 групи: 1 група – 22 особи з назофарингеальними проявами гастроєзофагеальної рефлюксної хвороби (ГЕРХ), 2 група – 22 особи з хронічною ЛОР патологією без ГЕРХ, 3 група – 22 дитини з ГЕРХ без патології з боку ЛОР органів, 4 група (група контролю) - 22 дитини без патології з боку шлунково-кишкового тракту та ЛОР органів. Серед дітей всіх груп, суттєвих гендерних відмінностей не було.

ГЕРХ діагностували на підставі наявності симптомів та добового моніторингування рН, проводили аналіз забезпеченості вітаміном D, визначали особливості мукозального імунітету та вивчали контамінацію слизових оболонок верхніх дихальних шляхів. Результати статистично опрацьовані з використанням програм Microsoft Office Excel і Statistica 13.

Результати. Діти з ГЕРХ з назофарингеальними проявами хворіють на гострі респіраторні вірусні інфекції (ГРВІ) достовірно частіше ніж діти з ГЕРХ без проявів з боку ЛОР органів (кількість загострень 8,5 (8,0;10,0) та 3,0 (2,0;3,0) відповідно ($p < 0,00001$)).

Контамінація слизових оболонок зіву та носу у дітей з назофарингеальними проявами ГЕРХ представлена умовно-патогенною мікрофлорою (у 95,5%) з превалюванням *Haemophilus influenzae*, а у 63,6% дітей висівалися більше двох мікроорганізмів ($p = 0,002$) та вказувало на значне порушення стану колонізаційної резистентності в піднебінних мигдаликах. До того ж, більша кількість тривалих кислих рефлюксів реєструвалась у дітей за наявності *Haemophilus influenzae* ($7,5 \pm 1,62$ проти $3,67 \pm 1,2$, $p = 0,04$). Ці дані підтверджують, що кислий вміст рефлюксату не тільки має подразнюючу дію на слизову оболонку ротової порожнини, але й призводить до порушення механізмів місцевого імунітету та складає благодійні умови для колонізації та росту бактеріальної мікрофлори, що в свою чергу призводить до розвитку уражень слизової оболонки глотки. Особливістю дітей з ГЕРХ з назофарингеальними проявами є наявність дефіциту вітаміну D у всіх дітей, що підтверджено більш низькими рівнями 25(OH)D₃. Це в свою чергу впливає на продукцію антимікробних пептидів (α дефензинів 1-3 та кателіцидинів LL 37). У дітей 1 групи визначено достовірно низькі рівні антимікробних пептидів (α дефензини 1-3 $2474,08 \pm 180,4$ пг/мл та кателіцидини LL 37 $18,89 \pm 2,84$ нг/мл, $p < 0,05$) на тлі більш низьких показників 25(OH)D₃ ($13,05 \pm 0,55$ нг/мл, $p = 0,00001$). Встановлені особливості обґрунтували включення в лікування ГЕРХ додатково вітаміну D₃.

Дітям основної групи до терапії додатково призначили вітамін D₃ в дозі 2000 МО/добу впродовж місяця та монотерапію вітаміном D₃ у дозі 1000 МО/добу впродовж року щоденно з вересня по квітень включно з перервою на період травень – серпень. У дітей основної групи до лікування кількість епізодів гострої респіраторно вірусної інфекції (ГРВІ) впродовж року склала 9,0 [8,0;10,0], а у дітей контрольної – 8,0 [7,0;10,0]. Через рік після початку лікування кількість епізодів ГРВІ склала 4,0 [3,0;4,0], що достовірно нижче ніж у дітей групи контролю (5,0 [4,0;6,0], p=0,008). Впродовж року загострення хронічного тонзиліту реєструвалось лише у 45,5% дітей контрольної групи (p=0,03), а симптоми ГЕРХ впродовж року відмічали лише 18,2% основної групи (p=0,03).

Отримані результати дослідження свідчать про доцільність включення вітаміну D₃ у схему лікування гастроєзофагеальної рефлюксної хвороби, а саме її назофарингеальних проявів, у дітей дошкільного віку.

Висновки. Особливістю дітей з ГЕРХ з назофарингеальними проявами є достовірно низькі рівні антимікробних пептидів на тлі більш низьких показників 25(ОН)D₃ (13,05±0,55 нг/мл, p=0,00001). Діти з ГЕРХ з назофарингеальними проявами хворіють на ГРВІ достовірно частіше ніж діти з ГЕРХ без проявів з боку ЛОР органів (кількість загострень 8,5 (8,0;10,0) та 3,0 (2,0;3,0) відповідно (p<0,00001)). Призначення вітаміну D₃ в комплексному лікуванні ГЕРХ з назофарингеальними проявами дозволяє зменшити кількість епізодів гострої респіраторно вірусної інфекції (ГРВІ) впродовж року (p=0,008) та досягти стійкого регресу клінічної симптоматики (p=0,03).

