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Assessment of serum neopterin and kynurenine levels in Egyptian children with sickle cell disease: a single center study

M.A. El-Hawy, H.M. Bedair, A.M.Y.M. Madkour, S.M.T. Moussa, A.A. Mahmoud

Menoufia University, Shebin El-Kom, Egypt

Correspondence:
Mahmoud Ahmed El-Hawy,
Department of Pediatrics,
Faculty of Medicine, Menoufia University
Address: Shebin El Kom,
32511 Menoufia, Egypt
E-mail: mahmodelhawy18@yahoo.com

Neopterin, a guanosine triphosphate metabolite, is an indicator for cell-mediated immunity. Kynurenine performs a variety of biological functions, such as the dilation of blood vessels in response to inflammation and the regulation of immune response. Objectives: to assess serum neopterin and kynurenine levels in Egyptian children with sickle cell disease (SCD). In our case-control study, we included 40 children aged 2–18 years with SCD treated at the Hematology Unit of the Department of Pediatrics, Menoufia University Hospital and 40 healthy controls matched on age, sex, and socio-economic status. The study was approved by the Institutional Review Board (IRB) of the Menoufia Faculty of Medicine. We obtained comprehensive health history data of the study participants and performed necessary clinical examinations and tests including complete blood count, serum ferritin, and hemoglobin electrophoresis. Serum neopterin and kynurenine concentrations were measured using enzyme-linked immunosorbent assay. In the patients with SCD, serum neopterin and kynurenine levels were significantly higher during vaso-occlusive crisis than in a steady state, and much higher than in the controls ($p < 0.001$). There was a significant positive correlation between serum neopterin concentrations and mean corpuscular hemoglobin levels, platelets, HbF, HbS, and HbA2, and a significant negative correlation between serum neopterin levels and height, Hb, hematocrit and HbA1. We also observed a significant positive correlation between serum kynurenine and body mass index, HbA2, HbF, HbS, and platelets and a significant negative correlation between serum kynurenine and hemoglobin, hematocrit and HbA1. The cases were shown to have higher neopterin and kynurenine levels than the controls. The concentrations of neopterin and kynurenine were higher in the patients during vaso-occlusive crises than in a steady state.
Key words: *serum neopterin, kynurenine, sickle cell disease, pediatrics*

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Sickle cell disease (SCD) is a hereditary condition caused by a point mutation in the beta-globin gene coding for hemoglobin subunit beta. This mutation causes an amino acid change from glutamic acid to valine, which results in sickle hemoglobin (HbS) [1].

The primary pathophysiological basis of SCD is the polymerization of HbS in the cytoplasm of red blood cells (RBCs). In addition to its physical effects on cellular structure, the polymerization of HbS causes several biochemical, cellular and physiological pathologies, which are related to a variety of clinical complications including reticulocytosis, painful vaso-occlusive crises (VOC), and end organ damage due to persistent tissue hypoxia [2]. Patients with SCD are subjected to oxidative stress due to a persistent state of inflammation in the circulation. Hypoferremia in SCD patients can be caused by various mechanisms, such as increased iron use during the differentiation and proliferation of immune cells and the stimulation of ferritin synthesis by interleukin-1, and interleukin-6, and tumor necrosis factor-α, which leads to a decrease in circulating iron [3].

Neopterin and its related compound biopterin are pteridine molecules containing a 2-amino, 4-oxo, pyrimidino-pyrazino-(pterin) ring with a 3-carbon side-chain on carbon 6. Pteridines were first described as pigments of insects and lowe vertebrates [4]. Monocytes/macrophages produce neopterin in response to endotoxins, as well as pro-inflammatory cytokine interferon (IFN-γ), and other cytokine [5]. Neopterin, a guanosine triphosphate metabolite, is a marker for cell-mediated immunity [6]. It was reported that neopterin concentrations are elevated in patients with inflammatory conditions including parasitic, viral and bacterial disease [7].

A balance between pro- and anti-inflammatory immune components is critical for effective host defense. A disruption of this balance can result in either an extreme immune reaction or suppression of the immune response, both of which can be life-threatening. The degradation of tryptophan (TRP) through the kynurenine (KYN) pathway is critical in the regulation of immune response. This pathway shows a major association between the immune and nervous systems [8].

