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IMMUNOMORPHOLOGICAL CHARACTERISTICS OF PERIPHERAL BLOOD IN CHILDREN WITH ACQUIRED IMMUNODEFICIENCY

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Abstract
Acquired immunodeficiency in pediatric populations demonstrates distinct alterations in peripheral blood cellular composition and immunophenotypic profiles. This investigation examined hematological and immunological parameters in 87 children aged 3 to 14 years with documented secondary immunodeficiency. Flow cytometric analysis revealed significant reductions in CD4+ lymphocyte populations and altered CD4/CD8 ratios compared to age-matched controls. Quantitative immunoglobulin assessments demonstrated variable patterns correlating with infection frequency. These findings establish specific immunomorphological markers that may facilitate early detection and monitoring of immunodeficient states in pediatric practice.

Keywords: immunodeficiency, lymphocytes, cd4, cd8, immunophenotyping, hematology, cytometry, immunoglobulins, pediatrics, leukocytes, neutrophils, monocytes, eosinophils, thrombocytes, erythrocytes.

Introduction

Acquired immunodeficiency in childhood represents a significant clinical challenge characterized by increased susceptibility to recurrent infections, prolonged recovery periods, and potential complications affecting growth and development. Unlike primary immunodeficiencies resulting from genetic defects, secondary immune dysfunction arises from environmental factors, nutritional deficiencies, chronic diseases, or pharmacological interventions. The peripheral blood compartment serves as an accessible window into systemic immune status, providing measurable indicators of cellular and humoral immunity. Contemporary clinical practice requires precise characterization of immunomorphological parameters to differentiate pathological immune suppression from physiological variations inherent to pediatric populations. Understanding the specific alterations in blood cell populations, lymphocyte subsets, and functional markers enables targeted therapeutic interventions and prognostic assessments. This study addresses the critical need for comprehensive immunomorphological profiling in children presenting with clinical manifestations suggestive of acquired immunodeficiency.

Literature Review

Makhkamova and colleagues demonstrated in 2019 that children with recurrent respiratory infections exhibited significantly reduced CD3+ and CD4+ populations, with mean values declining by 28% compared to healthy controls. Rashidova's 2020 investigation of 156 pediatric patients revealed correlations between absolute lymphocyte counts below 2.1×10^9/L and increased hospitalization

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rates. Yuldashev's comprehensive analysis in 2018 established age-specific reference ranges for Uzbek children, noting substantial variations from European standards particularly in CD16+ natural killer cell percentages. Tursunova documented in 2021 that immunoglobulin G concentrations below 6.8 g/L predicted severe bacterial infections with 73% sensitivity. Karimov's 2017 study identified neutrophil-to-lymphocyte ratios exceeding 2.8 as indicative of immune dysregulation in chronically ill children. These investigations collectively establish the clinical utility of immunomorphological assessment, yet gaps remain regarding integrated interpretation of multiple parameters and their temporal dynamics during disease progression.

Methodology

This prospective observational study was conducted at the Republican Specialized Scientific-Practical Medical Center of Pediatrics between January 2022 and November 2023. The investigation received ethical approval from the institutional review board, and written informed consent was obtained from all parents or legal guardians. The study population comprised 87 children aged 3 to 14 years who presented with clinical manifestations of acquired immunodeficiency, defined as four or more documented bacterial infections requiring antimicrobial therapy within the preceding 12 months, or two or more episodes of pneumonia, meningitis, or sepsis. Exclusion criteria included known primary immunodeficiency disorders, active malignancy, recent immunosuppressive therapy within 6 months, congenital anomalies, and unwillingness to participate. A control group of 62 age-matched healthy children without history of recurrent infections was enrolled from routine health screening programs. Venous blood samples were collected in EDTA-containing tubes between 08:00 and 10:00 hours following overnight fasting to minimize diurnal variation. Complete blood count analysis was performed using a Sysmex XN-1000 automated hematology analyzer within 2 hours of collection, measuring hemoglobin concentration, total leukocyte count, differential white cell percentages, absolute neutrophil count, lymphocyte count, monocyte count, eosinophil count, platelet count, and red blood cell indices.

Immunophenotyping was conducted using four-color flow cytometry on a BD FACSCalibur system. Lymphocyte subpopulations were identified using fluorochrome-conjugated monoclonal antibodies against CD3, CD4, CD8, CD16, CD19, and CD56 markers. Absolute counts were calculated by multiplying percentages by total lymphocyte counts obtained from hematology analysis. The CD4/CD8 ratio was computed for each subject. Serum immunoglobulin concentrations were measured by nephelometry using a Siemens BN ProSpec system, quantifying IgG, IgA, and IgM levels. Statistical analysis employed SPSS version 26.0 software. Continuous variables were expressed as means with standard deviations. Normality was assessed using the Shapiro-Wilk test. Independent samples t-tests compared parameters between immunodeficient and control groups. Pearson correlation coefficients evaluated relationships between variables. Statistical significance was established at $p<0.05$. Sample size calculation indicated that 80 subjects per group would provide 85% power to detect a 15% difference in CD4+ cell counts at alpha 0.05.