Ключові слова: гастроєзофагеальна рефлюксна хвороба; хронічний тонзиліт; діти; дошкільний вік; патологічний гастроєзофагеальний рефлекс; вітамін D; кальцидіол; кальцитріол; 25(ОН)D₃; 1,25(ОН)2D; ВДЗБ (вітамін D зв'язуючий білок); *Haemophilus influenzae*; мукозальний імунітет; антимікробні пептиди; кателіцидин; α-дефензини 1-3.

УСОВЕРШЕНСТВОВАНИЕ ЛЕЧЕНИЯ НАЗОФАРИНГЕАЛЬНЫХ ПРОЯВЛЕНИЙ ГАСТРОЭЗОФАГЕАЛЬНОЙ РЕФЛЮКСНОЙ БОЛЕЗНИ У ДЕТЕЙ НА ФОНЕ ДЕФИЦИТА ВИТАМИНА D

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Цель работы - обоснование назначения витамина D3 для лечения и профилактики назофарингеальных проявлений ГЭРБ у детей, путем определения основных факторов риска развития заболевания.

Материалы и методы. В исследовании взяли участие 88 детей, средний возраст которых составлял $4,6 \pm 0,14$ года. Пациентов разделили на 4 группы: 1 группа - 22 человека с назофарингеальными проявлениями гастроэзофагеальной рефлюксной болезни (ГЭРБ), 2 группа - 22 человека с хронической ЛОР патологией без ГЭРБ, 3 группа - 22 ребенка с ГЭРБ без патологии со стороны ЛОР органов, 4 группа (группа контроля) - 22 ребенка без патологии со стороны желудочно-кишечного тракта и ЛОР органов. Среди детей всех групп, существенных гендерных различий не было.

ГЭРБ диагностировали на основании наличия симптомов и суточного мониторингования pH, проводили анализ обеспеченности витамином D, определяли особенности мукозального иммунитета и изучали контаминацию слизистых оболочек верхних дыхательных путей. Результаты статистически обработаны с использованием программ Microsoft Office Excel и Statistica 13.

Результаты. Дети с ГЭРБ с назофарингеальными проявлениями болеют острыми респираторными вирусными инфекциями (ОРВИ) достоверно чаще, чем дети с ГЭРБ без проявлений со стороны ЛОР органов (количество обострений 8,5 (8,0; 10,0) и 3,0 (2,0 ; 3,0) соответственно ($p < 0,00001$)).

Контаминация слизистых оболочек зева и носа у детей с назофарингеальной проявлениями ГЭРБ представлена условно-патогенной микрофлорой (в 95,5%) с преобладанием *Haemophilus influenzae*, а в 63,6% детей высевались более двух микроорганизмов ($p = 0,002$), что указывало на значительное нарушение состояния колонизационной резистентности в небных миндалинах. К тому же, большее количество длительных кислых рефлюксов регистрировалось у детей при наличии *Haemophilus influenza* ($7,5 \pm 1,62$ против $3,67 \pm 1,2$, $p = 0,04$). Эти данные

подтверждают, что кислое содержимое рефлюксата не только имеет раздражающее действие на слизистую оболочку ротовой полости, но и приводит к нарушению механизмов местного иммунитета и составляет благоприятные условия для колонизации и роста бактериальной микрофлоры, что в свою очередь приводит к развитию поражений слизистой оболочки глотки.

Особенностью детей с ГЭРБ с назофарингеальными проявлениями является наличие дефицита витамина D у всех детей, что подтверждено более низкими уровнями 25 (ОН) D3. Это в свою очередь влияет на продукцию антимикробных пептидов (α дефензины 1-3 и кателицидин LL 37). У детей 1 группы определены достоверно низкие уровни антимикробных пептидов (α дефензины 1-3 $2474,08 \pm 180,4$ пг / мл и кателицидины LL 37 $18,89 \pm 2,84$ нг / мл, $p < 0,05$) на фоне более низких показателей 25 (ОН) D3 ($13,05 \pm 0,55$ нг / мл, $p = 0,00001$). Установленные особенности обосновали включение в лечение ГЭРБ с назофарингеальными проявлениями дополнительно витамина D3.

