ANNOTATION

of dissertation for the degree of Doctor of Philosophy (PhD)

8D10102 – «Medicine»

**Clinical and pathogenetic features and treatment of reactive arthritis in children with**

**connective tissue dysplasia**

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Work completion dates: 2019-2022.

**Relevance of the research topic.** In recent years, rheumatic diseases have been characterized by an increase in frequency, development among all age groups of the population, a tendency to chronicity and early disability [van Vollenhoven R.F., 2023; Albrecht K., Strangfeld A., 2023]. One of the pressing problems in pediatric rheumatology is reactive arthritis (ReA), which occupies a leading position in the structure of rheumatic diseases in children and adolescents [Nishibukuro M. et al, 2018]. The etiology and pathogenesis of rheumatic diseases are complex and diverse, and the underlying mechanisms have not been sufficiently studied. Recently, the importance of the composition of the body's microbiome has been studied [Konig M.F., 2020] in the development and progression of various rheumatic diseases. A link between microbiome disorders has been identified, and new data indicate a potential role for intestinal fungi and viruses in modulating immune responses in autoimmune and inflammatory diseases. A recent study showed that the composition of the intestinal microbiome can be altered by vitamin D [Tabatabaeizadeh S.A. et al., 2020]. In addition, modern studies pay serious attention to connective tissue dysplasia (CTD) as one of the risk factors for the development of musculoskeletal pathology. Although dysplasia is not a nosology, but a genetically determined disorder of connective tissue development, its presence can affect the course of various diseases [https://medvestnik.stgmu.ru/files/articles/694.pdf]. In particular, CTD can predetermine the development of inflammatory, degenerative and autoimmune diseases [Chelpachenko O.E. et al., 2021]. In general, studies on ReA are extremely few in number and concern mainly the adult contingent [Pisareva A.D. et al., 2018].

**Purpose of the study.**

Optimization of treatment and secondary prevention of relapses of reactive arthritis in children with connective tissue dysplasia.

**Research objectives:**

1. To determine the incidence rate and clinical characteristics of reactive arthritis in children with undifferentiated connective tissue dysplasia (CTD).

2. To study clinical and immunological parameters, metabolic indices of connective tissue in children with reactive arthritis depending on the presence of CTD.

3. To determine the composition of the microbiome in children with reactive arthritis and the role of its disorders in the development of this pathology.

4. To develop a pathogenetically substantiated approach to the treatment and prevention of relapses of reactive arthritis in children with CTD and to determine its effectiveness.

**Scientific novelty of the study:**

For the first time, the presence of an increased incidence rate and clinical features of the course of reactive arthritis in children with connective tissue dysplasia were revealed.

For the first time, the composition of the body's microbiome was studied using chromatograph mass spectrometry in children with reactive arthritis, its features that contribute to the development of the disease in CTD.

For the first time, a pathogenetically substantiated method for the complex treatment of reactive arthritis in children with CTD was developed, which allows preventing relapses of the disease in the future.

**Practical significance:**

The identified features of the body's microbiome parameters allow us to assess their role as factors influencing the clinical course and outcome of reactive arthritis against the background of CTD, which provides a comprehensive assessment of the risk and probable course of the disease. The application of the developed approach to the correction of the microbiome composition in the acute period of the disease in clinical practice improves the prognosis of reactive arthritis in children with CTD, and the complex correction of the pathogenetic factors of CTD (vitamin D, magnesium, calcium) and microbiome disorders in the post-arthritic period makes it possible to justify the prevention of recurrent arthritis associated with these disorders.

