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Assessment of Pyruvate Kinase Level in Sickle Cell Disease Patients in Basrah

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Abstract

Introduction. Anemia, red cell sickling, and aberrant hemoglobin polymerisation are all symptoms of sickle cell disease. While increased pyruvate kinase activity may improve red cell function, lower 2,3-diphosphoglycerate, and restore energy balance, altered metabolism exacerbates deformability.
Purpose. In Basrah, this research sought to assess pyruvate kinase activity in sickle cell disease patients, sickle cell trait carriers, and healthy persons.
Materials and methods. Between October 2024 and August 2025, a comparative case-control research was carried out at Al Basrah Hereditary Blood Disease Centre in Basrah, Iraq, to measure the pyruvate kinase activity of red blood cells. A total of 100 participants were recruited: 30 individuals with sickle cell disease (HbSS), 30 carriers of sickle cell trait (HbAS), and 40 healthy controls (HbAA). Pyruvate kinase activity was assessed using a quantitative Enzyme-Linked Immunosorbent Assay (ELISA), and clinical and laboratory data were acquired.
Results. Age and gender were statistically similar across the groups (p=0.923, p=0.803). Splenectomy (10%, p=0.027) and hydroxyurea administration (16.7%) were only seen in HbSS, which necessitated a higher frequency of transfusions (4.23±1.02 units/year). HbSS exhibited markedly reduced hemoglobin (8.89 g/dL, p=0.001), red blood cell count (3.41×10^{12}/L, p=0.001), packed cell volume (24.56%, p=0.001), and pyruvate kinase activity (42.47 ng/mg, p=0.021), with elevated reticulocyte levels (9.95%, p=0.001) and white blood cell count (10.55×10^{9}/L, p=0.027). Deficiency in pyruvate kinase was nonexistent in HbAA, 3.4% in HbAS, and 6.7% in HbSS. Pyruvate kinase activity was lower with hydroxyurea (p=0.047), greater with splenectomy (p=0.044), and positively linked with HbS% in HbSS (r=0.488, p=0.012).
Conclusion. In sickle cell disease, particularly HbSS, pyruvate kinase deficiency is associated with severity, splenic status, and therapy, suggesting that it may serve as a biomarker for risk assessment, disease surveillance, and treatment recommendations.

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Keywords: sickle cell disease, adenosine triphosphate, pyruvate kinase, diphosphoglycerate, anemia

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Оценка уровня пируваткиназы у пациентов с серповидноклеточной анемией в Басре

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Резюме

Введение. Анемия, серповидная трансформация эритроцитов и аномальная полимеризация гемоглобина – ключевые признаки серповидноклеточной анемии. Повышенная активность пируваткиназы может улучшить функцию эритроцитов, снизить уровень 2,3-дифосфоглицерата и восстановить энергетический баланс, однако нарушенный метаболизм усугубляет деформируемость клеток.
Цель. В Басре настоящее исследование было направлено на оценку активности пируваткиназы у пациентов с серповидноклеточной анемией, носителей гена серповидноклеточной анемии и здоровых лиц.
Материалы и методы. С октября 2024 по август 2025 г. в Центре наследственных заболеваний крови Аль-Басра (Басра, Ирак) проводилось сравнительное кейс-контрольное исследование для измерения активности пируваткиназы в эритроцитах. В исследование включено 100 участников: 30 человек с серповидноклеточной анемией (HbSS), 30 носителей серповидноклеточного гена (HbAS) и 40 здоровых контрольных лиц (HbAA). Активность пируваткиназы определяли с помощью количественного иммуноферментного анализа (ИФА), а также собирали клинические и лабораторные данные.
Результаты. Возраст и пол были статистически сходными между группами (p=0,923; p=0,803). Спленэктомия (10%, p=0,027) и терапия гидроксиуреей (16,7%) наблюдались только в группе HbSS, которая также требовала более частых переливаний крови (4,23±1,02 единицы/год). В группе HbSS отмечено значительное снижение гемоглобина (8,89 г/дл, p=0,001), количества эритроцитов ($3,41\times10^{12}$/л, p=0,001), гематокрита (24,56%, p=0,001) и активности пируваткиназы (42,47 нг/мг, p=0,021),

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а также повышение уровня ретикулоцитов (9,95%, p=0,001) и лейкоцитов (10,55×10^9/л, p=0,027). Дефицит пируваткиназы отсутствовал в группе HbAA, составил 3,4% в HbAS и 6,7% в HbSS. Активность пируваткиназы была ниже при терапии гидроксиуреей (p=0,047), выше после спленэктомии (p=0,044) и положительно коррелировала с процентом HbS в HbSS (r=0,488, p=0,012).

