Received: 03 August 2024 / Accepted: 27 October 2024 / Published online: 30 December 2024

DOI 10.34689/SH.2024.26.6.005

UDC 616.72-002-007.17.

REACTIVE ARTHRITIS IN THE CONTEXT OF CONNECTIVE TISSUE DYSPLASIA – THE ROLE OF THE ORGANISM'S MICROBIOME

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Abstract

Introduction: The course of reactive arthritis (ReA) depends on several factors related to its etiology and pathogenesis, but it is also determined by the condition of the organism in the period preceding the disease. Reactive arthritis in the context of connective tissue dysplasia and the characteristics of the microbiome may have a more severe course and an increased risk of the chronicity of the joint inflammatory process.

Research objective: To assess the impact of the organism's microbiome in children with reactive arthritis in the context of connective tissue dysplasia (CTD) on the course and outcomes of the disease.

Materials and Methods: Design: cross-sectional clinical study. Research was conducted at the clinical base of the University Hospital of the NCJSC "Semey Medical University" between 2019-2022. The population group (without ReA) consisted of 288 children aged 6 to 18 years. The main group of children with reactive arthritis included 146 patients, of whom 50 had connective tissue dysplasia, divided into two age categories: 6-12 years (49, including 23 with CTD) and 13-18 years (97 and 27, respectively).

The research methods included a comprehensive examination of children according to the Diagnostic and Treatment Protocol, determination of the presence of CTD based on a combination of clinical signs, and microbiome composition analysis using multi-ion chromatography mass spectrometry, as well as statistical methods.

Results: Significant differences were identified in the structure of the organism's microbiome and related parameters between the examined groups. In the presence of ReA, compared to the population group, a decrease in the content of the most common resident microorganisms and an increase in transient microorganisms, microscopic fungi, and organisms not normally found in the body were observed in the 13-18-year age group.

A comparative analysis of subgroups of children with ReA, with and without CTD, also revealed a lower content of resident microorganisms and an increase in the presence of non-normal forms of microorganisms in the latter group.

Higher levels of plasmalogen were found in both age groups of children with ReA without CTD. In contrast, the presence of CTD was associated with a higher level of endotoxin. When comparing these results with the control group, significant differences were noted in overall load indicators, related to microbiome imbalance, plasmalogen, and endotoxin levels.

Clinical progression analysis showed an increased duration of joint symptom persistence and a higher risk of chronicity in the presence of coexisting CTD combined with microbiome changes.

Conclusion: The data obtained allowed us to formulate the following conclusions:

- The state of the microbiome in children with reactive arthritis is characterized by an imbalance, which involves a decrease in the content of resident microorganisms and an absolute and relative increase in transient and potentially pathogenic microorganisms, a known risk factor.
- The presence of CTD determines a greater severity of the microbiome imbalance, including in terms of integral indicators, plasmalogen, and endotoxin content, which serves as a risk factor for the potentiation and persistence of the inflammatory process in the joints.
- This combination is clinically significant, as confirmed by the longer duration of the main clinical and laboratory signs of ReA in children with undifferentiated CTD.

Keywords: Reactive arthritis, children, connective tissue dysplasia, microbiome, multi-ion chromatography mass spectrometry.

Резюме

РЕАКТИВНЫЙ АРТРИТ НА ФОНЕ ДИСПЛАЗИИ СОЕДИНИТЕЛЬНОЙ ТКАНИ – РОЛЬ МИКРОБИОМА ОРГАНИЗМА

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Введение: Течение реактивного артрита (РеА) зависит от ряда факторов, связанных с его этиологией и патогенезом, но также определяется состоянием организма в период, предшествующий заболеванию. Реактивный артрит на фоне дисплазии соединительной ткани и особенности микробиома может иметь более тяжелое течение и повышенный риск хронизации суставного воспалительного процесса.

Цель исследования: Оценка влияния микробиома организма у детей с реактивным артритом на фоне дисплазии соединительной ткани (ДСТ) на течение и исходы заболевания.

Материалы и методы: Дизайн: поперечное клиническое исследование. Сроки выполнения - 2019-2022 гг., проведено на клинической базе Университетского госпиталя НАО «Медицинский университет Семей».

Популяционную группу (без РеА) составили 288 детей в возрасте от 6 до 18 лет. В основную группу детей с реактивным артритом было включено 146 пациентов, в том числе 50 – с наличием дисплазии соединительной ткани, распределённые на 2 возрастные категории: 6-12 лет (49, в том числе 23 с ДСТ) и 13-18 лет (97 и 27 соответственно).

Методы исследования включали комплексное обследование детей согласно Протоколу диагностики и лечения, определение наличия ДСТ на основании сочетания клинических признаков, определение состава микробиома организма с помощью мультиионнной хромато-масс-спектрометрии, статистические.

Результаты исследования: Определены существенные различия в структуре микробиома организма и связанных с ним параметров между обследованными группами. При наличии РеА в сравнении с популяционной группой определено снижение содержания наиболее распространённых резидентных и повышение – транзиторных микроорганизмов, микроскопических грибов и не встречающихся в норме организмов в возрастной группе 13-18 лет. При сравнительном анализе подгрупп детей с РеА без ДСТ и с наличием ДСТ также выявлено более низкое содержание резидентных и повышение не встречающихся в норме форм микроорганизмов во втором случае.