Детям основной группы в терапии дополнительно назначили витамин D3 в дозе 2000 МЕ / сут в течение месяца и затем монотерапию витамином D3 в дозе 1000 МЕ / сут в течение года ежедневно с сентября по апрель включительно с перерывом на период май - август. У детей основной группы до лечения количество эпизодов острой респираторно вирусной инфекции (ОРВИ) в течение года составило 9,0 [8,0; 10,0], а у детей контрольной - 8,0 [7,0; 10,0]. Через год после начала лечения количество эпизодов ОРВИ составило 4,0 [3,0; 4,0], что достоверно ниже у детей группы контроля (5,0 [4,0; 6,0], $p = 0,008$). В течение года обострение хронического тонзиллита регистрировалось только в 45,5% детей контрольной группы ($p = 0,03$), а симптомы ГЭРБ в течение года отмечали лишь 18,2% основной группы ($p = 0,03$).

Полученные результаты исследования свидетельствуют о целесообразности включения витамина D3 в схему лечения гастроэзофагеальной рефлюксной болезни, а именно ее назофарингеальных проявлений, у детей дошкольного возраста.

Выводы. Особенностью детей с ГЭРБ с назофарингеальными проявлениями являются достоверно низкие уровни антимикробных пептидов на фоне более низких показателей 25 (ОН) D3 ($13,05 \pm 0,55$ нг / мл, $p = 0,00001$). Дети с ГЭРБ с назофарингеальными проявлениями болеют ОРВИ достоверно чаще, чем дети с ГЭРБ без проявлений со стороны ЛОР органов (количество обострений 8,5 (8,0; 10,0) и 3,0 (2,0; 3,0) соответственно ($p < 0,00001$). Назначение витамина D3 в комплексном лечении ГЭРБ с назофарингеальными проявлениями позволяет уменьшить количество

эпизодов острой респираторно вирусной инфекции (ОРВИ) в течение года ($p = 0,008$) и достичь устойчивого регресса клинической симптоматики ($p = 0,03$).

Ключевые слова: гастроэзофагеальная рефлюксная болезнь; хронический тонзиллит; дети; дошкольный возраст; патологический гастроэзофагеальный рефлекс; витамин D; Кальцидиол; кальцитриол; 25 (ОН) D3; 1,25 (ОН) 2D; ВДЗБ (витамин D связующий белок); *Haemophilus influenzae*; мукозальный иммунитет; антимикробные пептиды; кателицидин; α -дефензины 1-3.

Introduction

Gastroesophageal reflux disease (GERD) is one of the most topical medical and social issues in the modern gastroenterology which is connected with the global growth of patients with this pathology. The development of GERD in children considerably worsens quality of life and leads to severe complications. The question of extra-esophageal signs of gastroesophageal reflux disease is opaque and multidimensional. It still requires a solution, and it is widely discussed by different medical specialists, including otolaryngologists [1]. For paediatricians, an important problem is the formation of a chronic ENT pathology with frequent recurrence in children. The influence of its development and progress will allow to improve the effectiveness of conservative therapy and to save important lymphoid organs of a child. For instance, in Europe and Northern America ENT specialists believe that GERD can result in tonsils overgrowth and the development of tonsillitis due to the close anatomical location of the oropharynx and gastrointestinal tract [2]. The human nasopharynx is the first line barrier of the mechanical and immunological protection against infectious pathogens and dust particles, present in the air. Owing to this barrier microbe colonization is regulated and strong immune protection against pathogenic microorganisms is maintained. The balance between the life-sustaining activity of pathogenic flora and protective capabilities of an organism can be upset under the influence of various factors, and, as a result, a sharp inflammatory process develops. Aggravation can occur both under the influence of pathogenic flora and as a result of non-specific impact [3]. Among them, there is, for example, the action of the refluxate at high GER. Permanent, but insufficient activation of non-specific protective mechanisms often provokes an inflammatory reaction, but this is usually not enough for the inhibition of the infectious germs. Thus, chronic (recurrent) tonsillitis is implemented. It is recurrent tonsillitis that finally makes a lot of patients undergo tonsillectomy [4].

In the recent two decades, vitamin D has attracted considerable clinical and academic interest. The important role of vitamin D in the function of the digestive system has been described. It has been proved that vitamin D binds with a lot of target tissues in the digestive system, including epithelial cells of the oral cavity mucous membrane. It also influences the work of muscle cells, including upper and lower esophageal constrictors, as well as decreases consumption of magnesium and calcium that take part in the regulation of muscle cells contracting function. This means that vitamin D deficiency in the organism can impair their function and provoke laxation which, in its turn, can lead to regurgitation of stomach and duodenum contents into the esophagus and contribute to the development of gastroesophageal reflux disease (GERD) [5, 6, 7]. In the presence of highly pathological refluxes, acid or alkaline refluxate alters the condition of the nasopharyngeal region which can contribute to the development of a chronic inflammatory process.

