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Innate Immunity in the Development of Connective Tissue Dysplasia: Pilot Study in Children with Congenital Hip Dislocation

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Abstract

Introduction: Observing and treatment of hip dysplasia in children have always been in the sphere of interest of modern molecular medicine. The role of molecular factors in the formation of connective tissue dysplasia in children is considered crucial for such multisystem disorders, and connective tissue dysplasia progressing involves immune system parameters and biochemical markers. The aim of this work was to establish the relationship between immune status indicators and biochemical markers of connective tissue using bioinformatics and modeling methods. **Materials and Methods:** 27 patients with congenital hip dislocation, admitted to the University Clinic of Privolzhsky Research Medical University, Department of children orthopedics for surgical treatment, were examined. Determination of 10 blood parameters was conducted by modern biochemical and immunological methods. Statistica 12.0 software from StatSoft was used for statistical data processing. Methods of nonparametric statistics were used since the samples in the control group partially follow the normal distribution. Correlation methods and regression modeling methods were used to evaluate the relationship of indicators. **Results and Conclusion:** In our investigation we have shown the presence of statistical and mathematical interactions between the parameters of innate immunity and indicators of connective tissue metabolism. The leading role of the immune system in the development of pathologies associated with connective tissue dysplasia is assumed. In further investigations it is necessary to clarify the role hypoxia in HIF-1 stimulated control of skeletal dysplasia, collagen modification, connective tissue dysplasia development.

Keywords

Congenital Dysplasia, Congenital Hip Dislocation, Connective Tissue

Metabolism, Biomarkers, Immune System

1. Introduction

According to modern data, many independent investigation groups have established a relationship between joint disorders and dysplasia of connective tissue [1]-[6]. Hip dysplasia is one of the most common disorders in pediatric orthopedics and a leading precursor of osteoarthritis and is seen in 20% to 40% of patients with osteoarthritis of the hip. Observing and treatment of hip dysplasia in children have always been in the sphere of interest of modern molecular medicine [7] [8] [9]. Recently, more and more attention in the scientific literature is paid to the role of molecular factors in the formation of connective tissue dysplasia in children. Such multisystem disorders as connective tissue dysplasia involve immune system parameters and biochemical markers in their mechanism [10] [11] [12] [13] [14].

Among the most important biochemical parameters, in connective tissue dysplasia some parameters of the immune status and indicators of connective tissue metabolism are considered the most significant in the modern literature [14]-[20]. Despite this fact there was no possibility to find any works devoted to establishment of statistical and numerical dependences between parameters of immune system and connective tissue metabolism, so in a sense, this study is absolutely groundbreaking.

The aim of this work was to establish the relationship between immune status indicators and biochemical markers of connective tissue using bioinformatics and modeling methods.

2. Materials and Methods

We examined 27 patients with congenital hip dislocation who were admitted to the University Clinic of Privolzhsky Research Medical University, Department of children orthopedics for surgical treatment. It should be noted that it was homogeneous group. The age of patients was 15.0 ± 1.7 months. All children initially had a severe pathology of the hip joints. The criteria for inclusion in the investigation were patients who had a dislocation of the hip of the III, IV or V degree (according to the classification of M. V. Volkov, 1978) on one or both sides. The non-inclusion criteria were patients whose primary diagnosis was hip dysplasia (grade I) or congenital hip subluxation (grade II).

All patients were admitted to the Department for reduction of hip dislocation in one way or another. Sampling of the investigation material was carried out before the start of medical measures.

The control group included 15 patients without pathology of the musculoskeletal system. The average age of patients was 24.0 ± 1.8 months.

In the patients of the main and the control groups, the content of monocytes

and Toll–like receptors of peripheral blood monocytes (TLR-2, 4, 5), growth and angiogenesis factors (FGF and VEGF), serum magnesium, type I and II collagen levels, and the content of hyaluronic acid in blood plasma, and aggrecan in blood serum were determined.