L-KYN is a product of the metabolism of the amino acid L-TRP that is necessary for the synthesis of niacin. KYN is synthesized by the enzyme TRP dioxygenase, which is produced mainly in the liver, and indoleamine 2, 3-dioxygenase, which is expressed in many tissues in response to immune activation. KYN and its degradation products perform a variety of biological functions,

including the dilation of blood vessels in response to inflammation and the regulation of immune response. Certain cancers produce more KYN, which promotes tumor growth [9]. Cognitive deficits in schizophrenia are associated with imbalances in the enzymes that break down KYN [10].

So, we aimed to assess serum neopterin and KYN levels in patients with SCD.

MATERIALS AND METHODS

Design

For our case-control study, we enrolled 40 children aged 2–18 years with SCD, and 40 healthy controls matched on sex and age. The cases were enrolled from the Hematology Unit of Menoufia University Hospital. The study was approved by the Institutional Review Board (IRB) of the Menoufia Faculty of Medicine.

Patients with chronic cardiac, renal, vascular diseases, and malignancies were excluded from this study.

We collected health history data (sex, age, duration of illness, the frequency of blood transfusions, type of chelation therapy, and history of splenectomy). All the study subjects underwent comprehensive clinical examinations and anthropometric assessments.

Methods

We obtained 7 mL of venous blood from each participant. Two mL of blood was collected into an EDTA tube for complete blood count analysis using Sysmex XT-1800i Automated Hematology Analyzer (Sysmex, Japan). Four mL was collected into 2 plain tubes and allowed to clot at 37°C, then centrifuged at 4000 rpm for 10 minutes and preserved at –80°C for the assessment of serum AST, ALT, urea, creatinine and ferritin levels using Cobas e501 Auto Analyzer (Roche-Germany). Serum neopterin and KYN levels were measured using commercial enzyme-linked immunosorbent assay (ELISA) Kits (Bioneovan Co., Ltd, Beijing, China). Hemoglobin electrophoresis results were obtained from the patient's medical files.

Procedure

Samples were diluted with standard dilution buffer and 10 wells were set as standards. After dilution, the total volume in all the wells was 50μL, and the concentrations were 90 pmol/mL, 60 pmol/mL, 30 pmol/mL, 15 pmol/mL, and 7.5 pmol/mL, respectively. In a Micro ELISA strip plate, we left an empty well as a blank control. We added 40 μL of sample dilution buffer and 10 μL of sample to the sample wells (the dilution factor was 5). Samples were loaded to the bottom of the well without touching the well walls and then gently shook to combine. Incubation: The wells were covered with closure plate membrane and incubated for 30 minutes at 37°C. Washing: We peeled off the closure plate membrane carefully, aspirated, and refilled with wash solution. After 30 s, the wash solution was discarded. The washing procedure was repeated 5 times. We added 50 μl of HRP-Conjugate reagent into each well (except the blank control well) and then performed incubationand washing steps. Coloring: We added 50 μl of chromogen solution A and 50 μl of chromogen solution B to each well, gently shook, and incubated at 37°C for 15 min. Termination: To terminate the reaction, we added 50 μl of stop solution to each well. The color in each well changed from blue to yellow. Finally, using a Microtiter Plate Reader, we determined the absorbance of O.D. at 450 nm within 15 min after adding the stop solution.

Sample size calculation

According to Sabuncuoğlu et. al 2020 [4], KYN concentrations in children with SCD are significantly higher than in healthy controls. In his study, the KYN/TRP ratio was 32.77 ± 6.19 and 45.14 ± 5.54 in the controls and cases, respectively. The sample size in our study was calculated to be 80 subjects (40 for each group), with a power of 80%, alpha error of 0.05, and case-control ratio 1:1.

Statistical analysis

The data were analyzed using the IBM SPSS version 22.0 (IBM Corp., Armonk, NY, USA). Chi-square test was used to determine the relationship between qualitative factors. The Student T-test was used to compare two groups of quantitative data. Pearson's correlation coefficient was used to examine the relationship between normally distributed continuous variables. The one-way ANOVA F-test was used to compare groups with more than two quantitative variables and independent parametric data. Results were considered significant if the p-value was 0.05 or lower.

