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Specific Biochemical Markers in the Early Diagnosis of Connective Tissue Dysplasia in Children

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Abstract. *Connective tissue disorders, particularly hereditary and secondary collagenoses manifesting during childhood, remain a global health challenge in modern pediatrics and biochemistry. The progression of these pathologies is directly linked to the extensive degradation of the extracellular matrix, which exacerbates mineral metabolism disorders and leads to profound pathological damage at the tissue level. Therefore, the primary objective of this study was to evaluate the role of specific biochemical markers in the early diagnosis of connective tissue dysplasias in children and to comparatively investigate the pathogenetic correlations between the end products of matrix degradation and the mineral-electrolyte profile across various clinical forms. To achieve this, 50 children aged 3–15 years with verified diagnoses were enrolled in the study and divided into two cohorts: the hereditary collagenosis group (n=18) and the secondary (rheumatic) form group (n=22). A control group comprising 10 healthy children was also established. Blood serum samples from all participants were analyzed using biochemical methods to determine the levels of matrix degradation markers, mineral metabolism indicators (Ca, P, Fe), and enzymatic reactivity (ALP). Our findings indicate that disease progression and structural genetic defects significantly accelerate tissue destruction. Notably, a definitive 1.6-fold increase in the levels of specific biochemical markers in the hereditary group compared to the control group demonstrates the intensity of the pathological process. While this intense degradation leads to severe calcium-phosphorus deficiency and a corresponding peak in compensatory bone reactivity in the hereditary group, the rheumatic group is characterized by a sharp decline (1.5-fold) in iron levels. Consequently, the parallel monitoring of these biochemical parameters holds substantial clinical significance for early diagnosis, prognosis, and differential analysis of pathogenetic forms of the disease.*

Keywords: *connective tissue, hydroxyproline, glycosaminoglycans, calcium, phosphorus, iron, alkaline phosphatase, children*

Introduction

Connective Tissue (CT) constitutes approximately 50% of the total body mass in adults, serving as a complex system that regulates structural integrity, trophic, protective, and regenerative functions (Cherkas et al., 2023). Its primary components collagen, elastin and proteoglycans (glycosaminoglycans – GAGs) form the extracellular matrix (Amirrah et al., 2022; Rosa Neto et al., 2024). The degradation of these macromolecular systems triggers severe systemic disorders in children, encompassing both acquired inflammatory and genetic pathologies (Sodhi & Panitch, 2021).

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In pediatric practice, acute rheumatic fever and juvenile idiopathic arthritis represent significant socio-medical challenges (Carapetis et al., 2016). Acute rheumatic fever is an autoimmune process triggered by Streptococcus pyogenes infection, leading to irreversible sclerotic damage to heart valves (Nigrovic et al., 2018; Sinitskaya et al., 2024; Lorenz et al., 2024). In juvenile idiopathic arthritis, chronic inflammation of the synovial membrane and the activity of enzymes such as matrix metalloproteinase-3 (MMP-3) drive progressive bone-cartilage destruction (Ahn, 2025; Romano et al., 2025; Wojdas et al., 2021).

Alongside acquired inflammatory conditions, hereditary collagenoses such as Marfan syndrome, Ehlers-Danlos syndrome, and osteogenesis imperfecta are prevalent in childhood (Rosa Neto et al., 2024). For instance, fibrillin-1 (FBN1) defects in Marfan syndrome cause hyperactivation of the TGF-β signaling pathway, resulting in multisystem skeletal and cardiovascular complications (Marelli et al., 2023). In Azerbaijan, particularly in southern regions, the high frequency of consanguineous marriages contributes to a significant incidence of these hereditary CT disorders.

Since both hereditary collagenoses and rheumatism often present with similar phenotypic features in their initial stages, differential diagnosis remains a clinical challenge. Therefore, analyzing serum levels of free hydroxyproline (a collagen breakdown product) and total GAGs, alongside mineral-electrolytes (Ca, P, Fe) and alkaline phosphatase (ALP) activity, is essential for accurately identifying the nature and intensity of tissue destruction (Tush et al., 2019). Evaluating this specific biochemical profile holds great scientific and practical significance for the differential diagnosis of hereditary versus secondary connective tissue disorders.