To determine the content of monocytes and innate immunity factors from peripheral heparinized blood (25 UNITS per 1 ml) using gradient centrifugation at 1500 rpm (ficollurographin p = 1.077 g/cm^3), mononuclear cells (MNCs) were isolated for 40 minutes and washed twice with RPMI 1640 medium at 1500 rpm for 10 minutes. To determine the expression of TLR2 and TLR4 on peripheral blood MNC monocytes, immediately after their isolation they were incubated by PC5-labeled antibodies to CD14 (Beckman Coulter, USA), PE-labeled antibodies to TLR2 (CD14+CD282+), APC-labeled antibodies to TLR4 (CD14+CD284+) and FITC-labeled antibodies to TLR-5 (CD14+CD285+) (BD, Bioscience (USA)) with corresponding isotypic controls (Beckman Coulter) for 30 minutes at 4˚C. The expression of CD14, TLR2, TLR4, and TLR5 was analyzed using a Navios flow cytometer (Beckman Coulter, USA). The percentage of monocytes carrying TLR2, TLR4, and TLR5 on their surface was evaluated.

To determine the content of fibroblast growth factor (FGFb), reagent set of R&D systems (USA) was used, to determine the content of vasculoendothelial growth factor (VEGF-A) we used reagent set (eBioscience (USA)). Type I and type II collagens were determined using the Cloud-Clone Corporation (USCN) Human COLLAGEN ELISA Kit. Aggrecan was determined using PG-ELISA BCM Diagnostics kits. This assay employs the competitive inhibition enzyme immunoassay technique. A monoclonal antibody specific to hyaluronic acid has been pre-coated onto a microplate. A competitive inhibition reaction is launched between biotin labeled hyaluronic acid and unlabeled hyaluronic acid (Standards or samples) with the pre-coated antibody specific to hyaluronic acid. After incubation the unbound conjugate is washed off. Next, avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. The amount of bound HRP conjugate is reverse proportional to the concentration of hyaluronic acid in the sample. After addition of the substrate solution, the intensity of color developed is reverse proportional to the concentration of hyaluronic acid in the sample [21]. The optical density value was registered on the "Sunrise" analyzer (Austria) using the "Magellan" program, which allows automatically constructing a calibration curve and determining the concentration of the studied substances. The content of hyaluronic acid was determined using ELISA kit for hyaluronic acid (HA) for enzyme immunoassay test system, the determination was carried out on a Tecan infinite F50 spectrophotometer, at a wavelength of 450 nm.

Official representatives of all patients gave their voluntary informed consent to participate in the study.

STATISTICA 12.0 software from StatSoft was used for statistical data processing. Methods of nonparametric statistics were used since the samples in the control group partially follow the normal distribution. Correlation methods and regres-

sion modeling methods were used to evaluate the relationship of indicators.

3. Results

Testing normality of all investigated factors distribution using the Shapiro-Wilkes normality test showed the absence of the Gaussian distribution in the control group ($p < 0.1$), which was an indication for usage of non-parametric statistical analysis for the group comparison.

When comparing the results obtained in patients of the experimental and control groups using the Wald-Wolfowitz test, statistically significant differences between the groups were found for all the studied parameters, except for the level of collagen type 2. In the experimental group the content of monocytes was reduced with an increase in the proportion of TLR-2, TLR-4 and TLR5 (Table 1).

The content of aggrecan in the urine was reduced in experimental group ($p = 0.001$), and the angiogenesis factor (FGF) was increased ($p = 0.001$) (Table 2).

Table 1. Parameters of innate immunity, experimental and control group comparison, Wald-Wolfowitz Runs Test: *the tests are significant at $p < 0.05000$.

Parameters of innate immunity							
Parameters	Experimental group N = 28			Control group N = 15			p
	Median	Confidence −95.000%	Confidence +95.000%	Median	Confidence −95.000%	Confidence +95.000%	
Monocytes	1.93	1.54	2.32	2.6	2.14	7.81	0.000042*
TLR-2	20.40	18.25	26.85	18.7	12.92	19.63	0.000042*
TLR-4	1.54	1.26	2.97	1.11	0.90	2.48	0.0021*
TLR-5	4.77	4.88	17.1	5.01	4.22	5.69	0.0021*

Table 2. Parameters of connective tissue metabolism, experimental and control group comparison, Wald-Wolfowitz Runs Test: *the tests are significant at $p < 0.05000$.