Более высокое содержание плазмалогена определено в обеих возрастных группах детей с РеА при отсутствии ДСТ. Напротив, при наличии ДСТ было выявлено более высокое содержание эндотоксина. При сравнении с контрольной группой следует выделить показатели общей нагрузки, имевшие ряд значимых различий, связанных с дисбалансом микробиома, плазмалогена и эндотоксина.

При анализе клинического течения определено повышение продолжительности персистирования симптоматики поражения суставов и риска хронизации при наличии сопутствующей ДСТ в сочетании с изменениями микробиома.

Заключение: Полученные данные позволили нам сформулировать следующие выводы:

- состояние микробиома организма у детей с реактивным артритом характеризуется дисбалансом, заключающимся в снижении содержания резидентных и абсолютном и относительном повышении – транзиторных и потенциально патогенных микроорганизмов, что является известным фактором риска;

- наличие ДСТ определяет большую выраженность дисбаланса микробиома, в том числе по интегральным показателям, содержанию плазмалогена и эндотоксина, что служит фактором риска потенцирования и персистирования воспалительного процесса в суставах;

- данное сочетание является клинически значимым, что подтверждается большей продолжительностью основных клинико-лабораторных признаков РеА у детей, имеющих недифференцированную ДСТ.

Ключевые слова: *реактивный артрит; дети, дисплазия соединительной ткани; микробиом; мультиионная хромато-масс-спектрометрия.*

Түйіндеме

ДӘНЕКЕР ТІН ДИСПЛАЗИЯСЫНЫҢ ФОНЫНДАҒЫ РЕАКТИВТІ АРТРИТ – АҒЗА МИКРОБИОМЫНЫҢ РӨЛІ

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Кіріспе: Реактивті артрит (РеА) ағымы бірқатар факторларға байланысты, оның этиологиясы мен патогенезіне, сонымен қатар ауру дамымай тұрған кезеңіндегі ағзаның жалпы күйімен анықталады. Дәнекер тіннің дисплазиясы фонындағы реактивті артрит ағза микробиомының ерекшелігіне байланысты ауыр өтіп, қабыну процесінің созылмалы қаупіне ие болуы мүмкін.

Мақсаты: Дәнекер тіннің дисплазиясы (ДТД) фонында реактивті артриті бар балаларда ағза микробиомының ауру ағымына мен нәтижесіне әсерін бағалау.

Материалдар мен әдістер: Дизайн: Көлденең клиникалық зерттеу 2019-2022 жж. аралығында «Семей медицина университеті» КеАҚ Университеттік ауруханаының клиникалық базасында жүзеге асырылды.

Популяциялық топты (РеА-сіз) 6 мен 18 жас аралығындағы 288 бала құрады. Реактивті артритпен ауыратын балалардың негізгі тобына екі жас санатына бөлінген 146 науқас, оның ішінде дәнекер тінінің дисплазиясы бар 50 науқас кірді: 6-12 жас (49, оның ішінде ДТД бар 23) және 13-18 жас (тиісінше 97 және 27).

Зерттеу әдістеріне диагностикалық және емдеу хаттамасына сәйкес балаларды кешенді тексеру, клиникалық белгілердің жинағы негізінде ДТД анықтау, мультиионды хромато-масс-спектрометрияны қолдану арқылы организмнің микробиом құрамын анықтау және статистикалық әдістер кірді.

Зерттеу нәтижелері: Зерттелетін топтар арасында ағза микробиомының құрылымында және сәйкесінше соған байланысты параметрлерде айтарлықтай айырмашылықтар анықталды. РеА болған жағдайда, популяциялық топпен салыстырғанда, ең көп таралған резидентті микроорганизмдер құрамының төмендеуі және 13-18 жас тобында әдетте кездеспейтін транзиторлы микроорганизмдердің, микроскопиялық саңырауқұлақтардың көбеюі анықталды. ДТД жоқ және РеА және ДТД бар балалардың кіші топтарын салыстырмалы талдау кезінде резидентті микроағзалардың төмен санын және қалыпты жағдайда табылмайтын микроорганизмдердің формаларының жоғарылауын анықтады.

РеА бар ДТД-сыз балалардың екі жас тобында да плазмалогеннің жоғары мөлшері анықталды. Керісінше, ДТД болған жағдайда эндотоксиннің жоғары мөлшері анықталды. Бақылау тобымен салыстыру кезінде микробиомның, плазмалогеннің және эндотоксиннің дисбалансымен байланысты бірқатар елеулі жалпы жүктеме көрсеткіштерін айырмашылықтары бар екенін атап өткен жөн.

Клиникалық ағымды талдау кезінде ДТД болған кезде микробиомның өзгеруімен үйлесетін буын зақымдану симптомдарының ұзақтығының жоғарылауы және созылмалы жағдайға ауысу қаупі анықталды.