Выводы. У пациентов с серповидноклеточной анемией, особенно HbSS, дефицит пируваткиназы ассоциирован с тяжестью заболевания, статусом селезенки и терапией, что предполагает его потенциал как биомаркера для оценки риска, мониторинга заболевания и рекомендаций по лечению.

Ключевые слова: серповидноклеточная анемия, аденозинтрифосфат, пируваткиназа, дифосфоглицерат, анемия

■ INTRODUCTION

Sickle cell disease (SCD) is a severe condition defined by a single-nucleotide mutation in the β-globin chain, resulting in the synthesis of an aberrant form of hemoglobin (Hb): hemoglobin S (HbS). HbS undergoes polymerisation during deoxygenation, resulting in the sickling of red blood cells (RBCs). Sickled red blood cells exhibit reduced deformability, resulting in vasoocclusion and hemolytic anemia. Further contributing to the intricate pathophysiology of sickle cell disease are enhanced red cell adhesion, endothelial dysfunction, inflammation, oxidative stress, hemostatic activation, and rheological abnormalities [1].

Factors influencing sickling include RBC metabolic intermediates, namely the concentrations of 2,3-diphosphoglycerate (2,3-DPG) and adenosine triphosphate (ATP). Elevated intracellular 2,3-DPG concentrations reduce oxygen affinity, facilitating the polymerisation of HbS during deoxygenation and thereby inducing sickling. ATP is essential for preserving the integrity and deformability of red blood cell membranes, with about 50% of the cell's ATP produced in the last phase of glycolysis, which is catalysed by pyruvate kinase (PK) [2].

Reduced ATP levels have been shown in SCD mice, and ATP depletion has been linked to a higher quantity of irreversibly sickled cells. It's interesting to note that these metabolic alterations closely resemble important metabolic alterations seen in PK deficiency, a rare genetic glycolytic enzymopathy linked to nonspherocytic hemolytic anemia and brought on by mutations in the PKLR gene [3].

Erythrocyte pyruvate kinase (PKR) is the red blood cell-specific isoform of the essential glycolytic enzyme pyruvate kinase. While producing adenosine triphosphate (ATP) from adenosine diphosphate, PKR catalyses the last and rate-limiting step of glycolysis from phosphoenol-pyruvate to pyruvate. While PKR activation increases RBC lifespan and Hb levels, decreasing PKR activity causes anemia in people with pyruvate kinase deficiency [4].

Enhanced PKR activity is anticipated to diminish the synthesis of 2,3-DPG and augment the creation of ATP inside red blood cells. PKR is a rational target for SCD treatment, since a reduction in 2,3-DPG levels would enhance hemoglobin's affinity for oxygen, hence decreasing the likelihood of hemoglobin S polymerisation and red blood cell sickling [5, 6].

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Moreover, the increase in ATP generation may improve overall erythrocyte function and vitality. Decreased ATP levels in RBCs negatively impact ion homeostasis, leading to cellular dehydration [7], membrane damage, and compromised RBC deformability, all of which contribute to the pathophysiological characteristics of SCD. Mitigating these detrimental consequences, an increase in ATP production via PKR activation in sickle RBCs might possibly have the dual advantage of alleviating the chronic anemia linked to SCD and decreasing the frequency of VOC [8, 9].

The potential of PK activators to target important pathophysiological elements of sickle cell disease (SCD) makes them a potentially new treatment approach. So this study aimed to assess activity of the enzyme pyruvate kinase among patients with sickle cell disease in Basrah, which is the target for novel therapies.

MATERIALS AND METHODS

A comparative case – control study was conducted at AlBasrah Hereditary Blood Disease Center, for the duration between October 2024 and August 2025. The participants were divided into three groups.

