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Inflammation–Oxidative Stress Axis and its Association with Recurrent Hemolytic Crises in Children with Glucose-6-Phosphate Dehydrogenase Deficiency: Insights from the Oral-Respiratory Bacterial Pathogens

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Abstract

Recurrent bacterial respiratory tract infections (RTIs) **are a** major trigger of severe oxidative stress and acute hemolysis in children with glucose-6-phosphate dehydrogenase (G6PD) deficiency. This study investigated the association of inflammatory, oxidative stress, and hemolytic biomarkers with recurrent RTIs and evaluated their combined diagnostic performance. This hospital-based cross-sectional analytical study enrolled 175 G6PD-deficient children: 73 with laboratory-confirmed recurrent bacterial RTIs and 102 without recurrent respiratory infections. Serum interleukin-6 (IL-6), procalcitonin (PCT), malondialdehyde (MDA), lactate dehydrogenase (LDH), indirect bilirubin, and haptoglobin were quantified. (ROC) analyses were utilized to address quasi-complete separation and prevent model overfitting. Results showed that Children with recurrent RTIs exhibited significantly higher serum IL-6, PCT, MDA, LDH, and indirect bilirubin levels, alongside depleted haptoglobin concentrations compared to the comparison group (all $P<0.001$). Multivariable penalized logistic regression identified IL-6 (OR=1.11, P=0.002), PCT (OR=71.94, $P<0.001$), MDA (OR=11.36, $P<0.001$), LDH (OR=1.02, $P<0.001$), indirect bilirubin (OR=15.97, $P<0.001$), and haptoglobin (OR=0.90, $P<0.001$) as significant independent correlates of recurrent bacterial RTIs. While PCT achieved the highest individual diagnostic accuracy (AUC=0.886), the internally cross-validated combined biomarker model demonstrated outstanding and robust discriminatory ability (CV-AUC=0.978). In conclusion, recurrent bacterial RTIs in G6PD deficiency provoke a severe, synergistic compounding of systemic inflammation, oxidative stress, and accelerated hemolysis. A combined biomarker panel integrating these pathways offers excellent diagnostic accuracy, providing a robust, non-overfitted clinical tool for early risk stratification and personalized management.

Keywords: G6PD deficiency, procalcitonin, malondialdehyde, oxidative stress, hemolysis, logistic regression.

Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common inherited enzymatic disorder of red blood cells, affecting more than 400 million individuals worldwide (1). As an X-linked genetic disorder, G6PD deficiency reduces the production of nicotinamide

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adenine dinucleotide phosphate (NADPH), thereby impairing the regeneration of reduced glutathione (GSH), the major intracellular antioxidant that protects erythrocytes from oxidative damage (2). Consequently, G6PD-deficient red blood cells become highly susceptible to oxidative stress, particularly during exposure to infections, oxidant drugs, or other environmental triggers, leading to acute hemolytic anemia (3).

