НЕОНАТОЛОГІЯ, ХІРУРГІЯ ТА ПЕРИНАТАЛЬНА МЕДИЦИНА
NEONATOLOGY, SURGERY AND PERINATAL MEDICINE
Т. XVI, №2(60), 2026
VOL. XVI, №2(60), 2026
ISSN 2226-1230 (PRINT) ISSN 2413-4260 (ONLINE)
KEY TITLE: NEONATOLOGÌÂ, HÌRURGÌÂ TA PERINATAL'NA MEDICINA (ONLINE)
ABBREVIATED KEY TITLE: NEONATOL. HÌR. PERINAT. MED. (ONLINE)

УДК: 616.831-009.11/.12:612.017.1-036
DOI: 10.24061/2413-4260.XVI.2.60.2026.11

IMMUNOPATHOGENETIC PARAMETERS IN PATIENTS WITH CEREBRAL PALSY

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Abstract.

Cerebral palsy (CP) represents a multi-etiological disorder where immunological mechanisms, specifically T- and B-cell immunity, contribute significantly to pathogenesis. TREC and KREC, molecular markers of thymic and bone marrow lymphocyte production, facilitate immune status assessment in this patient category.

Objective. *To analyze immunohematological and immunogenetic parameters within a general cohort of patients with CP and across gender subgroups, while examining the correlations between these variables.*

Materials and Methods. *This prospective study enrolled 210 patients with CP (U. K. Kurbanov Republican Children's Psychoneurological Hospital, 2022-2025). TREC and KREC levels were quantified via real-time PCR (ROSSAmed PID kit, ROSSA LLC, Uzbekistan; BioRad system), immunoglobulin levels were measured through immunoturbidimetry, and hematological indices were determined using an automated analyzer. The study protocol was approved by the Local Bioethics Committee of the Bukhara State Medical Institute, and the research was conducted in accordance with the Declaration of Helsinki (2013 revision), with written informed consent obtained from the parents or legal representatives of all participants. Statistical analysis utilized Student's t-test, the Mann–Whitney U test, and correlation analysis, with the significance threshold set at $p < 0.05$. Data processing was conducted using SPSS Statistics version 26.0. This research was conducted as part of the Bukhara State Medical Institute research plan entitled «Clinico-immunological and genetic aspects of the pathogenesis of cerebral palsy» (2022-2026).*

Results. *No significant sex-related differences were observed in TREC, KREC, or lymphocyte levels ($p > 0.05$). TREC and KREC concentrations in males nevertheless exceeded those in females by 44% and 58%, respectively. Immunoglobulin levels and hematological parameters similarly demonstrated no statistically significant intergroup differences, although a trend toward elevated IgM concentrations was noted in females. Correlation analysis identified seven significant associations, including positive correlations for IgA/IgM ($r = +0,74$), IgA/TREC ($r = +0,65$), IgA/KREC ($r = +0,68$), alongside inverse relationships for Hb/Plt ($r = –0,72$), WBC/IgG4 ($r = –0,61$), Hb/IgG2 ($r = –0,72$), and ESR/IgG2 ($r = –0,68$).*

Conclusions. *Reduced TREC and KREC values in a subset of patients with CP suggest potential immune system pathology underlying the disease pathogenesis. Primary immunohematological indices lack significant sex-related differences; however, the observed trends necessitate verification in larger cohorts*

Keywords: *Cerebral Palsy; TREC; KREC; Immunoglobulins; Immunoreactivity; T-Lymphocytes; B-Lymphocytes; Sex Differences; Correlation Analysis.*

Introduction

Cerebral palsy (CP) ranks among the most prevalent pediatric neurological disorders leading to permanent disability. Despite decades of research, the pathogenesis of CP remains partially understood. Neuroimaging data (neurosonography, CT, brain MRI), biochemical indices, and postnatal clinical manifestations indicate the involvement of autoimmune mechanisms, cytokine production, and other immunological mediators that sustain chronic inflammation [1, 2, 3, 4, 5].