- Group 1: 30 Patients with confirmed sickle cell disease HbSS under follow-up at the Center.
- Group 2: 30 patients with confirmed sickle cell trait (HbAS), identified through family screening.
- Group 3: 40 healthy controls with normal hemoglobin electrophoresis/HPLC.

Patients in the HbSS and HbAS groups were eligible if they had a confirmed diagnosis by hemoglobin electrophoresis or HPLC, were clinically stable for at least 14 days without vaso-occlusive crisis, infection, or hospitalisation, and provided informed consent or assent. They were excluded if they had advanced liver or kidney disease or if their blood samples were grossly hemolyzed. Healthy controls were recruited from patients' family members matched by age and sex. They were included if they had a normal complete blood count, normal HPLC, were clinically well with no acute illness in the preceding 14 days, and had not received a transfusion within the past 12 weeks for any other cause. Consecutive eligible SCD patients were recruited until the target sample size was reached.

Data were collected using a structured case report form during a single clinical visit. Demographic and clinical variables included age, sex, and contact information. For patients with sickle cell disease or trait, additional clinical data were obtained, including SCD genotype, age at diagnosis, history of hydroxyurea use (dose and duration), transfusion history within the preceding 12 months (dates and number of units), splenectomy status, and comorbidities such as asthma, diabetes, chronic kidney disease (CKD), and hepatitis B or C. Vital signs, including temperature and pulse, were recorded at the time of blood sampling.

Laboratory investigations performed during the same visit included a complete blood count (CBC) with reticulocyte count. Hemoglobin electrophoresis or high-performance liquid chromatography (HPLC) was used to determine HbS%, HbF%, and HbA2%, either on the day of sampling or from the most recent test within the preceding three months, provided the patient was clinically stable.

The plasma samples were collected using EDTA tubes, then centrifuged and stored at –80 C for a duration of 6 months.

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The red blood cell pyruvate kinase (PK) activity was measured using a commercial human PK enzyme-linked immunosorbent assay (ELISA) (MyBioSource, Cat. No. MBS161759). This is a quantitative sandwich ELISA specific for human PK. The kit had a standard curve range of 0.5–100 ng/ml with a sensitivity of 0.27 ng/ml, and intra- and inter-assay coefficients of variation were <8% and <10%, respectively. To establish a reference range, PK activity among healthy controls was analysed, and the 2.5th and 97.5th percentiles of the distribution were calculated to define the central 95% reference interval and its between (11.84–70.0 ng/ml).

Data were entered and analyzed using statistical software such as SPSS version 26. Descriptive statistics were presented as means and standard deviations for normally distributed continuous variables, medians and interquartile ranges for skewed data, and frequencies with percentages for categorical variables. Group comparisons for continuous variables were conducted using one-way ANOVA. Chi-square test was used for categorical data. Pearson correlation coefficients were used to assess the relationships between RBC PK levels and other continuous variables. A p-value <0.05 was considered statistically significant.

The study protocol was reviewed and approved by the Ethical Committee of the University of Basrah, College of Medicine. Written informed consent or assent was obtained in Arabic from all participants, and in the case of minors, consent was provided by a parent or legal guardian. All collected data were anonymised using coded identifiers to ensure confidentiality and participant privacy.

■ RESULTS

The demographic characteristics of the study groups are presented in Table 1. Age and gender were comparable across groups (p-value > 0.05). Splenectomy (10%) and hydroxyurea use (16.7%) were observed exclusively in the HbSS group, while the mean annual transfusion frequency was significantly higher in HbSS (4.23±1.02 units/year).

Hematological and biochemical parameters (Table 2) revealed significantly lower Hb, RBC count, PCV, and PK activity in HbSS compared with HbAA and HbAA (p-value <0.05), while reticulocyte percentage and WBC count were markedly higher in HbSS (p-value <0.05).