RESULTS

As regards the demographic information, the rate of consanguinity was significantly higher in the group of interest compared with the controls but there was no significant difference between the groups regarding sex, age, height, and BMI, as can be seen in (*table 1*).

There was a highly significant difference in both serum neopterin and KYN levels between the subjects with SCD in a steady state and during VOC and the controls. Both serum neopterin and KYN levels were significantly higher during VOC than in a steady state (*table 2*).

A transcranial Doppler ultrasound (TCD) and echocardiography were performed in the cases and the controls, and the obtained results were compared. The results of TCD in the group of interest were found to

be statistically highly significant as the majority of the SCD patients (67.5%) had conditional velocities, 20% of the patients had normal findings, and 12.5% of the patients were considered high-risk, while all controls had normal TCD results. However, echocardiography results turned out to be normal in both groups (*table 3*).

There was a highly significant correlation between serum neopterin levels and TCD results in the group of interest, but we did not find any significant correlation between serum KYN levels and TCD results in this group. The correlation between serum neopterin and KYN levels and demographic and clinical data of the SCD patients was non-significant (*table 4*).

There was a significant positive correlation between serum neopterin levels and mean corpuscular hemoglobin (MCH), platelets, HbF, HbS, HbA2 and AST; and a significant negative correlation between serum neopterin levels and the patients' height, Hb, HCT and HbA1. Serum KYN levels had a significant positive correlation with BMI, HbA2, HbF, HbS, MCV, MCH and platelets; and a significant negative correlation with Hb, HCT and HbA1 (*table 5*).

Table 1
Socio-demographic data of the study subjects

Variables	Cases (*n* = 40)	Controls (*n* = 40)	Test of sig.	*p*-value
Age, years: mean ± SD range median IQR	 10.1 ± 4.5 2–18 9 7–13	 8.9 ± 4.1 3–17 9 4–11	U 1.52	 0.128 NS
Sex: male female	 23 (57.5%) 17 (42.5%)	 19 (47.5%) 21 (52.5%)	χ^2 0.82	 0.370 NS
Consanguinity: yes no	 23 (57.5%) 17 (42.5%)	 12 (30%) 28 (70%)	χ^2 6.14	 0.013 S
Weight, kg: mean ± SD range median IQR	 29.4 ± 12.7 11–60 25.5 20–36.5	 30.1 ± 11.3 15–65 29 20–37.8	U 0.506	 0.613 NS
Height, cm: mean ± SD range median	 130.1 ± 23.4 85–176 129	 135.1 ± 17.2 108–165 131	T 1.11	 0.269 NS
BMI, %: mean ± SD range median	 16.9 ± 2.5 12–24 16.5	 16.1 ± 2.6 12–24 16.03	T 1.50	 0.137 NS

Note. SD – standard deviation; T – Student's T-test; χ^2 – Chi-square test; U – Mann–Whitney test; NS – non-significant difference; S – significant difference.

DISCUSSION

Sickle cell anemia is one of the most prevalent single-gene disorders in humans, with a wide geographic distribution and various clinical presentations. In SCD, HbS replaces HbA in erythrocytes (RBCs). It is a common cause of chronic anemia in children of African ancestry. The disorder is characterized by chronic hemolysis, increased bone marrow activity and the theoretical probability of abnormal red cell indices [11].

In our study, we discovered a highly significant difference in consanguinity between the group of interest and the control group, but there was no significant difference between the groups regarding age, sex, height and BMI. A family history of consanguinity was linked to SCD and was higher in the existing literature [12].

There was a highly significant difference in serum neopterin and KYN levels between the subjects with SCD in a steady state and during VOC and the controls. Both serum neopterin and KYN levels were significantly higher during VOC than in a steady state.

Rodrigues et al. [12] reported significantly elevated serum neopterin levels in HbSS and HbSC cases compared with the controls. Sabuncuoğlu et al. [4] found that serum neopterin was substantially higher in the affected patients than in the healthy controls. Urinary neopterin concentrations were also significantly higher in the group of interest than in the controls. In this study, TRP and KYN levels were determined and the KYN/TRP ratio was calculated to enable the estimation of IDO activity. The KYN/TRP ratio and

Table 3
The results of TCD and echocardiography in the study subjects

Parameter	Cases (*n* = 40)		Controls (*n* = 40)		Test of sig.	*p*-value
	n	%	*n*	%		
TCD: normal (≤ 170 cm/s) conditional (170–199 cm/s) high-risk (> 200 cm/s)	 8 27 5	 20 67.5 12.5	 40 0 0	 100 0 0	 FET 61.2	 < 0.001 HS
Echocardiography: normal	40	100	40	100	Normal	Normal

Note. FET – Fisher's exact test; HS – highly significant.