Parameters of connective tissue metabolism							
Parameters	Experimental group N = 28			Control group N = 15			p
	Median	Confidence −95.000%	Confidence 95.000%	Median	Confidence −95.000%	Confidence 95.000%	
Aggrecan. mcg/ml	7.236	2.089	3.595	7.996	7.61	11.091	0.000085*
FGF. pkg/ml	0.4	5.15	8.86	1.39	1.39	1.397	0.000000*
VEGF. pkg/ml	729.25	314.02	540.62	487.725	617.50	1105.22	0.001165*
Collagen type I. pkg/ml	2911.1	2182.63	3757.64	1932.5	1591.59	2352.43	0.000085*
Collagen type II. pkg/ml	30000	25505.172	43909.89	30000	18391.77	29943.09	0.059418
Hyaluronic acid. ng/ml	53.65	64.38	110.84	55.8	50.296	73.48	0.010281*

In the experimental group, compared with the control group, there was a twofold decrease in the level of VEGF ($p = 0.001$) in parallel with a twofold increase in the level of type I collagen ($p = 0.001$), while the level of type II collagen did not significantly change and did not significantly differ ($p = 0.059$).

In the first stage of analysing connections between characteristics of immune system and connective tissue parameters, in experimental group we tried to study correlations between parameters mentioned above. Based on the normality test, we used Kendall Tau Correlations coefficient (Table 3).

According to the correlation results, significant weak correlations were found between parameters of immune system and parameters of connective tissue metabolism, which was the possibility to implement further calculations.

To investigate the type of dependence of the innate immunity parameters from factors of connective tissue metabolism to predict the value of one variable by the value of one or more variables we used a non-linear regression analysis. The polynomial regression was applied to explore the effects of innate immunity on metabolism of connective tissue.

When performing the non-linear regression analysis, it is necessary to calculate whether and how the dependent variable and the set of independent variables are related.

General formula of the regression model is:

$$y = F\left(x_1, x_2, \cdots, x_n\right).$$

A common nonlinear model is the polynomial regression model. The main conceptual limitation of all regression analysis methods is that they only detect numerical dependencies, and not the underlying causal relationships.

According to the results of the regression analysis for the TLR5 variable, the R-squared coefficient was 0.770, so 77% of the variation of the dependent variable is determined by the variation of the independent variables. The R square of the correlation coefficient shows how much of the variance of the dependent variable can be explained by the variance of the independent variables. It is equal

Table 3. Correlation of innate immunity and connective tissue parameters, experimental group.

Parameters investigated (in correlation pairs)	Kendall Tau Correlations coefficient (significant at $p < 0.05000$)
Monocytes-TLR-2	−0.344
Monocytes-hyaluronic acid	0.271277555
TLR-5-TLR-2	0.369480891
TLR-5-collagen type II	−0.286078665
FGF-collagen type II	0.332105582
Hyaluronic acid-collagen type II	0.306034895
Hyaluronic acid-monocytes	0.271277555
Hyaluronic acid-TLR-2	−0.287235058

to 0.6, that is, it shows a strong relationship of features. The p-value for the model as a whole is less than 0.00174, so we can assume that the resulting model adequately describes the relationship of parameters (Table 4).

A common equation of the first regression model is:

$$\text{TLR-5} = 17.83 + 0.63\text{TLR-2} - 8.47\text{monocytes} + 0.07\text{hyaluronic acid}.$$

According to the results of the regression analysis for collagen type II variable, the R-squared coefficient was 0.772, so 77.2% of the variation of the dependent variable is determined by the variation of the independent variables. The R square of the correlation coefficient shows how much of the variance of the dependent variable can be explained by the variance of the independent variables. It is equal to 0.6, that is, it shows a strong relationship of features. The p-value for the model as a whole is less than 0.00014, so we can assume that the resulting model adequately describes the relationship of parameters. Figure 1 and Figure 2

Table 4. Regression model results.

Descriptive Statistics for experimental group											
Variables	Valid N	Mean	Confidence −95.0000%	Confidence 95.0000%	Median	Minimum	Maximum	Lower Quartile	Upper Quartile	Std.Dev.	Standard Error
Monocytes	28	1.93	1.54	2.32	1.93	0.200	3.7	1.150	2.65	1.00	0.188
TLR-2	28	22.55	18.25	26.85	20.40	9.218	60.7	16.605	24.20	11.09	2.096
TLR-4	28	2.12	1.26	2.97	1.54	0.351	8.9	0.890	2.22	2.20	0.415
TLR-5	28	10.99	4.88	17.10	4.77	0.837	71.4	3.248	11.66	15.77	2.980
Aggrecan. mkg/ml	28	8.52	7.49	9.54	7.99	4.989	15.1	6.955	9.62	2.64	0.499
FGF. pg/ml	28	4.14	1.62	6.67	0.00	0.000	25.2	0.000	4.32	6.51	1.231
VEGF. pg/ml	28	544.50	390.49	698.51	487.73	41.530	1629.3	250.020	632.48	397.18	75.060
Collagentype 1. pg/ml	28	4028.03	2957.56	5098.50	2911.10	310.560	9596.1	2196.100	5933.00	2760.66	521.716
Collagentype 2. pg/ml	28	25671.87	13162.85	38180.88	30000.00	2618.800	179350.0	7366.350	30000.00	32259.72	6096.515
Hylauronic acid	28	82.39	50.81	113.96	53.65	0.010	285.0	28.000	89.80	81.43	15.389