Table 1
Demographic and clinical characteristics of study participants by genotype

Variables	Group 1 (SS=30)	Group 2 (AS=30)	Group 3 (AA=40)	p-value
Age (years, mean±SD)	6.33±3.9	6.1±4.01	6.86±3.96	0.923
Gender				
Male, n (%)	16 (53.3)	18 (60.0)	21 (52.5)	0.803
Female, n(%)	14 (46.7)	12 (40.0)	19 (47.5)	
Splenectomy, n (%)	3 (10.0)	0 (0.0)	0 (0.0)	0.027
Treatment with hydroxyurea n(%)	5 (16.7)	0 (0.0)	0 (0.0)	NA
Frequency of Blood transfusion (units/ year, mean±SD)	4.23±1.02	0.0±0.0	0.0±0.0	NA

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Table 2
Hematological and biochemical parameters across genotypes

Variables	Group 1 (SS=30)	Group 2 (AS=30)	Group 3 (AA=40)	p-value
Hb (g/dL)	8.89±2.53	12.25±1.19	11.54±1.5	0.001
Retic (%)	9.95±1.47	1.93±0.93	1.63±0.61	0.001
RBC ($\times10^{12}$/L)	3.41±1.01	4.98±0.59	4.47±0.46	0.001
WBC ($\times10^{9}$/L)	10.55±5.15	7.63±1.03	7.8±3.44	0.027
PCV (%)	24.56±7.38	35.69±8.07	32.68±4.01	0.001
PK (ng/mg)	42.47±4.47	26.87±3.49	31.93±3.41	0.021

Table 3
Prevalence of PK deficiency by genotype

Variables	Group 1 (SS=30)	Group 2 (AS=30)	Group 3 (AA=40)
PK deficiency, n (%)	2 (6.7)	1 (3.4)	0 (0.0)
Normal PK level, n (%)	28 (93.3)	29 (96.6)	40 (100.0)

Table 4
Correlation between PK activity and hematologic variables by genotype

Pk level	Group 1 (SS=30)		Group 2 (AS=30)		Group 3 (AA=40)	
	R	P-value	R	P-value	R	P-value
Hb S (%)	0.488	0.012	0.394	0.031	0.000	1.000
Hb (g/dL)	0.108	0.568	0.084	0.660	–0.172	0.289
Retic (%)	0.095	0.618	0.066	0.729	0.190	0.241
RBC ($\times10^{12}$/L)	0.015	0.938	–0.061	0.747	–0.082	0.614
WBC($\times10^{9}$/L)	–0.229	0.223	–0.205	0.277	–0.026	0.874
PCV (%)	–0.102	0.593	–0.093	0.624	–0.068	0.676
MCHC (g/dL)	–0.064	0.743	0.057	0.768	–0.296	0.064

Table 5
Comparison of PK activity based on clinical subgroups among SS group 1

Variables		Pk level	p-value
Hydroxyurea	Yes	41.01±24.16	0.047
	No	49.78±27.69	
Splenectomy	Yes	53.03±24.6	0.044
	No	41.30±19.4	
Blood transfusion	≥4	42.26±24.6	0.941
	<4	42.99±25.7	
H, g/dL	<9 g/dL	50.08±24.8	0.020
	≥9 g/dL	38.08±23.9	
Reticulocyte, %	<10 %	41.42±25.06	0.007
	≥10 %	44.06±24.6	

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The prevalence of PK deficiency (Table 3) was 6.7% in HbSS and 3.4% in HbAS, with no cases among HbAA controls.

Correlation analysis (Table 4) showed that PK activity was positively correlated with HbS% in HbSS patients (r=0.488, p-value=0.012) and correlated to a lesser extent with HbS% in HbAS (r=0.394, p=0.031). Other correlations were weak and not statistically significant.

Among HbSS patients (Table 5), PK activity was significantly lower in those receiving hydroxyurea (p-value=0.047) and higher in splenectomized patients (p-value=0.044). Higher PK activity was also associated with Hb <9 g/dL (p-value=0.020) and reticulocyte percentage ≥10% (p=0.007), while transfusion burden showed no significant association (p-value=0.941).