Author's certificate KZ №51130 dated 06.11.2024 was received. "A method for predicting the risk of complications and chronic course of reactive arthritis based on determining the state of the microbiota and the combination of its disorders with connective tissue dysplasia"

Received the author's certificate KZ №51130 dated 06.11.2024. "A method for correcting risk factors for complications and chronic course of reactive arthritis in relation to the body's microbiome against the background of connective tissue dysplasia"

Received the certificate for rationalization proposal №2454 dated 26.03.2025. "Complex treatment and secondary prevention of reactive arthritis against the background of undifferentiated connective tissue dysplasia"

**The main provisions of the dissertation research submitted for defense:**

1. ReA in children with CTD occurs significantly more often compared to the general population and has characteristic clinical features.

2. The studied indicators of connective tissue metabolism in children with CTD affect the nature of the course of reactive arthritis

3. The composition of the microbiome in children with ReA with and without CTD is different.

4. The inclusion of various probiotics in the complex treatment of reactive arthritis is pathogenetically justified, which allows to reduce the time of hospital stay and prevent subsequent relapses.

**Research materials and methods**

Research deadlines: 2019-2022.

Clinical base: University hospital of NCJSC "SMU", pediatrics department.

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| Analysis of the presence of the problem  Review and assessment of modern scientific data regarding the etiology, pathogenesis and treatment of reactive arthritis in children |

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| Cross-sectional study (stages 1 and 2) |

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| Determination of the frequency of CTD in children with ReA in comparison with the prevalence in the population (main group 1 – 146, control group 1 – n=288) | Determination of the characteristics of the organism microbiome in ReA, including against the background of СTD | Study of clinical and immunological parameters and the role of vitamin D in ReA depending on CTD |
| (main group 2 – n=71, control group 2 – n=75) | |

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| Longitudinal (prospective) clinical study (stage 3) |

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| Formation of a correction and comparison group | | | |
| Correction group (children with ReA against the background of CTD, main group 3, n=31) | | Comparison group (children with ReA against the background of CTD, control group 3, n=40) | |
| Determining the impact of therapy on organism microbiome parameters | Determination of the effect of therapy on immunological parameters | | Determining the impact of therapy on clinical outcomes and prognosis |

Organizational structure of the study

Criteria for inclusion and exclusion of children by stages:

Cross-sectional study.

Inclusion: age 6-18 years; residence in Semey;

Exclusion - refusal to participate in the study;

Prospective clinical study:

Inclusion: age 6-18 years; diagnosis of ReA with clinical and laboratory confirmation; informed consent of the patient and/or parents/guardians

Exclusion: incomplete examination according to the Study Protocol; refusal to participate in the study at the stage before processing the results.

The cross-sectional study of stages 1 and 2 included children of 2 categories:

- a sample from the general population of children (control group 1);

- patients with ReA (main group 1).

Control group 1 consisted of 288 children aged 6 to 18 years.

The main group 1 with ReA included 146 children, then distributed depending on the presence of CTD into the main group 2 (71 children with CTD) and the control group 2 - 75 children with ReA without CTD.

At the 3rd stage, an additional distribution of the group of 71 children with ReA and CTD was carried out, associated with the additional use of the drug Linex in order to correct the composition of the microbiome (the main group 3 - 31 and the control group 3 - 40 children).

When analyzing the microbiome indicators, data were used obtained from 122 clinically healthy children who were part of the control group 1, aged 6 to 18 years, including 50 - aged 6 to 12 years and 72 - aged 13 to 18 years.

Research methods:

In the complex examination of children with ReA, generally accepted methods were used, corresponding to the current protocol, namely: visual examination and palpation of the joints; complete blood count; general urine analysis; biological analysis: ALT, AST, creatinine, urea, glucose, total and direct bilirubin, uric acid; CRP; rheumatoid factor; antistreptolysin - O), determination of the leukocyte intoxication index.

