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Impaired function of pyruvate kinase is associated with hemolysis-related inflammation, ineffective erythropoiesis, and endothelial dysfunction in adults with sickle cell anemia

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INTRODUCTION

Sickle cell anemia (SCA) is characterized by red blood cell (RBC) sickling due to hemoglobin S polymerization, leading to vaso-occlusive episodes and chronic hemolytic anemia. Intravascular hemolysis contributes to oxidative stress, endothelial dysfunction, and inflammation. Besides hydroxyurea (HU) and transfusion, new therapeutic approaches include pyruvate kinase (PK) activators, which increase ATP, improve membrane health, and reduce sickling.

AIM

To explore whether impaired PK function is associated with disease modifiers (fetal hemoglobin, α-thalassemia) and laboratory markers of hemolysis, ineffective erythropoiesis, inflammation, and endothelial dysfunction in adults with SCA.

METHOD

RBCs from adults (≥16y) with SCA without recent transfusion were obtained and analyzed:

- Complete blood cell count (Abbott Sapphire) & hemolysis parameters
- PK activity (enzymatic activity)
- PK thermostability (enzymatic activity at 53^0 C)
- Plasma levels of soluble transferrin receptor (sTFR), hemopexin, heme oxygenase-1 (HO-1), CD40 Ligand (CD40L, a platelet-associated pro-inflammatory molecule linked to endothelial cell activation), and VEGF (Meso Scale Discovery ELISA).
- Plasma proinflammatory cytokines (OLINK technology)
- RBC adhesion to laminin (Biolamina)

Statistical analysis:

- We stratified individuals on low PK-thermostability levels (≤ 70%, n = 31), intermediate levels (70–85%, n = 32), and high levels(> 85%, n = 14) to explore associations between PK=thermostability and erythropoiesis, endothelial and inflammatory parameters (**Figure 2**.)
- Correlations were tested using the Spearman's correlation or Pearson's correlation when appropriate ($p<0.05$, GraphPad Prism v10).
- Group comparisons with Mann-Whitney U test or ANOVA (Kruskal-Wallis test) ($p<0.05$, GraphPad Prism v10).

RESULTS

N = 77, median age 31 years
57% female, 91% HbSS

60% on hydroxyurea (HU) therapy

Neither HU use or concomitant α-thalassemia was associated with PK thermostability or PK/HK ratio.

- HbF >15% → higher PK thermostability and PK/HK ratio (**Figure 1**). Inflammatory and hemolysis parameters were significantly higher in SCA (**Table 1**).

Inflammatory and endothelial parameters	SCD (n = 77)	Controls (n = 20)	*p-value*
sTFR (µg/mL)	5.55 (1.91 - 12.02)	1.00 (0.68 - 2.32)	*p<0.0001*
Hemopexin (µg/mL)	103.5 (5.2 - 307.8)	289.0 (0.5 - 319.1)	*p<0.0001*
HO-1 (ng/mL)	10.4 (1.4 - 28.60)	1.0 (0.7 - 1.9)	*p<0.0001*
Interferon-Y (pg/mL)	0.4 (0.1 - 3.7)	0.2 (0.1-0.8)	*p<0.01*
TNF (pg/mL)	25.5 (9.3 - 77.4)	16.1 (10.8 - 24.9)	*p<0.0001*
IL-6 (pg/mL)	3.6 (1.3 - 47.5)	1.4 (0.8 - 4.8)	*p<0.0001*
CXCL-8 (pg/mL)	10.4 (1.4 - 53.0)	8.0 (2.7 - 17.5)	*p<0.05*
IL-10 (pg/mL)	7.2 (2.7 - 42.1	4.77 (2.6 - 8.1)	*p<0.001*
IL-18 (pg/mL)	670.9 (157.7 -2809.0)	235.9 (101.2 - 645.1)	*p<0.0001*
CD40 Ligand (CD40L, pg/mL)	385.7 (54.3 - 2491.0)	126.2 (33.6 - 882.9)	*p<0.0001*
P-selectin (ng/mL)	116.6 (52.2 - 263.3)	88.0 (29.4 - 201.7)	*p<0.01*
sVCAM1 (µg/mL)	1.1 (0.6 - 2.3)	0.5 (0.3 - 0.6)	*p<0.0001*
VEGF (pg/mL)	518.4 (229.5 - 3261.3)	366.9 (220.0 - 919.6)	*p<0.01*
Functional assays			
RBC Adhesion	11.1 (3.1 - 39.1)	4.95 (3.20 - 14.30)	*p<0.0001*

Table 1. Baseline characteristics
Numbers represent median (range); sTFR, soluble transferrin receptor; HO-1, hemoxygenase-1; TNF, tumor necrosis factor; IL, interleukine; sVCAM1, soluble vascular cell adhesion molecule-1; VEGF, vascular endothelial growth factor

Impaired PK function is associated with increased hemolysis and erythropoiesis:

- PK thermostability showed a negative correlation with erythroblasts (r= -0.325, p<0.01), sTFR (r=-0.381, p<0.001), ferritin (r=-0.329, p=0.004), and ARC (r=-0.429, p=0.0001, data not shown)
- PK/HK ratio associated with bilirubin (r=-0.392, p=0.001), hemopexin (r=0.254, p=0.026), and hemoglobin (r=0.364, p=0.001, data not shown)
- When stratified to level of PK thermostability impairment, significant associations were found with sTFR, ARC, erythroblasts were found (**Figure 2A-C**)