Қорытынды: Алынған деректер келесі қорытындыларды жасауға мүмкіндік береді:

- реактивті артриті бар балалардағы организм микробиомының жағдайы белгілі қауіп факторы болып табылатын резидентті микроағзалардың құрамының төмендеуінен және тразиторлы және потенциалды патогенді микроорганизмдердің абсолютті және салыстырмалы жоғарылауынан тұратын теңгерімсіздікпен сипатталады;
- ДТД болуы микробиомның теңгерімсіздігінің неғұрлым ауырлығын анықтайды, оның ішінде интегралды көрсеткіштер бойынша, плазмалоген мен эндотоксин құрамы, бұл буындардағы қабыну процесінің күшеюі мен тұрақтылығының қауіп факторы ретінде қызмет етеді;
- бұл комбинация клиникалық тұрғыдан маңызды және де дифференцирленбеген ДТД бар балалардағы РеА негізгі клиникалық-зертханалық белгілерінің ұзақтығымен дәлелденеді.

Түйін сөздер: реактивті артрит; балалар, дәнекер тінінің дисплазиясы; микробиом; мультиионды хромато-масс-спектрометрия.

For citation / Для цитирования / Дәйексөз үшін:

Tugelbayeva A.M., Ivanova R.L., Toktabayeva B.Zh., Lobanov Yu.F. Reactive arthritis in the context of connective tissue dysplasia – the role of the organism's microbiome // *Nauka i Zdravookhranenie* [Science & Healthcare]. 2024. Vol.26 (6), pp. 38-48. *doi 10.34689/SH.2024.26.6.005*

Тугелбаева А.М., Иванова Р.Л., Токтабаева Б.Ж., Лобанов Ю.Ф. Реактивный артрит на фоне дисплазии соединительной ткани – роль микробиома организма // Наука и Здравоохранение. 2024. Т.26 (6). С. 38-48-15. *doi 10.34689/SH.2024.26.6.005*

Тугелбаева А.М., Иванова Р.Л., Токтабаева Б.Ж., Лобанов Ю.Ф. Дәнекер тін дисплазиясының фонындағы реактивті артрит – ағза микробиомының рөлі // Ғылым және Денсаулық сақтау. 2024. Т.26 (6). Б. 38-48. *doi 10.34689/SH.2024.26.6.005*

Introduction

The course of reactive arthritis (ReA) depends on several factors related to its etiology and pathogenesis, but it is also determined by the condition of the body in the period preceding the disease. A number of factors have been described that can influence the risk of development and the severity of joint damage in ReA [4,11,21].

Among these factors are the characteristics of the microbiome, which may lead to a more severe course with subsequent chronicity of the joint inflammatory process [14,15].

In medical science and healthcare, the term "undifferentiated connective tissue dysplasia" (UCTD) is used, which combines several well-known syndromes in the world scientific community (mitral valve prolapse, joint hypermobility, etc.), which share many common pathogenetic mechanisms [17,20,22]. UCTD manifests as various morphological changes, clinical signs, and is considered by many researchers as a physiological condition. However, there is evidence that the presence of UCTD affects the course of many somatic diseases [3,7,9].

Connective tissue dysplasia (CTD) is defined by the presence of genetic changes in the tissues of the musculoskeletal system, which increases the risk of developing acute and chronic joint pathology.

The combination of the characteristics of the body's microbiome [24,25] and CTD may have a synergistic effect on both the risk of developing ReA and its course, which should be taken into account in clinical practice.

Aim: To assess the impact of the microbiome in children with reactive arthritis in the context of CTD on the course and outcomes of the disease.

Materials and Methods

Study design: Cross-sectional clinical study. The study was conducted from 2019 to 2022 at the pediatric department of the University Hospital of the NCJSC "Semey medical university".

Inclusion and exclusion criteria for children:

Inclusion: Age 6-18 years-old; residence in the city of Semey.

Exclusion: Incomplete examination according to the study protocol; refusal to participate in the study.

The population group (hereinafter – "without ReA", in the tables – "no ReA" and "without CTD", in the tables – "no CTD") consisted of 288 children.

The age and gender distribution of the population group corresponded to the distribution of children of different ages in the surveyed population (the method of simple randomization was used). The slight excess in the number of girls was insignificant (p=0.925) and generally corresponded to the population distribution.

The main clinical group of children with ReA included 146 patients, with no age or gender differences between the population and main groups.

The general population of such children during the study period was accepted as the main group. Table 1 presents the characteristics of the children in the main group.

Table 1.

Clinical characteristics of ReA in the examined children.