Immunoreactivity, particularly the status of T- and B-cell immunity, substantially influences CP outcomes. Peripheral blood levels of T-cell receptor excision circles (TREC) and kappa-deleting recombination excision circles (KREC) serve as molecular markers for the severity of T- and B-lymphopenia. These circular DNA structures, formed during the maturation of T- and B-lymphocytes in the thymus and bone marrow, respectively, constitute reliable markers of thymic and bone marrow lymphocyte production [6, 7, 8, 9, 10].

Integrated with immunoglobulins (IgA, IgM, IgG, IgG2, IgG4) and baseline hematological indices (Hb, WBC, Plt, ESR), TREC/KREC facilitate a comprehensive evaluation of immune status in children with CP [11, 12, 13, 14, 15]. The study cohort exhibited a predominance of males (60.5%) relative to females (39.5%), necessitating a comparative sex-based analysis of the investigated parameters.

Study Objective. To analyze immunohematological and immunogenetic parameters within a general cohort of patients with CP and across sex-based subgroups, while examining the correlations between these variables

Materials and Methods

Design and Setting. This prospective study involved 210 patients with CP hospitalized at the U. K. Kurbanov Republican Children's Psychoneurological Hospital between 2022 and 2025.

Inclusion Criteria: Enrollment required a clinically verified diagnosis of CP staged by GMFCS and the absence of acute infectious diseases for 2-3 weeks prior to study entry.

Laboratory Methods. TREC and KREC were quantified using real-time PCR with the ROSSAmed PID reagent kit (ROSSA LLC, Uzbekistan) on a BioRad system (2020). Immunoglobulin levels (IgA, IgM, IgG, IgG2, IgG4) were determined by immunoturbidimetry, while hematological indices (Hb, WBC, Plt, ESR, lymphocytes) were analyzed using an automated analyzer.

Statistical Analysis. Intergroup sex-related differences were assessed using Student's t-test and the Mann–Whitney U test. Correlations between parameters were evaluated by calculating Spearman's correlation coefficient (r). The statistical significance threshold was set at $p < 0.05$, with data processing performed using SPSS Statistics version 26.0

Ethical Considerations. The study protocol was approved by the Local Bioethics Committee of the Bukhara State Medical Institute, and the research was conducted in accordance with the Declaration of Helsinki (2013 revision), with written informed consent obtained from the parents or legal representatives of all participants.

Research Program Alignment. This work was conducted under the research plan of the Bukhara State Medical Institute entitled «Clinico-immunological and genetic aspects of the pathogenesis of cerebral palsy» (2022-2026).

Results and Discussion

Quantitative TREC, KREC, and lymphocyte levels for male and female patients with CP are summarized in Table 1

Table 1

Student's t-test and Mann–Whitney U test results for TREC, KREC, and lymphocyte levels in children with CP stratified by sex

Parameter	Mean (M)	Mean (F)	t-test (p)	Mann–Whitney U test (p)
TREC	180 988.6	125 525.8	0.290	0.271
KREC	195 281.8	123 722.8	0.238	0.170
Лимфоциты, ×10⁹/л	10.93	10.15	0.524	0.486

Note: M, males (n = 127); F, females (n = 83). P-values > 0.05 across all parameters indicate an absence of significant intergroup differences.

Intergroup differences were assessed using Student's t-test (parametric) and the Mann–Whitney U test (non-parametric). P-values exceeding the statistical significance threshold ($p > 0.05$) across all three instances indicate no significant disparities between male and female patients regarding the investigated parameters; nevertheless, a pronounced tendency toward higher TREC and KREC levels was observed in males with CP compared to females (Figure 1).