Instrumental studies: X-ray of the pelvic bones and hip joints with the capture of the sacroiliac joints, joints, CT or MRI of the joints and sacroiliac joints (as indicated)

Clinical methods for diagnosing CTD

Determination of alkaline phosphatase activity in blood plasma

Determination of calcium and magnesium content in the blood

Determination of vitamin D content in the blood

Study of cytokine content in the blood

Study of phagocytosis indicators

Measurements of pain intensity using a «Visual analog scale» (VAS)

Determination of the composition of the intestinal microbiome using multi-ion mass fragmentography

Statistical methods

In terms of the treatment provided, the children included in the study were divided into 2 groups. In one (n=40), standard treatment was carried out with correction of the pathogenetic mechanisms of CTD, in the other (n=31), a set of correction methods was used, including an effect on the microbiome. To correct dysplasia, sodium chondroitin sulfate was prescribed for 3 months. Magnesium preparations (Magne B6, Sanofi, France or Magnesium Chelate, Evalar, Russia) were used in a dosage depending on age for 6 months. To correct the state of the microbiome - specifically, the intestinal microbiota, Linex (Sandoz GmbH, Germany) was used in age-related dosages for 3 months.

**Results of the study**

A comparative analysis revealed a significant excess of the frequency of CTD in the presence of ReA compared to the control group 1, corresponding to the main group in terms of age and sex composition. The degree of excess was 37.7%.

Analysis of arthritis variants at the second stage of the study concluded that, despite some shift in frequency towards oligoarthritis in the presence of CTD, there were no significant differences in this feature between the groups. Joint syndrome was assessed by the presence of pain and swelling. The duration of persistence of pain syndrome was higher in children with CTD and amounted to 2.4 ± 0.2 days compared to 1.8 ± 0.1 days (p = 0.025). Analysis of the severity of pain syndrome according to “VAS” showed a significant excess in the group with CTD (4.3 ± 0.2 points versus 3.7 ± 0.2 points in the control group 2, p = 0.038). An excess of the CRP indicator was determined in children of the main group 2 over the control group 2. The average values ​​were, respectively, 7.9 ± 0.5 and 6.5 ± 0.4 mg / l (p = 0.035). The values ​​of LII in the same groups were 1.45 ± 0.11 and 1.28 ± 0.13 conventional units (p> 0.05).

Thus, when analyzing clinical and laboratory data, moderate differences were revealed between the groups of children with ReA against the background of CTD and without it. Statistical significance was determined in relation to pain syndrome and CRP content. The concentration of magnesium in the blood of the examined subjects of the main group 2 was lower than in the corresponding control group by 18.2% (p> 0.05) in the subgroup of 6-12 years and 23.3% - 13-18 years (p = 0.043). With regard to the content of the active metabolite 25(OH)D, a significant excess was determined in the main group over the control group 2 in the age category of 6-12 years (23.4%, p = 0.041). When analyzing the content of cytokines, an excess of most of them over the reference values ​​was revealed (with the exception of IL-17 in both age categories of the examined children with ReA). When comparing between the groups of children with ReA, the following features were determined depending on the presence of CTD. At the age of 6-12 years, the content of IL-1β in the presence of CTD significantly exceeded the indicator in the control group 2 by 27.3% (p=0.041). There were no significant differences between the groups for IL-4 and IL-10, with a tendency for an excess in CTD in both cases. A significant excess in CTD was determined by IL-6 (35.7%, p=0.035). In contrast, the content of IL-17 in the main group 1 was significantly lower (34.5%, p=0.015). An excess was revealed in the presence of CTD by IL-18 (35.2%, p=0.028).

In older children, no significant differences were found between the groups in the blood levels of IL-1β, IL-4, IL-6 and IL-10, but there was a tendency to exceed the values ​​in the presence of CTD. For IL-17 and IL-18, the differences between the groups were more pronounced. In the first case, a lower value of the indicator was found in CTD (by 40.5%, p = 0.011), in the second - an excess of 55.0% (p = 0.019).

In general, in both groups there is the same tendency to exceed the total cytokine load and the proinflammatory effect in children with CTD, against which ReA developed.