Regression equation 1 for variable TLR5:

TLR-5 = 17.83 + 0.63**TLR-2** − 8.47**monocytes** + 0.07**hyaluronic acid**.

Regression Equation (2) for variable collagen type II:

Collagen type II = 14642.0655 + 2406.46**FGF** + 166.01**hyaluronic acid**.

Beta (b*) coefficients for Regression Equation (1) and (2)			
Variables	Regression coefficients for TLR5	Regression coefficients for collagen type 2	
	beta (b*)	Variables	beta (b*)
TLR-2	0.440091	FGF. pg/ml	0.470224
Monocytes	−0.539864	Hylauronic acid	0.408837
Hylauronic acid	0.344713	TLR-2	−0.194239
VEGF pg/ml	−0.239741		
Collagentype 1 pg/ml	−0.203618		

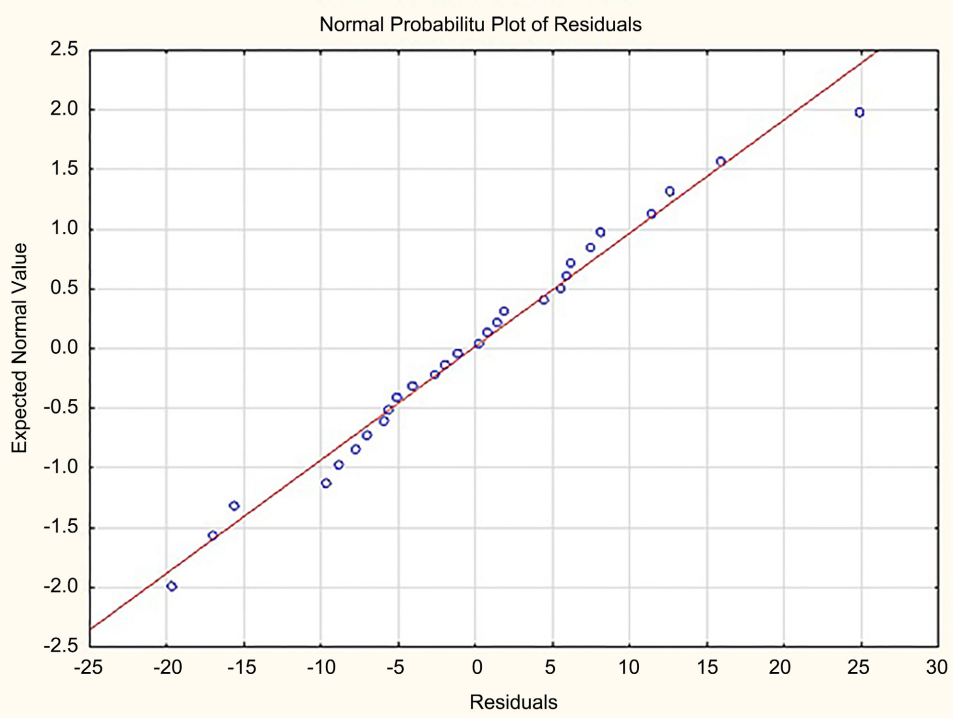

Figure 1. Normal probability plot of residuals for the independent variable TLR-5.