■ DISCUSSION

Sickle cell disease (SCD), especially in its homozygous form (HbSS), remains a globally significant genetic disorder characterized by chronic hemolysis, anemia, end-organ damage and increased morbidity. Understanding biochemical modifiers – such as red cell enzyme activities – that may contribute to the heterogeneity of disease severity is of high clinical importance [10]. Pyruvate kinase (PK), a key glycolytic enzyme, plays a foundational role in maintaining red cell ATP and membrane integrity; reduced PK activity may exacerbate hemolytic anemia and influence complications in SCD [11]. Given that treatment options (e.g. hydroxyurea), transfusion, and splenic function are already known to modulate disease course, studying PK activity in relation to genotype, clinical status, and hematologic markers can yield insights that might help in individualizing management and in prognostic stratification.

The SS genotype group had markedly reduced levels of hemoglobin, RBC count, packed cell volume (PCV), and PK activity compared to the AS and AA control groups ($p < 0.05$ for each). In contrast, the reticulocyte percentage and white blood cell count (WBC) were considerably raised in HbSS, indicating increased erythropoietic activity and inflammatory or stress reactions. The frequency of PK insufficiency was 6.7% in HbSS and 3.4% in HbAS, with no instances identified among AA controls. PK activity exhibited a significant correlation with HbS % in HbSS (r=0.488, p=0.012) and a weaker correlation in HbAS (r=0.394, p=0.031). In the HbSS cohort, reduced PK activity was substantially correlated with hydroxyurea therapy, whereas elevated PK activity was seen in splenectomised individuals and those with Hb <9 g/dL and a reticulocyte count ≥10%. The transfusion load, however, did not exhibit a significant correlation with PK levels.

These findings both support and clarify findings in the body of previous research. According to earlier research, SCD patients had less PK activity than healthy controls [11, 12]. The 6.7% rate of PK deficiency in HbSS seen in this research significantly exceeds the minimal baseline rates documented in general populations (typically <0.01%), indicating that the pathological environment in SCD may reveal latent enzyme deficiencies or diminish residual PK activity. The positive link between PK activity and HbS% aligns with the results of Rab et al. [13], who noted that an increased sickle hemoglobin load correlates with biochemical indicators of hemolysis and metabolic disruption.

The correlation between hydroxyurea administration and reduced PK activity in this population may first seem paradoxical, considering hydroxyurea's function in alleviating

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hemolysis and enhancing HbF levels. Nevertheless, hydroxyurea may indirectly change red cell metabolism in ways that impact enzyme stability or expression, or it may decrease erythropoiesis or reticulocyte production, decreasing the percentage of young red cells that may have greater PK activity. Various investigations have shown inconsistent effects of hydroxyurea on red cell metabolic enzymes, influenced by duration, dosage, and the patient's initial condition [14]. The elevated PK activity in splenectomised patients is credible, as splenectomy diminishes red cell destruction and extends red cell lifespan; older red cells may retain greater PK activity or enable the accumulation of cells with higher activity than would persist in a non-splenectomized condition [5, 15].

The correlation between elevated PK activity and more severe anemia (Hb<9 g/dL) alongside an increased reticulocyte percentage (≥10%) aligns with the principle of compensatory erythropoiesis: when haemoglobin levels are diminished, the bone marrow enhances reticulocyte production; reticulocytes, being immature red blood cells, typically exhibit heightened metabolic enzyme activity, which may artificially elevate measured PK levels. High reticulocyte counts were associated with metabolic resistance to hemolysis [16], which is consistent with results from other populations. Conversely, unlike many findings [17, 18], transfusion frequency in this research did not correlate with PK activity. Transfused red blood cells may dilute the patient's endogenous erythrocytes, obscuring enzyme deficits, or the limited variability in transfusion exposure within this sample may have restricted the identification of an effect.

This research was limited by its modest sample size and single-center methodology, potentially impacting generalisability. Moreover, dependence on cross-sectional measures limits the capacity to determine causal links between PK activity and clinical outcomes.

■ CONCLUSION

PK deficit is present in a non-trivial proportion of patients with SCD, especially HbSS, correlates with recognised indicators of disease severity, and is influenced by splenic status and therapy. These observations indicate that PK activity measurement may function as an additional biomarker in SCD evaluations, aiding in the stratification of patients for risk or in monitoring therapeutic responses. Regular evaluation of pyruvate kinase activity in individuals with sickle cell disease may assist in identifying those at increased risk of severe anemia and associated consequences. Extensive multicenter and longterm research are required to verify these results and investigate treatment implications.

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