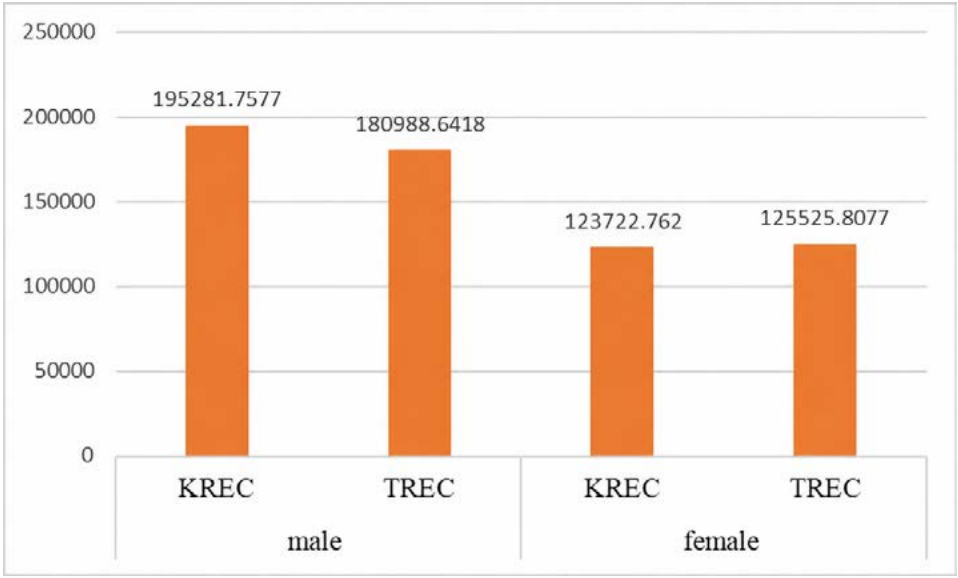

Figure 1. TREC and KREC levels in males with CP compared to females with CP.

Given that TREC and KREC function as markers of thymic and B-cell proliferation, the higher mean levels observed in males (by 44% and 58%, respectively) suggest a marginally increased production of «recent» T- and B-lymphocytes in male patients relative to females. Overlapping confidence intervals and substantial intragroup variability (indicated by wide SEM) preclude the characterization of these differences as significant, although the observed trend aligns with established data regarding more active thymic function in males during early childhood. The reduction in TREC/KREC levels among females might serve as an indirect marker of an earlier «thymic involutional shift» or stem from the limited sample size; consequently, determining population references and significant fluctuations in TREC/KREC levels necessitates further extensive research.

The general hematological profile in patients with CP reveals slightly elevated Hb and WBC levels in females; however, overlapping 95% confidence intervals indicate no substantial dispa–rities in erythropoiesis and leukopoiesis between sexes. Similar erythrocyte sedimentation rates (ESR ≈ 6.5-6.6 mm/h) in both subgroups reflect comparable levels of systemic inflammatory response between males and females.

Table 2

Comparison of hematological and immunological parameters in male and female patients with CP

Parameter	Mean ♂	Mean ♀	95% CI ♂	95% CI ♀	t-statistic.	p (two-tailed)
ESR, mm/h	6.55	6.60	5.83-7.26	5.76-7.44	-0.10	0.92
Hb, g/L	119.4	121.7	115.7-123.0	118.6-124.8	–0.95	0.35
IgA, g/L	1.42	1.22	1.06-1.78	1.03-1.42	0.93	0.36
IgG, g/L	10.92	9.80	9.88-11.96	8.22-11.38	1.17	0.25
IgG2, g/L	3.90	3.55	2.69-5.12	2.64-4.46	0.45	0.66
IgG4, g/L	0.38	0.36	0.25-0.51	0.22-0.50	0.12	0.91
IgM, g/L	1.29	1.62	1.14-1.43	1.29-1.95	–1.84	0.07
WBC, ×10^9/L	8.42	8.33	7.79-9.04	7.72-8.94	0.20	0.84
Plt, ×10^9/L	298.3	289.0	275.2-321.3	267.2-310.7	0.53	0.60

Note: Data are presented as mean ± 95% CI. P-values > 0.05 indicate no statistically significant intergroup differences. A trend toward elevated IgM levels was observed in females (p = 0.07).