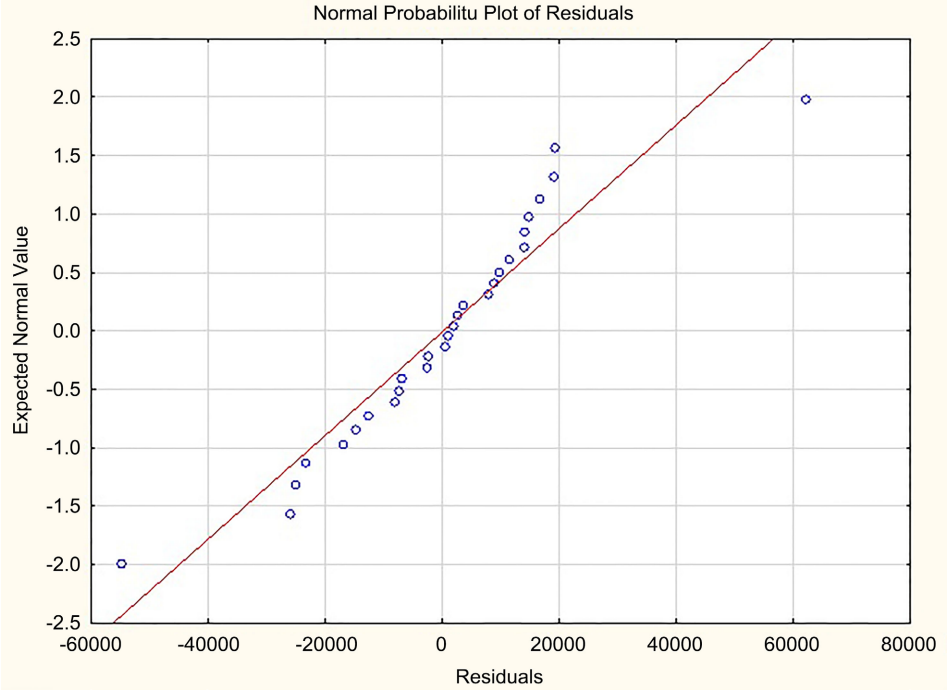

Figure 2. Normal probability plot of residuals for the independent variable collagen type II.

show Normal probability plot of residuals for Model 1 and 2 in order to suggests that the error terms are indeed normally distributed, approximately follow a straight line, so the models describe numerical dependence between parameters correctly.

The common equation of the second regression model is:

$$\text{Collagen type II} = 14642.0655 + 2406.46\text{FGF} + 166.01\text{hyaluronic acid}.$$

Figure 1 and Figure 2 show Normal probability plot of residuals for the independent variables for Equations (1) and (2).

So, numerical dependence of immune system parameters and parameters of connective tissue metabolism was established.

4. Discussion

According to statistical analysis we establish a difference between the experimental and control groups in all the studied parameters. As can be seen from Table 1, a significant increasing of TLR-2, TLR-4, and TLR-5 levels has been established.

TLR-4 is responsible for activating the MAPK signaling pathway, which leads to proliferation, the development of inflammation, and anti-apoptosis. One of the intracellular functions of the TLR-4 signaling pathway is the activation of the NF-kappa pathway, which also leads to inflammation and increased cell survival.

TLR-5 is known to induce inflammatory processes [9] and is associated with some types of tumors [10]. There is also evidence of TLR-5 dysregulation in neurodegenerative diseases, which is accompanied by an immunosuppressive response [11]. Several modern studies indicate the involvement of the TLR-5 signaling pathway in the pathogenesis of autoimmune diseases [12]. TLR5 is a trigger of the pro-inflammatory response, affecting antitumor immunity and tissue damage [13] [14] [15]. Increased level of TLR-5 leads to stimulation of apoptosis, expression of proinflammatory cytokines, enhances the response to oxidative stress, and activates TLR-5/NF-kB and TLR-5/MAPK signaling pathways [16].

TLR-2 acts via MYD88 and TRAF6, leading to NF-kappa-B activation, cytokine secretion and the inflammatory response [Uniprot], promotes cytokines synthesis, stimulates cell proliferation and vascularisation [22], participates differently in the carcinogenesis [23] [24]. Activation of TLR-2/NF-κB signaling pathway influences the occurrence of degenerative knee osteoarthritis [25].

TLR-4 is known to participate in carcinogenesis, inflammation, and increased levels of TLR-4, TLR-5 and IRF4 were proposed as diagnostic markers of knee osteoarthritis [25] and joint dysfunction [26]. In keratinocytes and epithelium, as well as in other connective tissue cells, in places of chronic and acute inflammation, under the influence of NFkB, the synthesis of HIF1 and VEGF/VEGFR pathway can be activated via the TLR-5-NOX4-ROS signaling pathway [20] [21] [22].