Materials and Methods

The study enrolled a total of 50 children aged 3–15 years, with 40 patients allocated to the main cohort and 10 to the control group. Within the main cohort, 22 children had a verified clinical diagnosis of rheumatism, while 18 presented with hereditary collagenoses. For comparative purposes, a control group consisting of 10 completely healthy children matched for age and sex was included. Blood serum samples obtained from all participants were investigated for ECM degradation markers, mineral profile indicators, and bone-metabolic enzymatic parameters to evaluate tissue metabolism characteristics. To assess the intensity of ECM destruction and the nature of tissue degradation, free hydroxyproline levels in the biological material were quantified spectrophotometrically using a classical method based on a specific color reaction. Concurrently, the concentration of total GAGs, a vital component of the amorphous ground substance of the matrix, was determined via enzyme-linked immunosorbent assay (ELISA) utilizing specific commercial test kits.

To evaluate bone-mineral homeostasis, serum concentrations of calcium (Ca), inorganic phosphorus (P), iron (Fe), and the activity of the alkaline phosphatase (ALP) enzyme were determined colorimetrically on an automated biochemical analyzer. Calcium levels were measured colorimetrically via a specific complex-formation reaction, phosphorus concentration was determined using the ammonium molybdate method, and iron ions were quantified photometrically using the ferrozine reagent. The total activity of ALP, an essential catalytic indicator of bone metabolism and osteoblast function, was determined using a standard colorimetric-kinetic method based on the rate of p-nitrophenyl phosphate (p-NPP) substrate cleavage. All numerical data obtained during the study were processed using SPSS software in compliance with modern statistical principles. The mathematical mean and standard error of the mean were calculated for each biochemical parameter. The statistical significance of intergroup differences was evaluated using the parametric Student's t-test, with results considered statistically reliable and significant at probability thresholds of $p < 0.05$, $p < 0.01$, and $p < 0.001$.

Results

To evaluate the response of core components governing the structural integrity and functional state of connective tissue to pathological processes, the serum dynamics of free hydroxyproline and total GAGs were comprehensively investigated. Immune-inflammatory cascades occurring during the active phase of rheumatism, alongside genetically progressive matrix destruction in hereditary collagenoses, induced sharp deviations in these parameters. The cross-statistical analysis among the three groups and the significance levels of intergroup differences are summarized in Table 1.

Table 1
Comparison of biochemical indicators reflecting tissue metabolism across the study groups

Biochemical markers	Unit	Control group (n = 10)	Rheumatic group (n = 22)	Hereditary collagenopathy group	P
Free hydroxyproline	mkg%	132,5 ± 7,1	182,6 ± 5,4	238,4 ± 6,2	$p_1 < 0,001$ $p_2 < 0,001$ $p_3 < 0,001$
GAG (Glycosaminoglycans)	mg/l	470,0 ± 14,5	712,3 ± 18,2	895,0 ± 22,4	$p_1 < 0,001$ $p_2 < 0,001$ $p_3 < 0,01$

Note. p_1-statistical significance between control and rheumatic groups, p_2-statistical significance between control and hereditary collagenopathy groups, p_3-statistical significance between rheumatic and hereditary collagenopathy groups.

Free hydroxyproline, which reflects the degree of collagen fiber destruction, was measured at 132.5 ± 7.1mkg% in the control group, where as it rose to 182.6 ± 5.4 mkg% in children with rheumatism due to active inflammation. In the hereditary collagenosis group, this indicator increased even more sharply, reaching 238.4 ± 6.2 mkg%. The total GAG concentration, characterizing the state of the extracellular amorphous ground substance, was 470.0 ± 14.5mg/l in healthy children, but increased 1.5-fold to 712.3 ± 18.2 mg/l in rheumatic patients as a result of proteoglycan depolymerization ($P_1 < 0.001$). In the group with hereditary connective tissue dysplasia, GAG levels surged approximately 1.9-fold compared to the control group, reaching a critical threshold of 895.0 ± 22.4 mg/l ($p_2 < 0.001$). The difference between the two diseased groups was also statistically significant ($p_3 < 0.01$).