Moreover, major studies confirm the influence of vitamin D on the immune system, in particular on the local nonspecific immunity. The innate immune system is the first barrier on the way of infections. It is represented not only by cells of the host but also by resident microorganisms (microbiota). The protection of a host includes physical barriers against the infection – i.e. skin, mucous surfaces, phlegm and endothelial vessel cells, enzymes, antimicrobial peptides and proteins, phlogotic humoral and cellular components [8, 9].

Antimicrobial peptides, in their turn, influence microbial colonization of nasopharynx. It is proved that children with vitamin D deficiency present with the insufficient synthesis of antimicrobial peptide cathelicidin in blood serum. Due to this, anti-infectious properties of cathelicidin are not implemented which supports the colonization of the respiratory tract by pathogenic agents [10].

It has been elucidated that oral administration of vitamin D results in the change of gastrointestinal microbiota composition and is accompanied by a decrease of opportunistic microorganisms and an increase of bacterial variety [11].

Thus, the development of chronic inflammation of pharyngeal lymphoid tissue ring can be associated, on the one hand, with a decrease of vitamin D as an immune reactions modifier, and, on the other hand, with the influence of refluxate (in case of GERD) on microbiocenosis of mucous membranes. The obtained data provide grounds for inclusion of vitamin D into the complex therapy for children with GERD.

A remarkable problem of GERD treatment is its longevity and the possibility of recurrence during the year. Therapy for GERD must be directed to the pathogenic mechanisms of GERD development. There are a lot of options of gastroesophageal reflux

disease treatment, i.e. instruction № 59 “Unified Clinical Protocols of Medical Care for Children with Gastrointestinal Diseases” issued by Ministry of Public Health of Ukraine, dated January 29, 2013; guidelines issued by North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) and by European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) (March 2018). However, they do not take into account the peculiarities of certain GERD signs (in particular, nasopharyngeal), which results in chronization and recurrence of the disease. Understanding of the connection between vitamin D level, impairment of motor evacuation function of the gastrointestinal tract in patients with GERD, a decrease of local immunity of nasopharyngeal mucous membrane, and its microbial contamination will allow improving therapy and prevention of GERD accompanied with nasopharyngeal signs in preschoolers at early stages. This will help to prevent chronization of the process and to avoid surgery, thus saving important lymphoid organs of a child.

One of the key tasks of the research was the adjustment of therapeutic and preventive dosages of vitamin D for children with nasopharyngeal signs of GERD. In order to fulfil this task, the most recent global guidelines concerning vitamin D prescription have been studied. Updated guidelines of the United States of America emphasize the necessity of supplemental vitamin D for children between 4 and 6 years of age, in the dosage of 400-800 IU per day [12]. The allowable upper limit of vitamin D consumption for children between 4 and 8 years of age is 3000 IU per day [13]. It is necessary to note that daily, weekly or monthly vitamin D dosages can be applied since they lead to similar concentrations of 25(OH)D₃ in patients' blood serum [14]. Nevertheless, some experts recommend to opt for daily dosages, as vitamin D half-decay takes about a day, and some researchers report on side effects, such as sharp increase or decrease of vitamin D concentration in blood serum, if vitamin D doses are high and intermittent [15].

Current meta-analyses have not revealed any side effects risks at 25(OH)D₃ blood serum concentrations higher than 125 nM/l, so at present, it is unclear what 25(OH)D₃ blood serum concentrations should be applied as lowest observed adverse effect level for vitamin D [16,17].

As for the choice between vitamins D and D₃, the majority of experts claim that vitamin D₃ is more preferable as it is an endogenic form that can be more effective for an increase of 25(OH)D₃ blood serum concentration than vitamin D₂ [18]. Recommended target levels for 25(OH)D₃ range from 25 to 50 nM/l (10 to 20 ng/ml) which corresponds to vitamin D dosage from 400 to 800 IU (from 10 to 20 mcg) per day [19]. Besides, literature view

enabled to define that the dose of about 2.5 mcg (100 IU) vitamin D per day can increase 25(OH)D₃ concentration up to 2.5-5 nM/l [20].

Taking into account all the above-mentioned information, optimal dose of vitamin D₃ for correcting its deficiency is 2000 IU of vitamin D₃ per day daily for a monthly period, with the transfer to a daily dose of 1000 IU per day from September to April, with a break from May to August.

The work aims to substantiate the prescription of vitamin D₃ for treatment and prevention of nasopharyngeal signs of GERD in children by means of determining the main risk factors of the development of the disease.

Materials and methods. Having signed informed consent, 88 preschoolers from Zaporizhzhia and Zaporizhzhia region were enrolled in the study. They were receiving inpatient care in the departments of otolaryngology and gastroenterology of the communal establishment "Zaporizhzhia Municipal Multi-Type Children's Hospital №5".