Assessment of the platelet lineage demonstrated marginally higher counts in females (297 × 10^9/L vs. 289 × 10^9/L), although this difference lacked statistical significance. Both values remain within the clinical reference range, indicating an absence of sex-based differences in megakaryocytic production among children with CP.

Assessment of humoral immunity parameters demonstrated that mean IgA and IgG levels, including IgG2 and IgG4 subclasses, were marginally higher in males (6-11%), though the 95% CI ranges overlapped entirely. These findings clinically indicate equivalent B-cell response maturity and comparable protective activity across primary antibody classes.

IgM levels conversely exhibited the most pronounced relative disparity, being approximately 28% higher in females. Although representing only a statistical trend ($p \approx 0.07$), this physiological observation may reflect a more robust primary (T-independent) B-cell response in young females, aligning with published data on female IgM predominance during puberty.

The absence of statistically significant differences between sexes clinically suggests that baseline inflammatory and immune parameters in children with CP develop through similar pathogenetic mechanisms.

Individually elevated IgM levels in certain females and high TREC/KREC values in specific males notably necessitate longitudinal monitoring, since elevated IgM concentrations may be associated with chronic antigenic stimulation, whereas high TREC/KREC levels alongside stable immunoglobulin profiles may represent compensatory reorganization of the innate immune system.

Patient sex should not be considered a determining factor when planning immunorehabilitation interventions; rather, adjustments should be based on individual marker values and clinico-anamnestic data.

Subsequent analysis of the correlations between the 11 hematological and immunogenetic parameters identified seven associations in patients with CP, comprising four positive and three inverse correlations. Correlation strength was moderate (r = 0.5-0.7) in two cases and high (r = 0.7-1.0) in five.

Significant positive correlations were identified between IgA and IgM (r = 0.74), IgA and TREC (r = 0.65), and IgA and KREC (r = 0.68), while inverse correlations were observed between Hb and Plt (r = –0.72), WBC and IgG4 (r = –0.61), Hb and IgG2 (r = –0.72), and ESR and IgG2 (r = –0.68) ($P \leq 0.05$) (Figure 2).