In the age category of 6-12 years, a significant excess in the control group 2 (in the absence of DST) was revealed for 6 types of resident microorganisms: Corineform CDC-group, Lactobacillus spp, Nocardia asteroids, Rhodococcus spp, Ruminococcus spp, Staphylococcus spp. At the same time, no significant differences were found between the groups for the integrated indicator. Significant differences of a similar direction were determined in the age category of 13-18 years for Actinomyces viscosus; Eubacterium spp; Lactococcus spp; Rhodococcus spp; Streptomyces spp. Of these, only one species (Eubacterium spp) is among the most widely represented in the small intestine. However, the total indicator also had an excess for this category of microorganisms in the absence of DST. The differences were 23.7% (p=0.018).

The differences in plasmalogen content between the main group 2 and the control group 2 in young children were moderate and amounted to 16.6%, however, they were statistically significant (p=0.045). In the older age group, these differences reached 23.2% (p=0.041). The differences in the endotoxin indicator were more pronounced - an excess of 66.5% in the ReA group with CTD in the group of young children (p=0.020) and 40.0% in the older age group (p=0.022).

The obtained data indicate the presence of differences in the microbiome structure in children with ReA associated with the presence of DST. The latter condition was associated with an increase in the content of transient organisms and other undesirable components of the microbiome, a decrease in the content of the main resident microorganisms, the plasmalogen index, and an increase in endotoxin. To assess the impact of the proposed treatment method, the dynamics of the main clinical, biochemical indicators, the state of the immune system and the microbiome were determined.

During the treatment, certain differences were formed related to the clinical group, the initial level of disorders and the age of the patients. Thus, the concentration of IL-17 had no significant differences between the groups aged 6-12 years on the 1st and 7th days of the study, but on the 30th day, a sharp (by 104.5%, p < 0.001) excess of the indicator was revealed during the implementation of complex therapy. The content of IL-18 in the correction group in relation to the comparison group gradually decreased. After 7 days, the differences were 10.5% (p > 0.05), and after 30 days - 19.6% (p = 0.020). In general, in both groups isolated depending on the treatment, there was a dynamic towards normalization of the content of the studied cytokines, but in the main group 3 it was significantly faster and deeper than in the corresponding control group. The content of resident microorganisms was characterized by a significant increase in those that were present in the administered preparation, Linex, which is a combination of Bifidobacterium and Lactobacillus, provided an increase in the content of these groups of microorganisms (by 124.7% and 121.6%, respectively). The total indicator reflecting the content of microorganisms of this category, when corrected, exceeded the values ​​of the comparison group by 44.2%. The content of transient microorganisms tended to decrease when using bacterial preparations. Given their small share in the structure, only an analysis of the total factor makes sense. With respect to the control group 3, it was lower after Linex by 82.4% (p = 0.017). A similar analysis conducted with respect to the indicator of the presence of microscopic fungi also revealed its decrease when using Linex - by 48.5% (p = 0.033). A decrease in the viral component in the microbiome after the use of Linex by 48.0% (p = 0.027) was also observed. The level of organisms not found in the norm was minimal in all cases, and after correction with the use of Linex - almost zero. The content of plasmalogen in children aged 6-12 years at the time of repeated examination when using the drug Linex determined the excess of the indicator over the control group 3 (p = 0.045). The endotoxin indicator in children of the younger group when using the probiotic was lower than in the corresponding control group. The differences were 30.4% (p = 0.042).

**Conclusions**

1. Comparison of the frequency of the presence of CTD in children with reactive arthritis and clinically healthy children revealed a significant excess of 37.7% (p=0.015). The structure of arthritis localization in the groups did not reveal significant differences. In children with arthritis against the background of CTD, an excess of the duration and severity of pain syndrome, as well as an excess of laboratory parameters of inflammation (CRP - by 21.5%, p=0.035) were determined.