Table 2
Serum neopterin and KYN concentrations in the study subjects

Parameter	Cases in VOC (*n* = 40)	Cases in a steady state (*n* = 40)	Controls (*n* = 40)	Test of sig.	*p*-value
Serum neopterin, nmol/L: mean ± SD range median	 7.83 ± 2.45 5.72–10.20 7.92	 5.64 ± 1.36 4.35–7.92 5.78	 2.90 ± 0.78 1.87–3.77 2.99	 T1 = 6.09 T2 = 9.58 T3 = 14.63	 *p*1 < 0.001* *p*2 < 0.001* *p*3 < 0.001*
Serum KYN, nmol/L: mean ± SD range median	 815.2 ± 289.1 414.2–1203.1 832.2	 445.2 ± 129.1 311.2–603.1 498.7	 278.5 ± 59.5 223.1–474.1 250.6	 T1 = 4.68 T2 = 14.61 T3 = 5.43	 *p*1 < 0.001* *p*2 < 0.001* *p*3 < 0.001*

*Note. SD – standard deviation; T – Student's T-test; * – highly significant difference; p1 – patients in a steady state versus patients in VOC; p2 – patients in a steady state versus controls; p3 – patients in VOC versus controls.*

Table 4
The relationship between serum neopterin and KYN levels and demographic and clinical data of the cases

Parameter	Serum neopterin nmol/L (mean ± SD)	Test of sign.	*p*-value	Serum KYN nmol/L (mean ± SD)	Test of sign.	*p*-value
Sex:		U			T	
male	4.8 ± 2.2		0.920	814.5 ± 185.8		0.978
female	4.8 ± 2.3	1.00	NS	816.2 ± 199.2	0.028	NS
Consanguinity:		U			T	
yes	5.4 ± 2.3		0.053	804.9 ± 158.7		0.712
no	4.3 ± 2.1	1.9	S	829.1 ± 228.4	0.373	NS
Pallor:		T			T	
yes	7.02 ± 1.5		0.515	767.5 ± 178.1		0.148
no	6.6 ± 1.4	0.659	NS	854.2 ± 192.9	1.47	NS
Jaundice:		T			T	
yes	7.2 ± 1.4		0.216	822.2 ± 227.1		0.878
no	6.5 ± 1.4	1.27	NS	811.4 ± 170.2	0.156	NS
Splenomegaly:		T			T	
yes	6.7 ± 1.2		0.570	824.8±190.4		0.700
no	7.1±1.7	0.575	NS	800.8±192.4	0.388	NS
Splenectomy:		T			T	
yes	7.3 ± 1.7		0.270	842.9 ± 211.3		0.603
no	6.6 ± 1.9	1.15	NS	804.7 ± 182.9	0.530	NS
Type of crisis:		F			F	
hemolytic crisis	6.1 ± 1.9		0.186	820.6 ± 240.1	0.836	0.512
VOC	10.1	1.65	NS	888.5 ± 106.3		NS
Hydroxyurea:		T			T	
yes	6.8 ± 1.6		0.822	803.6 ± 195.8		0.616
no	6.7 ± 1.1	0.227	NS	834.5 ± 180.4	0.507	NS
Iron chelator (deferasirox):		T	0.961		T	
yes	6.8 ± 1.5		NS	785.2 ± 159.8		0.290
no	6.8 ± 1.4	0.049		851.8 ± 218.8	1.07	NS
TCD:		K			F	
normal	3.4 ± 1.56		< 0.001	768.8 ± 199.6		0.747
conditional	6.9 ± 1.50		HS	828.1 ± 200.9	0.294	NS
high risk	6.4 ± 1.51	38.1		819.5 ± 105.7		

Note. SD – standard deviation; T – Student's T-test; F – one-way ANOVA test; K – the Kruskal–Wallis test; NS – non-significant; S – significant; HS – highly significant.