According to the results of clinical and anamnestic studies and instrumental examinations (24-hour esophageal pH monitoring), the patients were divided into 4 groups. Group 1 consisted of 22 children (54.5% boys and 45.5% girls; average age – 4.14±0.25 years) with diagnosed gastroesophageal reflux disease with nasopharyngeal signs. Group 2 comprised 22 children (50% boys and 50% girls; average age – 4.14±0.19 years) with chronic ENT pathology and without GERD. Group 3 was formed of 22 children (50% boys and 50% girls; average age – 4.8±0.25 years) with GERD and without any ENT pathology. Group 4 (control group) included 22 children (45.5% boys and 54.5% girls, average age – 4.66±0.22) without an ENT or gastrointestinal pathology. No substantial gender differences were observed in each of the groups ($p>0,05$).

GERD was diagnosed on the basis of the relevant esophageal symptoms and confirmed on the basis of 24-hour esophageal pH monitoring in the clinical setting of the communal establishment "Zaporizhzhia Municipal Multi-Type Children's Hospital №5". The monitoring was performed in the department of endoscopy using a gastrographic apparatus AG-1pH-M (manufactured by Start, Ltd., Ukraine). Transnasally located microprobe was used for pH monitoring during 16-24 hours. Microprobe detector was placed proximad at the point 5 cm away from the esophagogastric junction. The presence or absence of pathological refluxes was diagnosed according to DeMeester T.R. classification (1993).

The study of contamination of mucous membranes of the upper airways was performed with the help of the common bacteriological analysis, including inoculation of nasopharyngeal emissions (nasal and oral swabs).

Vitamin D provision was estimated by means of evaluation of the concentration of 25(OH)D₃, 1,25(OH)₂D and vitamin D-binding protein. The analysis was performed using enzyme immunoassay with the use of assay kits “25 OH Vitamin Total ELISA” manufactured by DAsource ImmunoAssays S.A. (Belgium), «1,25-Dihydroxy Vitamin D EIA» manufactured by Immunodiagnostic Systems Limited (the UK), «Vitamin-D-Bindungsprotein ELISA kit» manufactured by Immunodiagnostik AG (Germany), in the premises of the training medical and laboratory centre of the Zaporizhzhia State Medical University.

Vitamin D status was evaluated according to the guidelines of the European Society of Endocrinology (M. F. Holick, 2011): vitamin D deficiency was diagnosed if the level of its active metabolite 25(OH)D₃ in the blood was less than 20 ng/ml (< 50 nM/l), vitamin D insufficiency was diagnosed if the level of 25(OH)D₃ in the blood was 21-29 ng/ml (50-75 nM/l).

The presence of α -defensins 1–3 (Human Neutrophil Peptides 1–3, HNP 1–3) in the blood serum was estimated using enzyme immunoassay with the use of the commercial kit HNP 1–3 (ELISA, Bio Tech Lab-S). The level of cathelicidin LL-37 was estimated by means of enzyme immunoassay with the use of the commercial kit LL-37 (Hyculbiotech, the Netherlands).

In order to evaluate the treatment effectiveness, the children with nasopharyngeal signs of GERD were divided into two groups. The control group included 11 children who were treated according to the guideline of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) and European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) (March 2018): they took proton pump inhibitors once a day in over four weeks. The main group comprised 11 children who received, apart from the above mentioned therapeutic regimen, vitamin D₃ preparation at a dose of 2000 IU per day for a month. Both groups were gender, age, and diagnose uniform, and similar in terms of the clinical picture. After a month, a repeated examination was performed. The following stage presumed prescription of vitamin D₃ as monotherapy to the main group children, at a dose of 1000 IU per day for a year daily from September to April, with a break from May to August. The effectiveness was estimated by comparing the incidence of acute respiratory viral infections (ARVI), chronic tonsillitis aggravation and GERD recurrence during the year in children of the main and control groups.

The results were statistically processed with the use of Microsoft Office Excel and Statistica 13 software. The analysis of normality of indicia distribution was performed with Shapiro-Wilk test. The results are represented as the arithmetic mean (M) and standard

deviation (m) with the normal distribution of indicia, and as the median value (Me) and interquartile range [Q25; Q75] with the abnormal distribution of indicia. Qualitative indicia are represented as absolute frequencies and percent. Quantitative indicia in the groups were compared with the use of Mann-Whitney U-test, qualitative ones were compared with two-tailed Fisher's test. Spearman's correlation coefficient was used to reveal structural bonds.