Results

The immunodeficient cohort consisted of 48 males and 39 females with a mean age of 7.3±3.1 years, while controls included 34 males and 28 females with a mean age of 7.6±2.9 years. Hematological

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analysis revealed that children with acquired immunodeficiency demonstrated significantly lower mean hemoglobin concentrations (116.4±11.3 g/L) compared to controls (128.7±9.2 g/L, p<0.001). Total leukocyte counts were reduced in the immunodeficient group (5.8±1.4×10⁹/L versus 7.2±1.1×10⁹/L, p<0.001). Absolute lymphocyte counts showed marked depression, averaging 1.68±0.52×10⁹/L in affected children compared to 2.89±0.47×10⁹/L in controls (p<0.001). Neutrophil percentages were elevated at 64.2±8.7% versus 52.3±6.4% (p<0.001), though absolute neutrophil counts remained within normal ranges. Flow cytometric immunophenotyping demonstrated profound alterations in lymphocyte subset distribution. CD3+ T lymphocytes constituted 58.3±9.4% of total lymphocytes in immunodeficient children, significantly below the control value of 68.7±7.1% (p<0.001). Absolute CD3+ counts were markedly reduced at 0.98±0.34×10⁹/L versus 1.98±0.41×10⁹/L (p<0.001). The CD4+ helper T cell population showed the most pronounced decline, with percentages of 28.6±7.2% compared to 41.3±5.8% in controls (p<0.001), corresponding to absolute counts of 0.48±0.18×10⁹/L versus 1.19±0.28×10⁹/L (p<0.001). CD8+ cytotoxic T lymphocytes were less affected, comprising 24.7±6.3% in immunodeficient subjects versus 23.8±4.9% in controls (p=0.34). Consequently, the CD4/CD8 ratio was significantly diminished at 1.21±0.41 compared to 1.76±0.35 (p<0.001).

Natural killer cell populations marked by CD16+CD56+ demonstrated proportional increases to 18.4±5.7% in immunodeficient children versus 13.2±4.1% in controls (p<0.001), though absolute counts did not differ significantly. CD19+ B lymphocytes showed modest reductions at 16.8±4.3% versus 19.5±3.8% (p=0.003). Serum immunoglobulin analysis revealed heterogeneous patterns. Mean IgG concentration was 7.2±2.3 g/L in immunodeficient subjects compared to 9.8±1.7 g/L in controls (p<0.001). IgA levels were similarly decreased at 0.94±0.42 g/L versus 1.38±0.36 g/L (p<0.001). IgM concentrations showed no significant difference, measuring 1.12±0.38 g/L and 1.18±0.31 g/L respectively (p=0.28). Correlation analysis identified strong associations between CD4+ absolute counts and infection frequency (r=-0.67, p<0.001) and between IgG levels and hospitalization duration (r=-0.54, p<0.001).

Discussion

The observed immunomorphological alterations in peripheral blood of children with acquired immunodeficiency align with established concepts of immune dysregulation while revealing specific patterns relevant to pediatric populations. The preferential depletion of CD4+ helper T lymphocytes with relative preservation of CD8+ cytotoxic cells results in inverted or compressed CD4/CD8 ratios, a hallmark feature that distinguishes pathological immunosuppression from normal developmental variations. This pattern reflects disruption of thymic output and peripheral lymphocyte homeostasis, potentially mediated by chronic antigenic stimulation, nutritional deficiencies affecting lymphopoiesis, or regulatory mechanisms that disproportionately impact CD4+ subsets. The reduction in total lymphocyte counts below 1.8×10⁹/L observed in 63% of immunodeficient children corresponds to threshold values associated with increased infection risk documented in previous investigations. Yuldashev's reference ranges established that Uzbek children typically maintain lymphocyte counts between 2.5 and 4.2×10⁹/L during the preschool years, emphasizing the clinical significance of lymphopenia in our cohort. The compensatory increase in neutrophil percentages without proportional elevation in absolute counts suggests a relative shift rather than active

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granulopoiesis, consistent with bone marrow stress responses observed in chronic inflammatory states. Hypogammaglobulinemia, particularly affecting IgG and IgA isotypes, reflects impaired antibody production capacity despite relatively preserved B cell numbers. This dissociation between cellular presence and functional output indicates defects in T cell-dependent B cell activation, class switching, or plasma cell differentiation. The correlation between IgG concentrations below 7.0 g/L and increased hospitalization burden supports therapeutic consideration of immunoglobulin replacement in severely affected individuals. Rashidova's findings of similar immunoglobulin patterns in children with recurrent pneumonia validate these observations across different clinical contexts.

The elevation of natural killer cell proportions may represent a compensatory mechanism attempting to maintain innate immune surveillance when adaptive immunity is compromised. However, functional assays would be necessary to determine whether these cells retain cytotoxic capacity or represent anergic populations. Limitations of this investigation include the cross-sectional design preventing assessment of temporal evolution, absence of functional immune assays beyond quantitative measurements, and potential selection bias inherent to tertiary referral center populations. Longitudinal studies incorporating cytokine profiling, proliferative responses, and pathogen-specific antibody titers would provide comprehensive understanding of immune reconstitution dynamics.

Children with acquired immunodeficiency demonstrate characteristic immunomorphological alterations in peripheral blood, principally involving CD4+ lymphocyte depletion, compressed CD4/CD8 ratios, and hypogammaglobulinemia affecting IgG and IgA isotypes. These quantifiable parameters provide objective criteria for diagnosis, severity stratification, and therapeutic monitoring. Integration of hematological and immunophenotypic assessment enables clinicians to identify children at highest risk for severe infections and guide appropriate interventions.

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