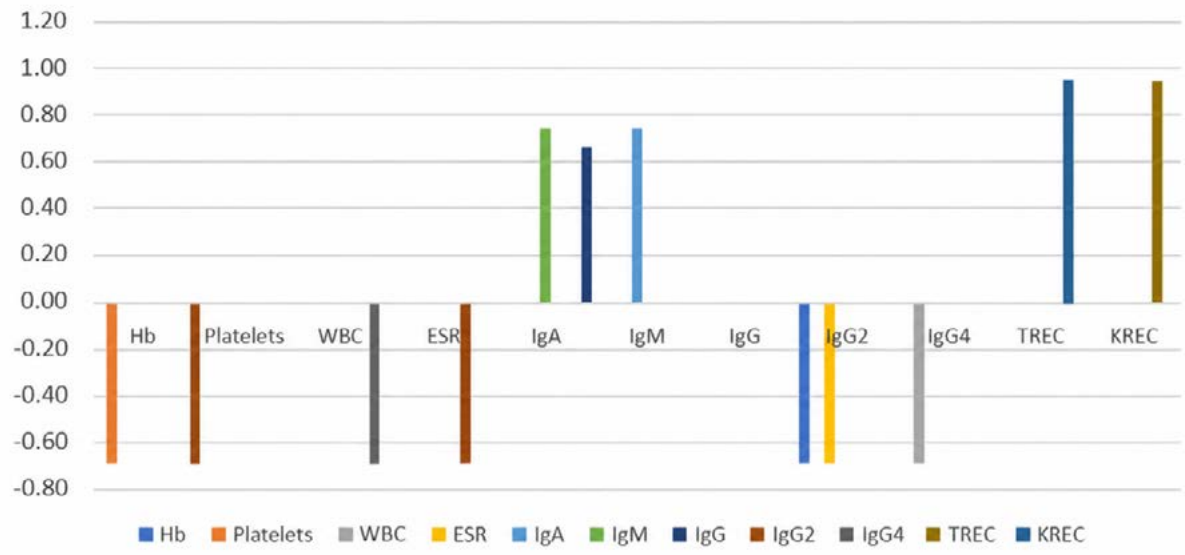

Figure 2. Significant correlations between hematological and immunogenetic parameters in children with CP (P ≤ 0.05).

Remaining correlations between hematological and immunogenetic parameters proved weak and statistically insignificant.

Discussion

The observed lack of significant sex-based differences in primary immunohematological parameters among children with CP aligns with existing literature [8, 16, 17]. Low TREC and KREC values in a subset of patients with CP nevertheless warrant particular attention, as they potentially indicate primary or transient immune deficiency underlying the disease pathogenesis. Although the role of immune dysregulation-specifically T- and B-lymphocyte deficiency-in CP progression has been investigated previously, available data remain insufficient for definitive conclusions [18, 19].

Identified positive correlations between IgA and both TREC and KREC may reflect integral lymphocyte maturation mechanisms, where active thymic and bone marrow production is associated with elevated IgA levels. Furthermore, inverse correlations for Hb/IgG2 and ESR/IgG2 are consistent with established data linking IgG2 subclass deficiency to chronic inflammation, anemia of inflammation, and elevated ESR [20].

Study limitations include the single-center design, the absence of a healthy control group, and unequal sex-based subgroups. The identified trends (IgM ↑ in females, TREC/KREC ↑ in males) necessitate verification in larger, stratified cohorts.

Conclusions

1. Reduced TREC and KREC values in a subset of patients with CP suggest the probable involvement of immune system pathology (either primary or transient) in the disease pathogenesis.

2. Primary immunohematological parameters (TREC, KREC, lymphocytes, immunoglobulins, and hematological indices) in children with CP exhibit no significant sex-related differences ($p > 0.05$).

3. Observed trends-including higher TREC (+44%) and KREC (+58%) levels in males and elevated IgM in females ($p = 0.07$)-require further investigation in larger stratified cohorts and may influence individualized immune correction strategies.

4. Correlation analysis identified seven significant associations: positive correlations for IgA/IgM ($r = +0.74$), IgA/TREC ($r = +0.65$), and IgA/KREC ($r = +0.68$), alongside inverse correlations for Hb/Plt ($r = –0.72$), WBC/IgG4 ($r = –0.61$), Hb/IgG2 ($r = –0.72$), and ESR/IgG2 ($r = –0.68$), reflecting integral relationships between immunogenetic and hematological parameters in CP.

Perspectives for Further Research

Further research perspectives involve the prioritization of multicenter studies utilizing expanded cohorts and age- and sex-matched healthy controls to establish TREC/KREC reference values in CP. The longitudinal evaluation of immunological parameters during comprehensive rehabilitation and the determination of their prognostic significance regarding GMFCS-staged motor deficit severity are warranted. Additionally, the mechanisms underlying the association between IgA and TREC/KREC, alongside the factors driving sex-based disparities in CP immunoglobulin levels, necessitate targeted investigation.

Author Contributions. AT Sharipov: study design, patient recruitment, results interpretation, manuscript drafting; Б BZ Khamdamov: scientific supervision, study conceptualization, critical manuscript revision; UP Nabieva: organization and implementation of immunological assays (TREC/KREC, immunoglobulins), immunological data interpretation; UA Sherbekov: statistical analysis, manuscript editing. UA Sherbekov: statistical analysis, manuscript editing. All authors have reviewed the final version of the manuscript and consented to its publication.