Results. The comparison of the acute respiratory viral infections (ARVI) incidence during a year in children with chronic tonsillitis did not reveal significant differences depending on the presence of chronic GER. In general, the total ARVI incidence in group 1 children (those with nasopharyngeal signs of GERD) is 8.5 (8.0; 10.0); in group 2 children (those with a chronic ENT pathology without GERD) it is 9.0 (7.0; 10.0). However, it has been noticed that children with GERD that is not accompanied by nasopharyngeal signs, have ARVI significantly more seldom – 3.0 (2.0; 3.0) ($p < 0.00001$).

In order to define the peculiarities of the course of GERD with nasopharyngeal signs, it is necessary to define the main risk factors of this disease development.

Our previous research has found out that all children with GERD accompanied by nasopharyngeal signs are characteristic of vitamin D deficiency, and in 9% of them vitamin D deficiency is lower than 10 ng/ml. The analysis of vitamin D metabolites in the blood serum has estimated that children with GERD tend to have significantly lower levels of 25(OH)D₃ than children from the control group do ($p = 0,00001$) (Table 1). It has also been found out that children with nasopharyngeal signs of GERD (group 1) demonstrate lower indicia than children of the other groups under study ($p = 0,00001$).

Table 1

The level of vitamin D metabolites in the blood serum of the examined children

	Group 1 (GERD and ChT) n=22	Group 2 (ChT) n=22	Group 3 (GERD) n=22	Group 4 (control) n=22
25(OH)D ₃ , ng/ml	13.05±0.55*^#	17.91±0.45*	22.23±0.82*	25.46±0.68
1,25(OH) ₂ D pg/ml	142.28±6.99*^#	76.63±1.73*	109.06±4.68*	46.38±2.61
VDBP, ng/ml	38.61±4.94	37.50±4.69	32.80±3.49	31.29±2.88

Note: *- significant difference relative to control group ($p < 0,05$), ^- significant difference relative to group 2 ($p < 0,05$), #- significant difference relative to group 3 ($p < 0,05$).

Main group children with GERD accompanied with nasopharyngeal signs tend to have significantly low levels of antimicrobial peptides (α -defensins1-3 2474.08±180.4 pg/ml and

cathelicidins LL 37 18.89 ± 2.84 нг/мл, $p < 0,05$) (Table 2) against the background of lower 25(OH)D₃ levels (13.05 ± 0.55 ng/ml, $p = 0,00001$).

Table 2

α - defensins and 1-3 cathelicidins count in the blood serum of the children under study

	Group 1 (GERD and ChT) n=22	Group 2 (ChT) n=22	Group 3 (GERD) n=22	Control group n=22
α -defensins, pg/ml	$2474.08 \pm 180.4^{*\wedge\#}$	$9769.66 \pm 828.01^*$	$5242.78 \pm 343.4^*$	3311.82 ± 125.3
Cathelicidins, ng/ml	$18.89 \pm 2.84^{*\wedge\#}$	$65.32 \pm 1.72^*$	$48.66 \pm 0.96^*$	36.71 ± 1.23

Note: *- significant difference relative to control group ($p < 0,05$), ^- significant difference relative to group 2 ($p < 0,05$), #- significant difference relative to group 3 ($p < 0,05$).

The contamination of oral and nasal mucous membranes in children with nasopharyngeal signs of GERD is represented by opportunistic microflora (in 95.5%) with prevailing *Haemophilus influenzae*. Besides, it has been revealed that microflora was hardly ever inoculated as a monoculture. For example, more than two microorganisms were inoculated in 63.6 children from group 1, which is significantly more frequent than in group 2 children (13.6%), $p = 0.002$. The discovery of combined microflora can indicate considerable impairment of colonization resistance in the palatal tonsils.

The analysis of levels of vitamin D metabolites and antimicrobial peptides in children with nasopharyngeal signs of GERD, depending on the microbial contamination of the nasopharynx, has demonstrated that in children with combined microflora (subgroup A), levels of cathelicidins LL 37 are significantly higher than in those children whose microflora had been inoculated as monoculture (subgroup B) (24.63 ± 2.9 ng/ml vs. 8.87 ± 4.03 ng/ml, $p = 0,008$) (Table 3). According to other indicia, no significant differences have been noticed in the framework of group 1.

The following stage included defining vitamin D metabolites level and the peculiarities of mucosal immunity depending on the reflux character in children with GERD accompanied by nasopharyngeal signs. Besides, the specifics of nasopharyngeal contamination, depending on reflux character against the background of vitamin D deficiency and mucosal immunity features, were studied. Levels of α -defensins and cathelicidins LL 37 in children's blood serum did not differ significantly, depending on the refluxate composition (Table 4).