Indicator	6-12 years, n=49		13-18 years, n=97		Total, n=146	
	abs.	%	abs.	%	abs.	%
Presence of monoarthritis, including:	40	81,6	82	84,5	122	83,6
- knee joint	16	32,7	33	34,0	49	33,6
- ankle joint	9	18,4	17	17,5	26	17,8
- joints of the foot	5	10,2	12	12,4	17	11,6
- hip joint	4	8,2	9	9,3	13	8,9
- wrist joint	3	6,1	6	6,2	9	6,2
- elbow joint	3	6,1	5	5,2	8	5,5
Presence of oligoarthritis, including:	9	18,4	15	15,5	24	16,4
- knee and ankle joint	2	4,1	5	5,2	7	4,8
- symmetrical knee joint	2	4,1	4	4,1	6	4,1
- joints of the hand	2	4,1	4	4,1	6	4,1
- symmetrical ankle joint	2	4,1	1	1,0	3	2,1
ankle+foot joints	1	2,0	1	1,0	2	1,4
Clinical symptoms:						
Pronounced local signs of joint inflammation	37	75,5	52	53,6	89	61,0
Increased body temperature, incl.	44	89,8	67	69,1	111	76,0
- within subfebrile levels	31	63,3	55	56,7	86	58,9
- febrile levels	13	26,5	22	22,7	35	24,0
- less than 5 days	23	46,9	16	16,5	39	26,7
- 5-10 days	20	40,8	46	47,4	66	45,2
- more than 10 days	6	12,2	0	0,0	6	4,1
Severe inflammatory reaction of the blood, incl.	31	63,3	56	57,7	87	59,6
- according to C-reactive protein (CRP)	27	55,1	48	49,5	75	51,4
- by leukocyte intoxication index (LII)	19	38,8	39	40,2	58	39,7
Availability of CTD	23	46,9	27	27,8	50	34,2

The majority of children exhibited clinical manifestations in the form of monoarthritis (83.6%). The most commonly affected joint was the knee, which is consistent with the findings of other authors [1]. Less frequently, the ankle joint (22.6%) and the hip joint (6.2%) were affected. Other localizations in monoarthritis were rare. Oligoarthritis was diagnosed in 16.4% of cases. It typically involved both knee joints and combinations of knee and ankle joint or hand joint involvement.

Severe local symptoms of joint inflammation were observed in 61.0% of children with ReA. The disease was accompanied by hyperthermia in 76% of children.

The children were divided into two groups within each age category – those with a set of clinical and laboratory signs of undifferentiated CTD and those without such signs. A total of 50 patients (34.2% of the total group) were included in the first group.

CTD signs were more frequently identified in the younger age group (23 subjects, 46.9%) compared to 27 (27.8%) in the older group ($\chi 2 = 5.276$, $p = 0.022$).

The degree of inflammatory response in the body was determined by the concentration of CRP and the level of the leukocyte intoxication index (LII). Changes in these indicators were found in 59.6% of the subjects with ReA.

Research methods

The methods for diagnosing reactive arthritis included all necessary clinical and instrumental studies in accordance with the EULAR guidelines and the protocol for the treatment of reactive arthritis. To diagnose CTD, a combination of clinical and instrumental data was used in accordance with Russian recommendations [8].

Determination of the intestinal microbiome composition

The analysis was performed using multi-ion chromatography mass spectrometry, with control over the sequence determined by an automatic program. Thirty-three characteristic ions from the mass spectra of fatty acids specific to microorganisms were identified. The AT-5973 system from Agilent Technologies (USA) was used. Chromatographic separation of the sample was performed on a capillary column with a methylsilicone bonded phase HP-5 ms Agilent Technologies, 25 mm in length and 0.25 mm internal diameter. This method is widely accepted in science and clinical practice and is comparable to sequencing in terms of informativeness.

The identified microorganisms were classified into the following groups:

Resident microorganisms (identified in more than 50% of cases) included: actinomycetes; alkaligenes; bifidobacteria; clostridia; coryneforms; egertella; eubacteria; fusobacteria; lactobacilli; lactococci; nocardia; prevotella; propionibacterium; pseudonocardia; rhodococcus; ruminococcus; staphylococci; streptococci; streptomycetes.

Transient microorganisms (identified in less than 50% of cases): bacilli; bacteroides; clostridia; Helicobacter pylori; kingella; acinetobacter; peptostreptococci; prevotella; stenotrophomonas; streptococci; enterobacteria.

Microscopic fungi: candida; aspergillus; mycomycetes.

Microorganisms not normally present: Bacillus megaterium; Bacteroides hypermegase; Campylobacter mucosalis; Enterococcus; Flavobacteria; Mycobacteria; Porphyromonas; Propionibacterium; Pseudomonas aeruginosa; Streptomyces pharmamarenensis.

At the same time, the content of plasmalogen, total endotoxin, total normal flora (microbiotic core), and the ratios of various microbiome components relative to the overall microbial load were determined.

Statistical methods

The data obtained as a result of the study were entered into the database in the MS Excel program. The quality of information preparation was checked, the results were processed and the data was grouped according to the specified criteria. The analysis was carried out directly using the SPSS program (v.27.0).

The direct values of the studied feature and the assessment of their accuracy and reliability were determined. The differences were analyzed using the Mann-Whitney criterion for the content of specific microorganisms in the microbiome and the Student's t-test for the analysis of plasmalogen and endotoxin [19]. $P < 0.05$ was taken as an indicator of the presence of statistical significance.

Results

Tables 2-5 present the features of the microbiome in clinical groups, depending on its main components, the age of children and their clinical status.