As appears from the above, increased levels of TLRs in the experimental group, compared with the control one, can be a sign of the presence of inflammation and disruption of MAPK and NF-kappa-B signaling pathways, which can pathologically affect the metabolism and formation of connective tissue. Modern scientific data indicate an increase in the level of TLRs in joint patholo-

gies, and this may explain the high level of these molecules in the experimental group of children with congenital hip dislocation and connective tissue dysplasia.

The decrease in the level of monocytes in the experimental group may be caused by non-specific reasons, such as an infectious or inflammatory process, autoimmune pathology, prolonged hypoxia. Objective co-presence of increased TLRs expression and decreased monocyte levels in connective tissue dysplasia requires further investigation.

Parameters of connective tissue metabolism were evaluated in experimental and control group and statistically significant difference was found for all studied variables. Level of aggrecan in urine was significantly lower in experimental group, when collagen type II content in blood was higher. Aggrecan is a major proteoglycan of the extracellular matrix in cartilage, and it is a large chondroitin-sulphated proteoglycan, the most common non-collagenic protein in cartilage and is essential for its functioning and maintenance of normal cartilage structure. It binds type 2 collagen and, like many proteoglycans, retains water, providing turgor and increasing the resistance of cartilage to heavy loads. aggrecan binds tightly to hyaluronic acid and forms stabilized link [27] [28]. It is known that with mutations of the ACAN gene (a gene expressing aggrecans), aggrecan molecules released during the degradation of cartilage tissue in increased amounts lead to a wide phenotypic spectrum of non-lethal skeletal dysplasia, including spondyloepimetaphyseal dysplasia, spondyloepiphyseal dysplasia and various unspecified short-growth syndromes associated with accelerated bone maturation. a decrease in the level of aggrecan indicates the predominance of anabolic processes and a decrease in the level of cytokines [29] [30]. It has already been shown [31] that the level of aggrecan in the cartilage matrix was significantly reduced in patients with acquired hip dysplasia. interestingly, the decreased level of aggrecan in the experimental group was accompanied by a sharp increase in the level of hyaluronic acid, a major component of cartilage extracellular matrix, polyanionic natural polymer occurring as linear polysaccharide composed of glucuronic acid and N-acetylglucosamine repeats via a β-1,4 linkage, the molecule, aggrecan forms a stable link with. It has been shown that hyaluronic acid normalises the rheologic properties of the synovial fluid, decreases inflammation, and could have a structure-modifying effect on human cartilage in osteoarthritis [32] [33] [34]. It has been shown experimentally that hyaluronic acid promotes human chondrocyte regeneration *in vitro* [34] and *in vivo* [35]. It can be assumed that in this case, the increased level of hyaluronic acid is explained by its regenerative properties, and the increase in its synthesis is a peculiar protective mechanism that decelerates the destruction of cartilage tissue.

Collagen, as the main component of connective tissue, is found everywhere in the human body, and many different diseases are associated with mutations of collagen genes.

The first type of collagen, which has the greatest mechanical strength, is found

in the skin, bones and cartilage, providing a normal structure and strength of the joints [36] [37] [38] [39]. The increased content of type I collagen in some forms of dysplasia may be associated with the germination of pathologically altered cartilage by vessels, or with its fibrous replacement, associated with the processes of dedifferentiation of chondrocytes and their switching from the synthesis of type II collagen to type I collagen [40].

FGFs regulate cellular processes, angiogenesis, immune response, and metabolism, and their dysregulation causes several pathologies, in particular, connective tissue pathologies [23]. Diseases of the skeleton and musculoskeletal system caused by mutations and dysfunction of FGF receptor genes (FGFR-1, 2, 3) are widely known [24]. FGFs are known to be involved in the regulation of proliferation and differentiation of osteoblasts and fibroblasts, in the processes of angiogenesis and tissue regeneration, they activate osteoblast differentiation and inhibit osteoclast differentiation. Individual FGF fractions positively regulate bone growth and density [25] [26]. An increased level of FGF stimulates angiogenesis in the cartilage tissue, so this is one of the possible causes of the formation of defective cartilage. FGF reduces the absorption of phosphorus, so it is a promising marker of the state of phosphorus-calcium metabolism, since its excessive signaling reduces the absorption of phosphorus, leading to chronic hypophosphatemia and the formation of mineral and bone disorders. FGF23 is significantly increased in osteomalacia.