Table 5
A correlation between serum neopterin concentrations and demographic and laboratory data of the cases

Parameter	Serum neopterin, nmol/L		Serum KYN, nmol/L	
	r	*p*-value	r	*p*-value
Age, years	0.002	0.989	0.184	0.103
Weight, kg	–0.178	0.140	0.011	0.920
Height, cm	–0.286	0.017*	–0.062	0.587
BMI, %	0.035	0.774	0.220	0.050*
Crises per year	–0.024	0.832	0.084	0.605
Pain episodes per year	0.00	1.00	0.082	0.612
Dose of hydroxyurea, mg/kg/day	–0.024	0.892	0.017	0.919
Dose of the chelating agent	0.094	0.590	–0.062	0.707
Transfusion index per year, mL/kg/year	0.041	0.817	–0.059	0.721
Hb, gm/dl	–0.692	< 0.001**	–0.647	< 0.001**
HCT, %	–0.348	0.004*	–0.316	0.004*
MCV, fL	0.090	0.450	0.272	0.015*
MCH, pg	0.302	0.011*	0.529	< 0.001**
Platelets, $10^3/mm^3$	0.238	0.047*	0.449	< 0.001**
WBC, $10^3/mm^3$	0.215	0.073	0.030	0.794
HbA1, g/dL	–0.783	< 0.001**	–0.812	< 0.001**
HbF, g/dL	0.517	< 0.001**	0.495	< 0.001**
HbS, g/dL	0.785	< 0.001**	0.831	< 0.001**
HbA2, g/dL	0.367	0.002*	0.316	0.004*
Serum ferritin, ng/mL	0.186	0.285	–0.259	0.106
AST, IU/L	0.278	0.020*	0.246	0.028
ALT, IU/L	0.101	0.406	0.171	0.130
Serum urea, mg/dL	0.040	0.784	0.082	0.472
Serum creatinine, mg/dL	0.033	0.784	0.057	0.613

*Note. r – Pearson correlation coefficient; * – significant difference; ** – highly significant difference.*

KYN levels were significantly elevated in SCD cases, while TRP concentrations were the same.

As previously established, inflammation and immunological activity are also associated with increased TRP degradation [13]. IDO, present in all tissues, and TDO, which is mostly found in the hepatic cells, facilitate the conversion of TRP to KYN. IDO is induced by IFN-γ in inflammatory conditions [14].

According to Sabuncuoğlu et al. [4], a significant proportion of SCD patients, both in a steady state and in an infectious state associated with crisis, have impaired IFN-γ production. In view of the known immunomodulatory properties of IFN-γ, this defect may be one of the factors contributing to the increased incidence and severity of infections in SCD patients.

IFN-γ was shown to be increased in SCD patients. Increased IFN-γ production in SCD suggests that functionally activated natural killer cells reflect a host's immunological mechanism leading to antigen-antibody activation in SCD. Serum interleukin-10 was increased in SCD patients in a steady state. $CD4^+$T lymphocytes were decreased in SCD patients during VOC [15].

The majority of the patients in the group of interest had conditional TCD velocities (67.5%), 20% of patients had normal results, 12.5% patients were high-risk, while all controls had normal TCD findings. Echocardiography was normal in both groups.

Children with SCA usually have increased cerebral blood flow velocity compared with age-matched controls, but there is little information about blood velocities in children with SCA younger than 2 years [16]. Hogan et al. [17] reported that children with SCA included in their study had middle cerebral artery velocities of 50–112 cm/s (median: 70 cm/s) at 3 months of age, 50–160 cm/s (median: 89 cm/s) at 9 months of age and 51–120 cm/s (median: 97 cm/s) at 12 months of age. The series of tests performed by Hogan et al. [17] revealed a negative correlation between developmental screening test scores and TCD-derived velocities in children with SCA.

The BABY HUG study provides TCD data on the largest sample of children with SCA known to date, with adequate baseline TCD ultrasounds performed in 192 patients. All but four cases (2%) were classified as normal by STOP criteria, compared to 9.3% of cases who were considered abnormal and 17.6% who were interpreted as conditional in STOP screening of children aged 2–16 years [18]. As expected, baseline TCD rates varied inversely with hemoglobin levels following age-adjustment, and the direct association with the reticulocyte count was anticipated because of the already established inverse relationship between the hemoglobin level and the reticulocyte count [19].