In children with ReA in the younger age group, in the absence of CTD, the content of microorganisms in this category was significantly lower compared to the control group for 6 species: Corineform CDC-group, Nocardia asteroids, Rhodococcus spp., Staphylococcus spp. In the presence of concomitant CTD, the following microorganisms should additionally be noted: Lactococcus spp., Prevotella spp., and Ruminococcus spp. In some cases (for the aforementioned species), significant differences were also observed between the groups of children with ReA, usually indicating lower content in the presence of CTD.

For the integrated bacterial load indicator in this category, a significantly lower value was found in the group of children with CTD (21.2% lower, $p = 0.028$) compared to the control.

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For the integrated bacterial load indicator in this category, a significantly lower value was found in the group of children with CTD (21.2% lower, $p = 0.028$) compared to the control.

Between the groups of healthy children and children with ReA without CTD, differences in favor of a lower content in the latter were observed in the older age group for: Actinomyces viscosus, Eubacterium spp., Lactococcus spp., Rhodococcus spp., and Streptomyces spp.

In the group with background CTD, Prevotella spp. and Streptococcus mutans were added to this list.

Significant differences were also noted between the groups of patients for Actinomyces viscosus, Eubacterium spp., Lactococcus spp., Rhodococcus spp., and Streptomyces spp. The overall bacterial load index in this group was significantly lower than in both the control group and the group with baseline CTD (30.0% lower, p = 0.021, and 19.2% lower, p = 0.040, respectively).

Table 2.

Indicators of the content of resident microorganisms in children with reactive arthritis depending on age and availability of CTD.

Microbiome component	6-12 years						13-18 years					
	«no ReA»		«ReA+CTD»		«ReA+ no CTD»		«no ReA»		«ReA+CTD»		«ReA+ no CTD»	
	Me	Q	Me	Q	Me	Q	Me	Q	Me	Q	Me	Q
Actinomyces spp	25	14	29	14	27	17	24	5	15	6	22	5
Actinomyces viscosus	622	52	492	91	531	49	714	141	395*#	100	585*	127
Alcaligenes spp	53	10	45	8	38	8	56	11	36	11	29	6
Bifidobacterium spp	2720	548	2311	624	2563	568	3699	1054	2741	854	3463	1085
Clostridium coccoides	13	5	9	7	21	9	33	8	20	8	33	9
Clostridium propionicum	81	21	80	63	75	67	131	22	64*	13	87*	16
Clostridium perfringens	25	6	33	6	30	8	78	12	69	12	63	11
Clostridium ramosum	2145	207	1628	206	2249	239	2005	699	1322	638	1487	570
Clostridium tetani	492	51	439	43	415	47	492	71	376	68	417	66
Corineform CDC-group XX	41	5	24*#	3	55	10*	79	10	38*	5	51*	7
Eggerthella lenta	238	61	201	88	243	69	311	52	174*	45	218*	40
Eubacterium spp	5066	717	4316	1018	5189	808	6137	715	4255*#	738	5614*	720
Fusobacterium/ Haemophylus	3	2	0	0	0	0	5	2	5	2	6	3
Lactobacillus spp	2382	405	1735*#	296	2400	349	2218	429	1833	685	2170	462
Lactococcus spp	612	56	309	57	218*	22	715	127	429*#	102	556	109
Nocardia asteroides	471	62	461#	67	291*	49	899	155	835	136	801	152
Prevotella spp	36	8	8*	6	13	3	25	10	7*	3	10	4
Propionibacterium	29	5	17	7	29	6	26	7	20	6	16	5
Propionibacterium freudenreichii	1423	490	1055	357	1580	598	2078	515	1146	384	1411	385
Propionibacterium jensenii	66	14	62	13	53	12	106	31	59	18	79	25
Pseudonocardia spp	31	8	11	4	27	8	19	7	15	9	9	4
Rhodococcus spp	79	15	17*#	5	68*	14	77	11	27*#	4	43	7
Ruminococcus spp	580	84	325*#	73	582	93	469	56	404	46	505	66
Staphylococcus spp	441	66	241*#	50	498	82	441	66	309	62	336	55
Staphylococcus epidermidis	35	4	25	7	36	4	74	19	77	37	66	19
Streptococcus mutans (anaerobic)	225	33	189	34	263	42	169	30	112*	22	175	34
Streptomyces spp	85	10	131	13	58*	7	171	29	82*#	13	143*	27
Overall load Index	18019	977	14193*	1171	17552	1324	21251	1408	14865*#	997	18395	1200

Notice – here and further * - differences with the group «no ReA» are significant,
- differences between ReA groups depending on the availability of CTD are significant, p<0,05

Table 3.

Indicators of the content of transient microorganisms in children with reactive arthritis, depending on age and the presence of CTD.