Table 3

The level of vitamin D metabolites and antimicrobial peptides in children, depending on microflora

	Group 1 (GERD and ChT) n=22		Control group n=22
	Subgroup A n=14	Subgroup B n=8	
25(OH)D ₃ , ng/ml	13.25±0.56#	12.69±1.21#	25.46±0.68
1,25(OH) ₂ D pg/ml	141.18±8.31#	144.19±13.35#	46.38±2.61
α-defensins, g/ml	2555.57±223.4#	2331.47±318.8#	3311.82±125.3
cathelicidins, ng/ml	24.63±2.9*#	8.87±4.03#	36.71±1.23

Note: *- significant difference relative to subgroup B (p<0,05), ^- significant difference relative to group 2 (p<0,05), #- significant difference relative to control group (p<0,05).

Table 4

Vitamin D metabolites, α-defensins 1-3, and cathelicidins count in the blood serum of the children under study depending on the refluxate composition

	Children with acid refluxes n=4	Children with alkaline refluxes n=7	Children with combined refluxes, n=11
25(OH)D ₃ , ng/ml	13.89±0.84	13.25±0.87	12.61±0.93
1,25(OH) ₂ D pg/ml	135.69±9.8	128.06±12.7	153.71±10.31
VDBP, ng/ml	33.69±12.87	41.33±9.35	38.66±7.05
α-defensins, pg/ml	2298.60±736.84	2525.31±722.85	2505.29±1006.02
cathelicidins LL 37, ng/ml	13.44±10.87	23.10±11.91	18.21±15.05

However, it has been elucidated that the more the number of acid GERs, more than 5 minutes long, is, the lower cathelicidins LL 37 levels in the blood serum are. Besides, a bigger number of prolonged acid refluxes was registered in those children who had *Haemophilus influenza* (7.5±1.62 vs. 3.67±1.2, p=0.04). These data confirm that acid refluxate composition not only irritates the oral cavity but also leads to the impairment of the local immunity mechanisms and creates favourable conditions for bacterial microflora colonization and growth. This, in its turn, results in the development of destructive and non-destructive lesions of the pharyngeal mucous membrane.

Thus, the analysis of the results of the conducted studies has displayed that it is typical of children with GERD with nasopharyngeal signs to have vitamin D deficiency which has been proved by lower 25(OH)D₃ levels. This, in its turn, influences the production of

antimicrobial peptides (α -defensins 1-3 and cathelicidins LL 37). This means that in the presence of vitamin D level decrease, the activation of adequate immune response does not happen to its full which leads to a decrease of inflammatory immunoreactivity of the organism, and makes one of the causes for a more severe course of the disease. The determined peculiarities grounded the introduction of vitamin D₃ into therapy for GERD.

Before initiation of treatment, the main complaints of children with nasopharyngeal signs of GERD were: belching (in 54.5%), wet spot sign (in 86.4%), exasperation and difficulties with falling asleep (in 68.2%), loss of appetite (in 91%). Reexamination was performed after a month. It was noticed that regression of clinical symptoms in the course of the treatment did not vary in different groups depending on the addition of vitamin D₃ (at a dose of 2000 IU per day) to the main therapy.

At the following stage children of the main group (n=11) continued taking vitamin D₃ as monotherapy at a dose of 1000 IU per day for a yearly period, daily from September to April, with a break from May to August. Before the treatment, the yearly incidence of the acute respiratory viral infection (ARVI) in the main group children was 9.0 [8.0; 10.0], while in the control group children it was 8.0 [7.0; 10.0] (Table 5).

Table 5

Comparative analysis of the ARVI aggravation depending on the implementation of preventive treatment with vitamin D₃

	Main group (n=11)	Comparative group (n=11)
Number of aggravations within a year prior to hospitalization (Me [25-75%])	9.0 [8.0;10.0]	8.0 [7.0;10.0]
Number of aggravations within a year after hospitalization (Me [25-75%])	4.0 [3.0;4.0]*^	5.0 [4.0;6.0]^

Note: ^- statistically significant difference (p<0.05) within the group, *- statistically significant difference (p<0.05) between groups.

After a year since the beginning of the therapy, ARVI incidence in children of the main group was 4.0 [3.0; 4.0] which is significantly lower than in the control group children (5.0 [4.0; 6.0], p=0.008). Annual aggravation of chronic tonsillitis was noted by all the children before the initiation of the therapy. During a year, chronic tonsillitis aggravation was registered only in 45.5% of control group children (p=0.03). Occasional complaints of belching, difficulties with falling asleep and wet spot sign were made by 2 children (18.2%) of the main group and 8 children (72.7%) of the control group (p=0.03).

Obtained results prove the advisability of including vitamin D₃ in the therapeutic regimen of gastroesophageal reflux disease, and its nasopharyngeal signs in particular, in preschoolers.