Connective tissue pathologies are accompanied not only by $_{ECM}$ destruction but also by profound alterations in the body's mineral homeostasis and bone tissue remodeling processes. To evaluate this pathological course at the molecular level, serum calcium (Ca), inorganic phosphorus (P), iron (Fe) levels, and the activity of ALP were studied. The results and intergroup statistical significance levels are summarized in Table 2.

Table 2

Comparison of biochemical indicators reflecting mineral-electrolyte balance across the study groups

Biochemical markers	Unit	Control group (n = 10)	Rheumatic group (n = 22)	Hereditary collagenopathy group	P
Calcium (Ca)	mg/dl	9,15 ± 0,18	8,28 ± 0,16	7,03 ± 0,14	$p_1 < 0,001$ $p_2 < 0,001$ $p_3 < 0,001$
Phosphorus (P)	mg/dl	4,52 ± 0,20	3,84 ± 0,12	3,23 ± 0,11	$p_1 < 0,01$ $p_2 < 0,001$ $p_3 < 0,001$
Iron (Fe)	mkmol/l	15,8 ± 1,1	10,8 ± 0,7	13,4 ± 0,9	$p_1 < 0,001$ $p_2 < 0,001$ $p_3 < 0,05$
Alkaline Phosphatase (ALP)	U/L	215,0 ± 12,5	305,0 ± 14,2	345,0 ± 15,6	$p_1 < 0,001$ $p_2 < 0,001$ $p_3 < 0,05$

Serum calcium and phosphorus levels decreased significantly compared to controls in both rheumatism (Ca: 8.28 ± 0.16 mg/dl; P: 3.84 ± 0.12 mg/dl) and hereditary collagenosis (Ca: 7.03 ± 0.14 mg/dl; P: 3.23 ± 0.11 mg/dl). The most severe bone mineralization defect was recorded in the hereditary collagenosis group ($p_2 < 0.001$). Such a pronounced drop in calcium and phosphorus within the hereditary cohort proves that inorganic components fail to fix properly within a defective collagen matrix. The difference between the two diseased groups was highly significant ($p_3<0.001$). An interesting finding was noted in iron metabolism. Compared to the control group (15.8 ± 1.1 mkmol/l), the iron concentration in children with rheumatism decreased 1.5-fold, dropping to 10.8 ± 0.7 mkmol/l ($p_1<0.001$). This sharp decline is attributed to the sequestration of iron ions with in storage sites during chronic inflammation. In hereditary collagenosis, iron levels decreased only slightly (13.4 ± 0.9 mkmol/l). The difference in iron levels between the two patient groups was statistically significant ($p_3<0.05$). ALP activity, which reflects metabolic stress in bone tissue, was sharply elevated in both cohorts compared to the control (215.0 ± 12.5 U/L). However, the highest enzymatic activity was found in the blood of children with hereditary collagenosis (345.0 ± 15.6 U/L). This biochemical status indicates an increase in the internal functional strain of osteoblasts attempting to compensate for and repair the genetically fragile bone framework. The intergroup difference was mathematically confirmed ($p_3 < 0.05$).

Discussion

The results obtained in this study clearly demonstrate the internal molecular pathogenesis and distinct metabolic profiles of hereditary collagenoses and rheumatism a systemic immune-inflammatory disease whose clinical presentations (joint syndrome, limited mobility, asthenia) sometimes overlap. The status of collagen, the main structural protein of connective tissue, and its surrounding amorphous ground substance determines the rate of tissue metabolism. The serum dynamics of free hydroxyproline, utilized as a specific marker in our study, indicate profound damage in both pathologies, albeit occurring via different pathways. In the rheumatic group, the statistically significant 1.4-fold increase in free hydroxyproline (182.6 ± 5.4 mkg%) relative to controls is directly related to the active immune-inflammatory process ($p<0.001$). Cytokines (IL-1, TNF-α) accumulating in the synovial membranes and joint capsules during rheumatic fever activate matrix metalloproteinases (MMPs) in the extracellular environment. These enzymes degrade mature collagen fibers, causing free hydroxyproline, a product of hydrolysis, to be released into systemic circulation. A completely different scenario is observed in the hereditary collagenosis group, where