Microbiome component	6-12 years						13-18 years					
	«no ReA»		«ReA+CTD»		«ReA+ no CTD»		«no ReA»		«ReA+CTD»		«ReA+ no CTD»	
	Me	Q	Me	Q	Me	Q	Me	Q	Me	Q	Me	Q
Bacillus cereus	0	0	12	11	0	0	0	0	3	2	2	1
Bacteroides fragilis	1	1	2	2	0	0	0	0	3	3	0	0
Bacteroides hypermegas	0	0	1	1	5	0	0	0	0	0	0	0
Clostridium hystolyticum	2	1	19	7	10	6	5	2	13#	7	0	0
Enterococcus spp	0	0	2	2	0	0	1	1	5	3	1	1
Helicobacter pylori	0	0	2	1	2	0	1	1	5	4	2	2
Acinetobacter spp	0	0	0	0	0	0	0	0	3	3	0	0
Peptostreptococcus anaerobius	0	0	1	1	1	0	0	0	55*	15	18	7
Porphyromonas spp	0	0	0	0	0	0	0	0	3	3	0	0
Prevotella ruminicola	0	0	4	2	1	1	0	0	0	0	1	1
Streptococcus spp	33	10	128*	38	88*	29	2	1	3	2	2	1
сем. Enterobacteriaceae (E.coli etc.)	0	0	0	0	0	0	0	0	1	1	2	1
Overall load Index	36	10	171*#	24	107*	16	12	6	94*#	20	28*	9

According to the indicators of transient microorganisms, the differences between the groups were moderate. In the group of patients with CTD, there was an excess of Streptococcus spp. over the control group, and in the older age group – Peptostreptococcus anaerobius.

It should be pointed out the presence of statistical significance of the total indicator in both age categories (excess in the presence of CTD by 59.8% in children 7-12 and by 235.7% - 13-18, years-old p = 0.037 and p=0.029, respectively).

Table 4.

Indicators of the content of microscopic fungi in children with reactive arthritis, depending on age and the presence of CTD.

Microbiome component	6-12 year						13-18 year					
	«no ReA»		«ReA+CTD»		«ReA+ no CTD»		«no ReA»		«ReA+CTD»		«ReA+ no CTD»	
	Me	Q	Me	Q	Me	Q	Me	Q	Me	Q	Me	Q
Candida spp	608	69	918	149	558	70	305	78	523*	146	457*	244
Aspergillus spp	103	21	189	53	135	30	160	55	158	66	216	82
Micromycetes spp (campesterol)	1202	279	1945*#	400	1126	287	499	148	985*	310	1111*	362
Micromycetes spp (sitosterol)	417	75	802*	117	935*	185	794	225	1377	347	1197	373
Overall load Index	2330	307	3854*	376	2754	290	1758	229	3043*	296	2981*	247

There were also moderate differences when comparing groups of children with ReA, depending on the presence of CTD in the content of microscopic fungi. Only one strain showed an excess in the development of ReA against the background of CTD (Micromycetes spp (campesterol)) in the age group of 6-12 years-old. At the same time, the excess over the control group was revealed by a number of indicators.

The overall load index was exceeded in the presence of CTD in both age categories (by 65.4% in the younger and 73.1% in the older, p=0.022; p=0.014, respectively)

Table 5.

Indicators of the content of non-normalized microorganisms in children with reactive arthritis, depending on age and the presence of CTD.

Microbiome component	6-12 year						13-18 year					
	«no ReA»		«ReA+CTD»		«ReA+no CTD»		«no ReA»		«ReA+CTD»		«ReA+no CTD»	
	Me	Q	Me	Q	Me	Q	Me	Q	Me	Q	Me	Q
Bacillus megaterium	0	0	2	1	0	0	0	0	0	0	0	0
Campylobacter mucosalis	0	0	0	0	0	0	0	0	127	92	0	0
Flavobacterium spp	0	0	2	1	2	1	0	0	41*#	21	17*	0
Porphyromonas spp	0	0	0	0	6	2	6	6	0	0	0	0
Pseudomonas aeruginosa	0	0	2	2	0	0	0	0	0	0	0	0
Overall load Index	0	0	6	4	8*	3	6	6	168*#	70	17*	0

According to the number of microorganisms that do not occur normally, significant differences were determined in relation to one species (Flavobacterium spp) and the total index in the age group of 13-18 years-old (by 141.0% and 888.2%, p=0.042, p=0.007, respectively) in excess in children with concomitant CTD.

Figures 1 and 2 show integral indicators of the effect of microbiota on the body – plasmalogen and endotoxin in children with reactive arthritis, depending on the presence of CTD.

The differences in plasmalogen content in the compared groups of young children were moderate and amounted to 16.6%, however, they had statistical significance (p=0.045). In the older age group, these differences reached 23.2% (p=0.041).