Impaired PK function is associated with inflammation and endothelial dysfunction:

- PK thermostability correlated with leukocytes (r=-0.316, p<0.011), neutrophil count (r=-0.433, p<0.001), monocytes (r=-0.235, p<0.05) and CD40L (r=-0.337, p=0.003).
- When stratified to level of PK thermostability impairment, significant associations were found with neutrophils, CD40L, and RBC adhesion (**Figure 2E-H**)

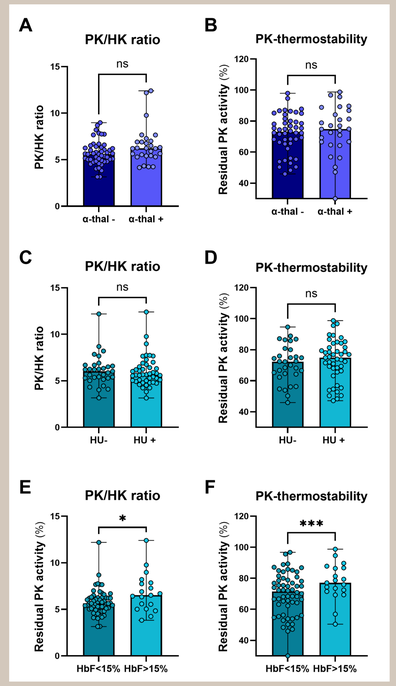

Figure 1. Effect of disease modifiers on PK characteristics. *Neither concomitant α-thalassemia or hydroxyurea (HU) therapy are associated with improved PK function in a cohort of 77 sickle cell anemia patients. Panel E and F display significant associations with fetal hemoglobin (HbF) levels.*
PK, pyruvate kinase; HK, hexokinase.
**p<0.05, ***p<0.001*

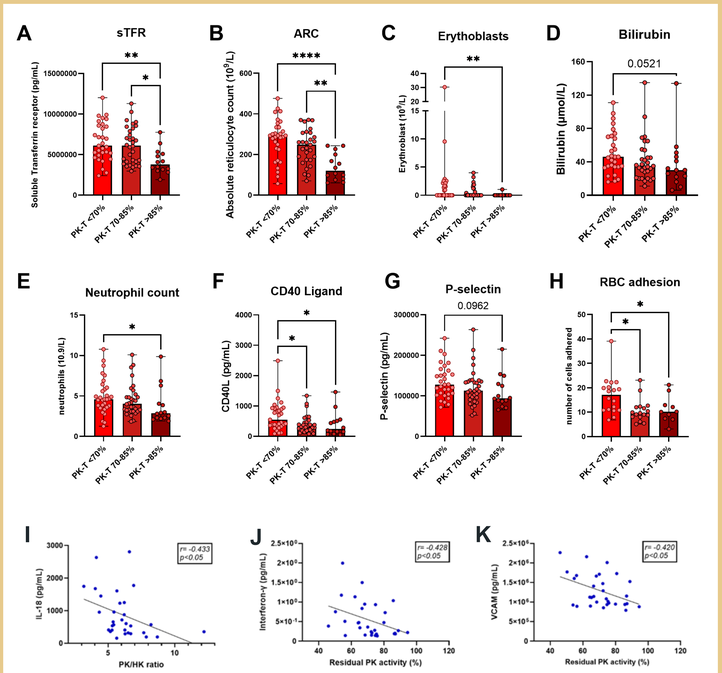

Figure 2. Associations between relative pyruvate kinase (PK) deficiency, reflected by a reduced PK/ hexokinase (HK) activity ratio or PK thermostability with markers of hemolysis, ineffective erythropoiesis, red blood cell (RBC) adhesion and inflammation. *Panel A-H display associations found in a cohort of 77 sickle cell anemia patients when stratified according to level of loss of thermostability. Panel I-K display significant associations in a subgroup of 31 SCA patients without hydroxyurea therapy.*
sFTR, soluble transferrin receptor; IL-18, interleukin 18; sVCAM-1, vascular cell adhesion molecule-1.
**p<0.05, **p<0.01, ****p<0.0001*

In adults without HU therapy impaired PK function was associated with inflammatory and adhesion markers:

- Higher P-selectin → Lower PK thermostability (r=-0.242, p<0.05)
- Higher IL-18, that has been linked to cardiomyopathy → lower PK/HK ratio (r=-0.433, p<0.05, (**Figure 2 panel I**)
- Higher interferon- γ (r=-0.428, p<0.05) and sVCAM (r=-0.420, p<0.05) → lower PK-thermostability (**Figure 2 panel J-K**)

No associations were found with IL-6, CXCL-8, IL-10, TNF, VEGF) and PK function.

CONCLUSIONS

Impaired PK function, reflected by reduced PK thermostability and lower PK/HK ratio, is associated with several markers of increased hemolysis, ineffective erythropoiesis, inflammation, and endothelial dysfunction in adults with SCA. Future research is needed to determine if these findings translate to changes in these markers after in vivo activation of PK.

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