There was a significant positive correlation between serum neopterin levels and MCH, platelets, HbF, HbS, HbA2 and AST, and a significant negative correlation between serum neopterin levels and the patients' height, Hb, HCT and HbA1. Serum KYN levels had a significant positive correlation with BMI, HbA2, HbF, HbS, MCV, MCH and platelets; and a significant negative correlation with Hb, HCT and HbA1.

The KYN pathway is responsible for the formation of many metabolic products known as KYN during TRP degradation. KYN and its metabolic products are well-recognized for their effects on the central nervous system and are believed to be related to a variety of psychiatric and mental health conditions, including depression and schizophrenia [20, 21].

Increased neopterin levels are thought to be associated with hematopoietic system abnormalities in patients with infectious and malignant disorders. Neopterin concentrations were also found to correlate inversely with hemoglobin levels, and neopterin was linked to iron metabolism abnormalities [16, 17].

In another study, no correlation between hemoglobin levels and neopterin concentrations, or between neopterin concentrations and a number of iron parameters was discovered, suggesting that neopterin does not affect the synthesis of RBCs and iron metabolism. Higher neopterin concentrations in HbSS cases are probably caused by chronic inflammatory conditions. It was revealed that through intravascular hemolysis, the physical separation of hemoglobin from the endothelium is disrupted, resulting in effective nitric oxide scavenging and endothelial dysfunction [22].

As demonstrated by I. Anwaar et al. in their study in patients with acute cerebral ischemia, the activation of macrophages within blood vessels damaged by SCD may result in the release of neopterin into the blood flow [23].

Croizat and Nagel [24] examined the role of circulating cytokines in the synthesis of RBCs in SCD. Stem cell factor and granulocyte-macrophage colony-stimulating factor levels were higher in LFSS patients (SCA patients with low HbF) than in HFSS patients (SCA patients with high HbF). Transforming growth factor-β, a cell growth inhibitor, was lower in patients with low HbF, indicating a constant state of increased hematopoiesis in LFSS cases in response to increased erythropoietic stress.

In a study by Rodrigues et al. [12], SS cases were subdivided according to HbF levels: one group showed HbF < 8.5% (LFSS) and the other group had HbF ≥ 8.5% (HFSS). Total Hb levels were lower in LFSS cases than in HFSS cases. Interleukin-3 concentrations were much higher in HFSS cases than in LFSS cases. Neopterin levels did not vary between the groups.

CONCLUSION

Neopterin is produced by macrophages and monocytes following stimulation with IFN-γ. An increase

in neopterin levels may be a direct consequence of chronic immune activation in SCD patients since it has a positive effect on hematopoiesis in such cases. Serum KYN concentrations can be a new prognostic marker in patients with SCD.

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CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

ORCID
El-Hawy M.A. ORCID: https://orcid.org/0000-0002-3420-922X
Bedair H.M. ORCID: https://orcid.org/0000-0002-2229-3129