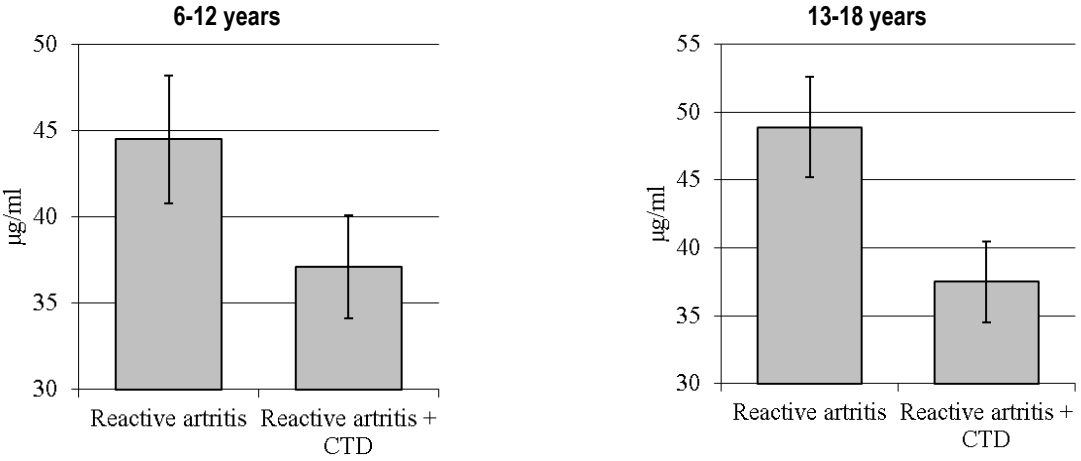

Figure 1. Plasmalogen content in children with ReA, depending on age and the presence of CTD.

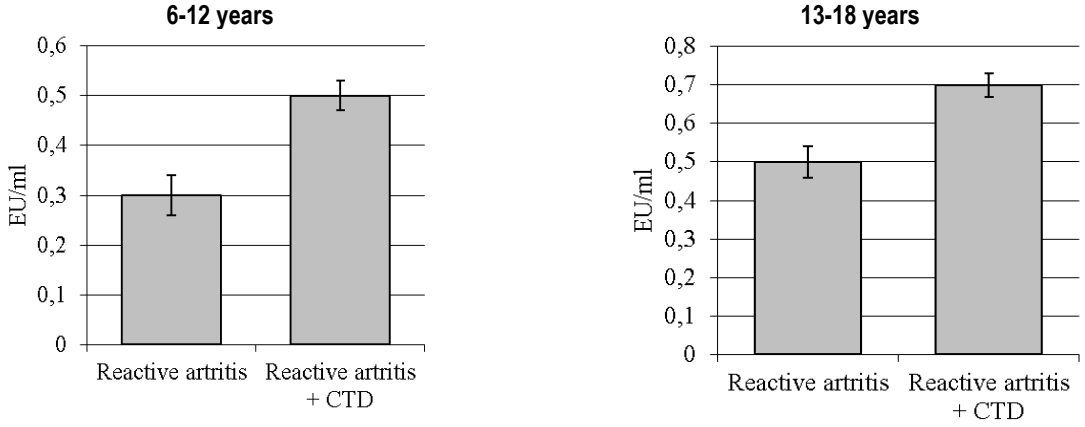

Figure 2. Endotoxin content in children with ReA depending on depending on the age and availability of CTD.

The differences in the endotoxin index were more pronounced. The excess in the group of reactive arthritis in combination with CTD was 66.5% in the group of young children (p=0.020) and 40.0% in older children (p=0.022).

To analyze the effect of the state of the microbiota in children with CTD on the background of ReA on the course and outcome, the plasmalogen index was selected in association with the dynamics of inflammatory parameters during treatment, the state of joint function and the preservation of the proinflammatory status (taking into account the cytokine content) during examination after 3 months.

The data obtained during the distribution into groups are shown in Figures 3 and 4.

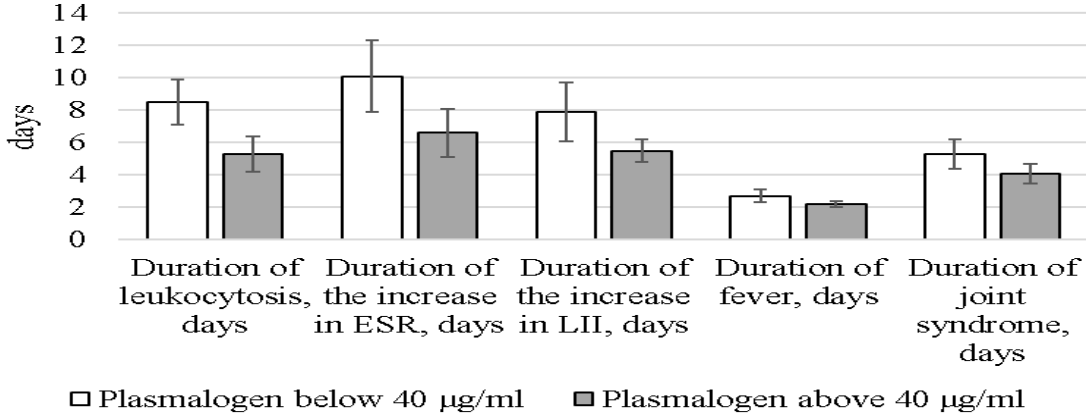

Figure 3. Ratios of plasmalogen content and clinical course indicators in children aged 6-12 years-old.

The differences in the duration of leukocytosis, depending on the plasmalogen content, were significant and amounted to 60.4% by median (p=0.018). The differences in the duration of the period of increase in ESR were slightly lower (53.0%, p=0.023). The increase in LII persisted in the group of reduced plasmalogen content by 43.6% longer (p=0.037). The duration of fever and joint syndrome did not have significant differences, although there was a tendency to exceed them in both cases.