VEGF is widely localized in hypertrophic zones of chondrocytes. During the early development of the skeleton, VEGF is responsible for the survival of chondrocytes in the conditions of hypoxia of the forming bones, vascularization and proliferation and differentiation of osteoblasts. In the postnatal period, VEGF supports bone homeostasis, stimulates the differentiation of mesenchymal stem cells into osteoblasts, and suppresses their differentiation into adipocytes [39] [40] [41] [42]. A decrease in the level of VEGF may be associated with the development of dysplasia, impaired formation of bone and cartilage tissue, and endothelium. There is evidence of a significant role of VEGF in the remodeling of cartilage tissue [43]. The observed decrease in VEGF levels may be a sign of the development of dysplasia, impaired formation of bone and cartilage tissue and endothelium.

TLR as a main component of innate immunity plays a significant role in regulating the survival and recovery of tissues and blood vessels under hypoxia [5]. It is known that in some pathological conditions (tumors), hypoxia can activate HIF1 and trigger the mechanisms of vasculogenic mimicry [7].

Regression model provided showed linear dependence between immune system and connective tissue parameters. According to the two our models, numerical dependence was found between TLR-5 and TLR-2, monocytes and hyaluronic acid; as well as for Collagen type II, FGF and hyaluronic acid.

Hyaluronic acid is a major component of the extracellular matrix of the skin and plays an important role in the metabolism of the dermis, serves as water attractant to maintain tissue turgor, and performs spring function in joints [44].

Recent animal experiments have shown that hip dysplasia and other joint abnormalities may be a consequence of hyaluronic acid deficiency in synovial fluids [45] [46]. It has also been shown that the intra-articular hyaluronic acid implementation alone or associated with ozone allowed improvement of hip dysplasia in animal model [47]. The role of hyaluronic acid is found to be crucial according to both regression models describing the connection between innate immunity and connective tissue as well as between some connective tissue parameters. These findings need further investigation in order to find more links in this chain of events, but one theoretical suggestion still can be made. After a thorough analysis of medical histories and careful collection of anamneses, it was possible to assume the presence of hypoxia in the intrauterine period and during childbirth in most patients of the experimental group. According to the new data oxygen regime is found to be an additional regulator of connective tissue development, both through the innate immunity (TLR) [48] [49] and HIF signaling pathways [3] [50]. TLRs are responsible for activating the MAPK signaling pathway, which leads to proliferation, the development of inflammation, and anti-apoptosis. One of the intracellular functions of the TLR signaling pathway is the activation of the NF-kappa pathway, which also leads to inflammation and increased cell survival. Activation of these signaling pathways leads to activation of HIF-1 synthesis, and then, under conditions of continued hypoxia, leads to activation of the VEGF signaling pathway and angiogenesis, an increase in p53 activity and cell survival via the PI3K signaling pathway. All above-listed leads to upregulation of keratinocyte proliferation and stress-induced neuronal death and ROS synthesis [Uniprot database, KEGG database]. It has been shown that prolonged hypoxia leads to upregulation of HIF-1 expression and increased apoptosis of fibroblasts [1]. In cell culture models, hypoxia via HIF-1 stimulation stimulated angiogenesis and VEGF synthesis in stromal and endothelial cells [2]. HIF-1 metabolically controls the synthesis and modification of collagen in chondrocytes. Prolonged HIF-1 signaling leads to skeletal dysplasia, super modification of collagen, and decreased collagen synthesis [3]. HIF-1 transcription is a central component of collagen hydroxylation under hypoxic conditions, mediating the hydroxylation and folding reactions of collagen and stimulating the synthesis of prolyl-4-hydroxylase [4]. Considering all the above mentioned, it becomes obvious that it is necessary to further study the influence of innate immunity on the formation of connective tissue and the relationship of the immune system parameters with the conditions of hypoxia during the development of the body. Indeed, these studies will certainly be continued in this direction in order to prove or disprove this hypothesis.

5. Conclusion

In this article, we have shown the presence of statistical and mathematical interactions between the parameters of innate immunity and indicators of connective tissue metabolism. The leading role of the immune system in the develop-

ment of pathologies associated with connective tissue dysplasia is assumed. In further investigations it is necessary to clarify the role hypoxia in HIF-1 stimulated control of skeletal dysplasia, collagen modification, connective tissue dysplasia development.

Ethical Statement

All experiments were conducted with the human subjects' understanding and given and signed informed consent. The responsible Ethical Committee of Privolzhsky Research Medical University has approved the experiment.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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