Conflict of Interest. The authors declare no conflicts of interest regarding the preparation and publication of this article.

Use of Artificial Intelligence. Artificial intelligence was not utilized in the preparation of this manuscript.

Funding. This research received no external funding.

Acknowledgements. The authors express their gratitude to the staff of the U. K. Kurbanov Republican Children's Psychoneurological Hospital for their assistance in organizing the study and collecting clinical material.

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ІМУНОПАТОГЕНЕТИЧНІ ПОКАЗНИКИ У ХВОРИХ З ДИТЯЧИМ ЦЕРЕБРАЛЬНИМ ПАРАЛІЧЕМ

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Резюме.

Дитячий церебральний параліч (ДЦП) є поліетіологічним захворюванням, у патогенезі якого важливу роль відіграють імунологічні механізми, зокрема стан Т- та В-клітинного імунітету. Молекулярні маркери тимічної та кістковомозкової продукції лімфоцитів – TREC і KREC – представляють інтерес для оцінки імунного статусу у даної категорії пацієнтів.

Мета. Аналіз імуногематологічних та імуногенетичних параметрів у загальній когорті хворих на ДЦП та в гендерних підгрупах, а також вивчення кореляційних взаємозв'язків між ними.

Матеріали та методи. Проспективне дослідження із залученням 210 пацієнтів з ДЦП (Республіканська дитяча психоневрологічна лікарня ім. У. К. Курбанова, 2022-2025 рр.). Визначено рівні TREC та KREC методом ПЛР у режимі реального часу (набір ROSSAmed PID, ТОВ «ROSSA», Узбекистан; апарат BioRad), імуноглобулінів – імунотурбідиметрично, гематологічних показників – на автоматичному аналізаторі. Дослідження виконано відповідно до принципів Гельсінської декларації ВМА (редакція 2013 року). Протокол схвалено локальним комітетом з біоетики Бухарського державного медичного інституту. Інформовану згоду батьків (законних представників) усіх пацієнтів отримано в письмовій формі. Статистичні методи дослідження: t-критерій Стьюдента, U-критерій Манна–Уїтні, кореляційний аналіз ($p<0,05$). Поріг статистичної значущості – $p<0,05$. Обробка даних – SPSS Statistics версії 26.0. Робота виконана в рамках плану НДР Бухарського державного медичного інституту за темою «Клініко-імунологічні та генетичні аспекти патогенезу дитячого церебрального паралічу» (2022-2026 рр.).

Результати. Достовірних статевих відмінностей за рівнями TREC, KREC та лімфоцитів не виявлено ($p>0,05$). Проте TREC у хлопчиків був вищим на 44%, KREC – на 58% порівняно з дівчатками. Щодо імуноглобулінів та гематологічних показників також не встановлено статистично значущих міжстатевих відмінностей, хоча відзначалася тенденція до вищих значень IgM у дівчаток. Кореляційний аналіз виявив 7 значущих взаємозв'язків: прямі – IgA/IgM ($r=+0,74$), IgA/TREC ($r=+0,65$), IgA/KREC ($r=+0,68$); обернені – Hb/Plt ($r= –0,72$), WBC/IgG4 ($r= –0,61$), Hb/IgG2 ($r= –0,72$), ШОЕ/IgG2 ($r= –0,68$).

Висновки. Низькі значення TREC і KREC у частини пацієнтів з ДЦП свідчать про можливу патологію імунної системи, що лежить в основі патогенезу захворювання. Ключові імуногематологічні показники не мають достовірних міжстатевих відмінностей; виявлені тенденції потребують верифікації на розширених вибірках.

Ключові слова: дитячий церебральний параліч; TREC; KREC; імуноглобуліни; імунореактивність; Т-лімфоцити; В-лімфоцити; гендерні відмінності; кореляційний аналіз.

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Received: March 17, 2026
Accepted: May 28, 2026
Published: June 29, 2026