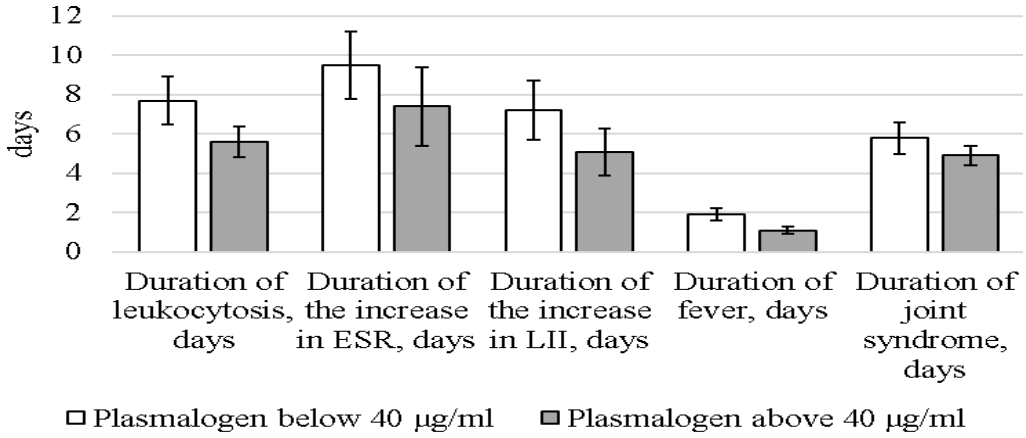

Figure 4. Ratios of plasmalogen content and clinical course indicators in children aged 13-18 years-old.

Differences in the duration of leukocytosis (37.5%, p=0.040) and fever (72.7%, p=0.016) were significant in older children. It should also be noted that all registered differences were unidirectional, i.e., violations were more pronounced with reduced plasmalogen content.

Discussion

The rationale for the study was the expediency of determining the presence and potential aspects of the influence of the characteristics of the microbiome of the body of children during the development of ReA [9,12] and the potential effect of CTD on it [13].

There are results of a number of studies indicating the role of this condition on the development of inflammatory joint diseases, however, the association of intestinal dysbiosis with CTD has been noted only in isolated studies by Russian scientists [2,6].

For many inflammatory diseases of the joints, a change in the microbiome is a known risk factor, which is described in a number of papers [18,26]. However, in these studies, the combined effect of changes in the microbiome and CTD has not been studied.

The data obtained by us allowed us to conclude that there is a general trend of deviations in the composition of the microbiome in both ReA and CTD. These changes were expressed in a decrease in the content of the most common resident microorganisms and the total capacity of the microbiome and an increase in the representation of transient bacteria, fungi and pathogens in the microbiome.

At the same time, in the presence of background CTD, more pronounced deviations were revealed, in a number of cases, including integral indicators of the general load index, plasmalogen and endotoxin content, which indicates a likely greater negative impact of microbiome factors on the condition of joints and the course of ReA.

A certain disadvantage of the study is that we did not provide an analysis of the state of the microbiome in children with CTD without ReA and, accordingly, we cannot, based on the data provided, judge the primary or secondary nature of the identified abnormalities in the development of arthritis. However, the published results confirm the presence of differences between groups of children without CTD and with the presence of such in this parameter outside the presence of inflammatory diseases [5].

The corresponding confirmations of this hypothesis were obtained as a result of statistical analysis.

On the other hand, the presence of these disorders was associated with deterioration in the results of ReA treatment. Accordingly, the assessment of the mutual effect of CTD on the state of the microbiome and the totality of pathogenetic factors in both cases – on the course of ReA shows increased effects and significantly worse results: delays the onset of clinical and laboratory remission of ReA, leads to the need for longer treatment, including those associated with hospital stay. For certain factors, similar data were obtained by a number of authors [6, 16, 23].

As a result, a combination of negative factors can increase the likelihood of chronic joint process, the need for long–term treatment and rehabilitation, and in extremely negative cases, the development of irreversible joint changes associated with disability or the need for orthopedic interventions.

Conclusion

The obtained data allowed us to formulate the following conclusions:

- The state of the microbiome in children with reactive arthritis is characterized by a microbiota imbalance, which involves a decrease in the content of resident microorganisms and an absolute and relative increase in transient and potentially pathogenic microorganisms, which is a known risk factor;
- The presence of CTD determines a more pronounced imbalance of the microbiome, including integral indicators, plasmalogen, and endotoxin content, which serves as a risk factor for potentiating and persisting the inflammatory process in the joints;
- This combination is clinically significant, which is confirmed by the longer duration of the main clinical and laboratory signs of ReA in children with undifferentiated CTD.

***Contributions of authors**: All authors equally participated in the study and writing of this article.*

***Conflict of interest**: The authors declare no conflicts of interest.*

***Funding**: No external funding was provided for this study.*

***Publication information**: This article has not been previously published and has not been considered by any other publication.*

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