2. For the content of cytokines in children with reactive arthritis, a dependence on the presence of CTD was revealed, consisting in an excess of the activity of proinflammatory cytokines. In the older age group, a pronounced imbalance in the content of IL-17 and IL-18 was determined. In the first case, with CTD, a reduced value was revealed (by 40.5%, p=0.011), in the second - an excess of 55.0% (p=0.019). Differences were revealed in the parameters related to the formation of connective and bone tissue - alkaline phosphatase activity, calcium, magnesium (23.3% in the 13-18-year-old group, p=0.040) and vitamin D, characteristic of CTD.

3. The microbiome of the body in children with reactive arthritis is characterized by the presence of a number of differences from healthy ones. There is a decrease in the number of resident microorganisms, an increase in transient and abnormal ones, as well as an excess of endotoxin. The differences were more characteristic of the older group of subjects. When comparing children with reactive arthritis against the background of CTD and without it, in the first case, a lower content of resident microorganisms was found in the older group, a higher content of transient ones in both, viruses in the younger group, and abnormal ones in the older one. The plasmalogen content was significantly lower (23.2%; p=0.041), and the endotoxin content was significantly higher in the presence of CTD (66.5%, p=0.020).

4. The use of the method of correcting the body's microbiome at the level of intestinal microbiota in the acute period of reactive arthritis against the background of CTD ensures a decrease in the content of proinflammatory cytokines and phagocytosis activity, which contributes to a faster clinical recovery. The use of a probiotic improves the balance of intestinal microflora, which is manifested in changes in the microbiome parameters of the whole body (an increase in plasmalogen by 41.3%; p=0.040, a decrease in endotoxin by 58.2%, p=0.014).

**Practical recommendations**

1. When diagnosing reactive arthritis in children, signs of CTD are necessary at the same time, since in combination with CTD, a more severe and prolonged course of the disease is predicted.

2. For the treatment of reactive arthritis against the background of CTD, it is recommended to include in the treatment complex drugs for immunocorrection and probiotics to improve the state of the intestinal microbiome and, therefore, increase the effectiveness of treatment and prevent subsequent relapses.

**Testing of the work**

The main results of the dissertation were presented and discussed at:

1. Republican scientific and practical conference with international participation "Modern pediatrics, achievements and prospects. Modern perinatal medical technologies in solving demographic security problems" dedicated to the 80th anniversary of Doctor of Medical Sciences, Professor G.A. Tuleutaeva (Semey, 05/21/2021)

2. All-Russian therapeutic congress with international participation "Botkin Readings" (April 23-24, 2021, Russian Federation, St. Petersburg)

3. XV International scientific and practical conference "Ecology. Radiation. Health" dedicated to the 30th anniversary of the closure of the Semipalatinsk nuclear test site. (Semey, August 28, 2021)

4.I International Scientific and Practical Conference «Concepts for the development of society’s scientific potential» (November 21-22, 2021 Prague, Czech Republic)

5.I International MED-Congress «Man and Health. Multidisciplinary approach in medicine» (Semey, October 18-19, 2022)

6.XVI International Scientific and Practical Conference «Ecology. Radiation. Health», named after B.A. Atchabarov (August 28 - 29, 2023)

**Publications**

18 scientific papers have been published on the topic of the dissertation, including 3 articles in the journal "Science and Healthcare", 2022.4 (T24), 2024.6 (T26), 2024.4 (T26) recommended by the Committee for Quality Assurance in Science and Higher Education of the Ministry of Science and Higher Education of the Republic of Kazakhstan, where the dissertation candidate is the first author and corresponding author, 1 article in a journal indexed in Scopus: "Georgian Medical News" No.2 (311) 2021. CiteScore equal to 0.6 and percentile - 31% (Q3) at the time of dissertation defense, 1 article in the proceedings of an international scientific conference, 2 copyright certificates of the Republic of Kazakhstan, 1 rationalization proposal No.2454, 4 theses, where the applicant is the author and 7 implementation acts.