free hydroxyproline levels rose 1.8-fold compared to controls, reaching a critical peak of 238.4 ± 6.2 mkg% ($p < 0.001$). In hereditary connective tissue dysplasias, genetic mutations disrupt the initial synthesis of collagen protein, the folding of its triple helix structure, and the molecular cross-linking mechanisms. Being structurally fragile and unstable, these congenital fibers undergo progressive degradation, failing to withstand normal mechanical loads (Nickell et al., 2025).

A similar pathogenetic dependence was tracked in the concentration of GAGs. The 1.5-fold increase in GAGs during rheumatism indicates that the inflammatory process depolymerizes proteoglycan aggregates. In hereditary collagenosis, GAG levels increased 1.9-fold compared to the control group ($p<0.001$). This serves as an essential differential diagnostic criterion proving that genetic defects cause a systemic breakdown of both the fibers and the amorphous zone of the ECM.

A statistically significant decrease in both calcium and inorganic phosphorus levels was recorded in both pathological groups relative to controls. However, the most profound hypocalcemia was noted in the hereditary collagenosis cohort ($p < 0.001$). In hereditary dysplasias, the intrinsic capacity for calcium and phosphorus ion fixation within bone is impaired because the organic matrix is fundamentally defective. In rheumatism, the mineral imbalance is secondary, primarily driven by chronic inflammation activating osteoclasts and accelerating bone resorption.

Iron analysis stands out as another crucial biochemical indicator differentiating the pathogeneses of these two diseases. Iron concentrations dropped sharply to 10.8 ± 0.7 mkmol/l in rheumatic children ($p < 0.001$). This severe hyposideremia is explained by the activation of hepcidin (synthesized in the liver during systemic inflammation), which functionally blocks iron ions within the macrophage system and storage depots. Conversely, in hereditary collagenosis, iron decreased only slightly (13.4 ± 0.9 mkmol/l), pointing to a general microelement imbalance rather than an inflammatory blockade. ALP activity increased sharply in both patient cohorts compared to controls. In rheumatism, this elevation represents a local compensatory response of osteoblasts to inflammation-induced bone-cartilage damage. In the hereditary collagenosis group, the enzyme reached its absolute peak, reflecting internal adaptive strain. The body maximally stimulates the catalytic activity of osteoblastic cells in an effort to rebuild a genetically fragile, defective, and progressively collapsing bone matrix.

Conclusion

Comprehensive biochemical investigations have successfully elucidated the distinct pathogenetic mechanisms of hereditary and acquired connective tissue pathologies in pediatric patients. Matrix destruction observed in hereditary collagenoses is significantly more severe than that seen in rheumatism. The 1.6- to 1.9-fold increase in free hydroxyproline and GAG indicators in the hereditary cohort confirms that tissue structure is systemically dismantled due to genetic factors. While calcium and phosphorus metabolism disorders were apparent in both pathologies, the deep hypocalcemia and hypophosphatemia recorded in hereditary collagenoses are directly linked to the loss of mineralization capacity within the defective collagen matrix. The sharp decline in iron levels during rheumatism uniquely reflects the "functional blockade" mechanism characteristic of chronic inflammation. Conversely, the higher ALP activity in hereditary collagenoses serves as evidence of a maximal compensatory-adaptive process enacted by the body to restore compromised bone tissue. A laboratory panel consisting of free hydroxyproline, GAG, Ca, P, Fe, and ALP parameters is proposed as a highly informative system of differential diagnostic criteria for the early-stage differentiation of hereditary collagenoses from rheumatic lesions.

Declaration of Competing Interests

The authors declare that they have no competing interests.

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