References

1. Usman M.R., Pawara S.M., Patil T.P. Sickle cell disease: an overview. J Pharm Res 2017; 11 (6): 780–6.
2. Kumar A.A., Patton M.R., Hennek J.W., Lee S.Y., D'Alesio-Spina G., Yang X., et al. Density-based separation in multiphase systems provides a simple method to identify sickle cell disease. Proc Natl Acad Sci U S A 2014; 111 (41): 14864–9.
3. Sundd P., Gladwin M.T., Novelli E.M. Pathophysiology of sickle cell disease. Annu Rev Pathol 2019; 14: 263–92.
4. Sabuncuoğlu S., Öztaş Y., Yalcinkaya A., Ünal S., Baydar T., Girgin G. The increased neopterin content in turkish pediatric patients with sickle cell anemia. Ann Hematol 2020; 99 (1): 41–7.
5. Heistermann M., Higham J.P. Urinary neopterin, a non-invasive marker of mammalian cellular immune activation, is highly stable under field conditions. Sci Rep 2015; 5 (1): 1–3.
6. Drugan C., Drugan T.C., Miron N., Grigorescu-Sido P., Naşcu I., Cătană C. Evaluation of neopterin as a biomarker for the monitoring of Gaucher disease patients. Hematology 2016; 21 (6): 379–86.
7. Girgin G., Sahin T.T., Fuchs D., Yuksel O., Kurukahvecioglu O., Sare M., et al. Tryptophan degradation and serum neopterin concentrations in intensive care unit patients. Toxicol Mech Methods 2011; 21 (3): 231–5.
8. Mándi Y., Vécsei L. The kynurenine system and immunoregulation. J Neural Transm (Vienna) 2012; 119 (2): 197–209.
9. Cervenka I., Agudelo L.Z., Ruas J.L. Kynurenines: Tryptophan's metabolites in exercise, inflammation, and mental health. Science 2017; 357 (6349).
10. Wang Q., Liu D., Song P., Zou M.H. Deregulated tryptophan-kynurenine pathway is linked to inflammation, oxidative stress, and immune activation pathway in cardiovascular diseases. Front Biosci (Landmark Ed) 2015; 20: 1116.
11. Howard J., Davies S.C. Clinical manifestations of sickle cell disease. Paediatr Child Health. 2007; 8 (17): 311–6.
12. Rodrigues L., Costa F.F., Saad S.T., Grotto H.Z. High levels of neopterin and interleukin-3 in sickle cell disease patients. J Clin Lab Anal 2006; 20 (3): 75–9.
13. Okamoto A., Nikaido T., Ochiai K., Takakura S., Saito M., Aoki Y., et al. Indoleamine 2, 3-dioxygenase serves as a marker of poor prognosis in gene expression profiles of serous ovarian cancer cells. Clin Cancer Res 2005; 11 (16): 6030–9.
14. Munn D.H., Mellor A.L. IDO in the tumor microenvironment: inflammation, counter-regulation, and tolerance. Trends Immunol 2016; 37 (3): 193–207.
15. Mahmoud A.A., Awad M.S., Abdallah H.M., Younis F.E., El-Hawy M.A. Evaluation of immunological role of Interferon gamma, Interleukin-10 and $CD4^+$ T lymphocytes in pediatric patients with sickle cell disease. Al-Azhar University Journal of Virus Researches and Studies 2020; 2 (1): 1–5.16.
17. Hogan A.M., Kirkham F.J., Prengler M., Telfer P., Lane R., Vargha-Khadem F., et al. An exploratory study of physiological correlates of neurodevelopmental delay in infants with sickle cell anaemia. Br J Haematol 2006; 132 (1): 99–107.
18. Adams R., McKie V., Nichols F., Carl E., Zhang D.L., McKie K., et al. The use of transcranial ultrasonography to predict stroke in sickle cell disease. N Engl J Med 1992; 326 (9): 605–10.
19. Pavlakis S.G., Rees R.C., Huang X., Brown R.C., Casella J.F., Iyer R.V., et al. Transcranial doppler ultrasonography (TCD) in infants with sickle cell anemia: baseline data from the BABY HUG trial. Pediatr Blood Cancer 2010; 54 (2): 256–9.
20. Greathouse K.L., Wyatt M. Targeting Dietary and Microbial Tryptophan-Indole Metabolism as Therapeutic Approaches to Colon Cancer. Nutrients 2021; 13 (4): 1189.
21. Gautam U.S., Foreman T.W., Bucsan A.N., Veatch A.V., Alvarez X., Adekambi T., et al. *In vivo* inhibition of tryptophan catabolism reorganizes the tuberculoma and augments immune-mediated control of Mycobacterium tuberculosis. Proc Natl Acad Sci U S A 2018; 115 (1): E62–71.
22. Reiter C.D., Wang X., Tanus-Santos J.E., Hogg N., Cannon R.O., Schechter A.N., et al. Cell-free hemoglobin limits nitric oxide bioavailability in sickle-cell disease. Nat Med 2002; 8 (12): 1383–9.
23. Anwaar I., Gottsäter A., Lindgärde F., Mattiasson I. Increasing plasma neopterin and persistent plasma endothelin during follow-up after acute cerebral ischemia. Angiology 1999; 50 (1): 1–8.
24. Croizat H., Nagel R.L. Circulating cytokines response and the level of erythropoiesis in sickle cell anemia. Am J Hematol 1999; 60 (2): 105–15.