Discussion

In recent years, there have been studies of the influence of gastrointestinal pathologies on the progress and chronization of ENT pathologies. Considerable attention is focused on extra-esophageal otolaryngological signs of GERD. European and American otolaryngologists believe that GERD can contribute to tonsillitis development due to the close anatomical location of the oropharynx and gastrointestinal tract [2]. Regular reflux of the stomach content into the nasopharynx, which takes place in GERD, results in the impairment of the local immunity mechanisms. This, in its turn, contributes to the growth and colonization of pathogenic bacterial microflora. An important role of vitamin D in the digestive system functioning has been described. According to academic sources, vitamin D stimulates the production of antimicrobial peptides, in particular α -defensins and cathelicidins LL37. Antimicrobial peptides, in their turn, influence microbial colonization of nasopharynx. It has been proved that sufficient synthesis of cathelicidin does not take place in children with vitamin D insufficiency. In this case, anti-infectious properties of this antimicrobial peptide are not implemented which supports the colonization of the respiratory tract by pathogenic agents [10].

We have discovered that children with GERD with nasopharyngeal signs tend to have significantly low levels of antimicrobial peptides against the background of even lower levels of 25(OH)D₃. A decrease of the mucosa local immunity influences microbial contamination of the nasopharynx which, at the presence of pathological GER, impairs the mucosa. Oral and nasal mucous membranes of children with nasopharyngeal signs of GERD were contaminated by opportunistic microflora (9 in 95.5%) with the prevalence of *Haemophilus influenzae*. Moreover, it has been found out that microflora was hardly ever inoculated as a monoculture. The discovery of combined microflora can indicate considerable impairment of colonization resistance of oral and nasal mucous membranes. Besides, children with *Haemophilus influenzae* presented with a bigger number of prolonged acid refluxes. These data confirm that the acid content of the refluxate not only irritates the mucous membrane of the oral cavity but also leads to the impairment of local immunity mechanisms and creates favourable conditions for the colonization and growth of bacteria which results in the development of destructive and non-destructive lesions in the pharyngeal mucous membrane.

Thus, the analysis of the study results has proved that children with GERD accompanied with nasopharyngeal signs are characteristic of vitamin D deficiency which is confirmed by lower levels of 25(OH)D₃. This, in its turn, influences the production of antimicrobial peptides (α defensins 1-3 and cathelicidins LL 37). This means that in the presence of vitamin D level decrease, the activation of adequate immune response does not happen to its full which leads to a decrease of inflammatory immunoreactivity of the organism, and makes one of the causes for a more severe course of the disease. The determined peculiarities grounded the introduction of vitamin D₃ into therapy for GERD.

We recommend the additional prescription of vitamin D₃ at a dose of 2000 IU per day during 30 days. Afterwards, vitamin D₃ is suggested as monotherapy, at a dose of 1000 IU per day for a yearly period, daily from September to April, with a break from May to August. This therapeutic regimen allowed to decrease the incidence of ARVI and to achieve regress of clinical symptoms in most of the children. Obtained results prove the advisability of including vitamin D₃ in the therapeutic regimen of gastroesophageal reflux disease, and its nasopharyngeal signs in particular, in preschoolers.

Conclusions

1. Children with GERD accompanied with nasopharyngeal signs tend to have significantly low levels of antimicrobial peptides (α -defensins 1-3 2474.08±180.4 pg/ml and cathelicidins LL 37 18.89±2,84 pg/ml, p<0.05) against the background of lower 25(OH)D₃ levels (13.05±0.55 ng/ml, p=0.00001).

2. The contamination of mucus membranes of oropharynx and nose of the children with nasopharyngeal signs of GERD is represented by opportunistic microflora (in 95.5%) with prevailing *Haemophilus influenzae*. More than two kinds of microorganism have been inoculated in 63.6% of children (p=0.002), which is significantly more frequent than in group 2 children (13.6%, p=0.002). This also indicates considerable impairment of colonization resistance in palatal tonsils.

3. A bigger number of prolonged acid refluxes has been registered in children with the presence of *Haemophilus influenza* (7,5±1,62 against 3,67±1,2, p=0,04). Children with a bigger number of acid GERs, more than 5 minutes long, tend to have lower cathelicidins LL 37 levels in the blood serum (R=-0.45; p=0.03). This, in its turn, explains the development of mucous membrane lesions in children with nasopharyngeal signs of GERD.

4. The prescription of vitamin D₃ in the complex therapy for GERD with nasopharyngeal signs at a monthly dose of 2000 IU per day, and as yearly monotherapy at a dose of 1000 IU per day (daily from September to April, with a break from May to August),

allows to decrease the incidence of the acute respiratory viral infection (ARVI) over a year ($p=0.008$) and to gain stable regression of the clinical symptomatology ($p=0.03$).

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Conflict of interests: none.

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