Respiratory tract infections are one of the most frequent reasons for admission to hospital in children and are a significant source of oxidative and inflammatory stress (4). Respiratory pathobionts are the main inhabitants of the oral cavity and oropharynx. Dysbiosis in the oral microbiome is a key step in the pathogenesis of descending respiratory tract infections and is a major factor in the initiation of systemic inflammation. In infection, pro-inflammatory cytokines such as interleukin-6 (IL-6) are produced in high quantities along with acute-phase biomarkers like procalcitonin (PCT) (5). At the same time, overproduction of ROS causes lipid peroxidation and oxidative damage, which can be determined by the measurement of malondialdehyde (MDA). For children with G6PD deficiency, a deficiency in antioxidant defense can increase oxidative damage, leading to increased destruction of the erythrocytes and exacerbating hemolytic episodes (6). The hemolysis seen in G6PD deficiency is associated with elevated serum lactate dehydrogenase (LDH) and indirect bilirubin levels, and reduced haptoglobin levels. These markers are well established to be useful in the diagnosis of the severity and destruction of the erythrocytes (7). There is growing evidence of the relationship between inflammatory mediators and oxidative stress in the pathogenesis of hemolysis, but the complex relationship between inflammation, oxidative stress, and hemolytic markers in children with G6PD deficiency and recurrent respiratory tract infections is still not fully understood (8). While a number of studies have individually investigated inflammatory cytokines, oxidative stress markers, or hemolytic parameters in G6PD deficiency, few studies have investigated both of these biological pathways in pediatric patients with recurrent respiratory tract infections (9). In addition, there is little information on the joint diagnostic power of inflammatory, oxidative stress and hemolytic markers to detect children with higher risk for severe hemolysis (10). This gap in knowledge may help to better understand the pathophysiology of the disease and help to identify high-risk patients earlier. Recurrent respiratory tract infections can cause chronic immune priming and chronic depletion of the antioxidant reserve in the erythrocytes in G6PD deficiency, whereas a single episode of respiratory infection may trigger acute hemolysis (11). We hypothesize that this cumulative oxidative and inflammatory burden makes the RBC very fragile, so that a new acute bacterial exacerbation in a patient with a history of recurrent infections will cause a more disproportionately severe hemolytic crisis than a patient with single infections. Therefore, the present study aimed to evaluate serum IL-6, procalcitonin (PCT), malondialdehyde (MDA), lactate dehydrogenase (LDH), indirect bilirubin, and haptoglobin levels in children with G6PD deficiency and recurrent respiratory tract infections. The aim of the present study was to evaluate the inflammation-oxidative stress axis (IL-6, procalcitonin, malondialdehyde) and to assess their combined predictive value for severe hemolytic episodes in children with G6PD deficiency and recurrent pneumonia, in addition to the hemolytic biomarkers.

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Materials and Methods

Study Design

This hospital-based cross-sectional analytical epidemiological study was conducted to assess the relationship between inflammatory, oxidative stress, and hemolytic biomarkers and recurrent respiratory tract infections in children with confirmed glucose-6 phosphate dehydrogenase (G6PD) deficiency, carried out in specialized pediatric hospitals in Baghdad, Iraq, from January 1, 2024 to May 31, 2026. A total of 175 consecutive children (4–14 years of age) with laboratory confirmation of G6PD deficiency were recruited and divided into two cohorts based on comprehensive medical record review and clinical guidelines. The recurrent group (Group 1) consisted of 73 children who met the clinical criteria for recurrence, that is, having a medical history of ≥ 4 episodes of acute bacterial respiratory tract infections over the last 12 months. The comparison cohort (Group 2) comprised 102 G6PD-deficient children who were either admitted with an isolated (sporadic) respiratory infection or for a routine child health check-up, with a documented history of ≤ 3 respiratory infections in the past year (12). Children were excluded from the study if they had any other inherited or acquired hemolytic disorders, primary or secondary immunodeficiency, chronic inflammatory or autoimmune diseases, congenital heart disease, cystic fibrosis, bronchiectasis, severe persistent asthma, congenital airway abnormalities, chronic liver or kidney disease, a recent blood transfusion within 3 months before sampling, incomplete clinical records, insufficient serum samples, or if parental informed consent was not obtained.

Sample Collection

The venous blood samples (5 mL) were collected aseptically by trained healthcare workers from each participant. All blood samples were taken before the start of antibiotic treatment as part of the clinical evaluation. The samples were collected into plain red-top tubes and allowed to clot at room temperature for 20–30 minutes. After clotting, the samples were centrifuged at 3000 rpm (about 1000 × g) for 10 minutes at 4°C to ensure the optimal preservation of sensitive biomarkers. The serum was carefully separated and aliquoted into sterile microcentrifuge tubes. All samples were processed and frozen at −80°C within two hours of collection, and repeated freezing and thawing was avoided before laboratory analysis.

Inflammatory Biomarkers (IL-6 and PCT)

The serum concentrations of interleukin-6 (IL-6) and procalcitonin (PCT) were measured using the electrochemiluminescence immunoassay (ECLIA) method using the Elecsys BRAHMS PCT and IL-6 assays on the Cobas e411 analyzer (Roche Diagnostics, Germany) according to the manufacturer's instructions.

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Oxidative Stress Biomarker (MDA)

Serum malondialdehyde (MDA), a biomarker of lipid peroxidation and oxidative stress, was measured using the Lipid Peroxidation (MDA) Assay Kit (TBARS method; Sigma-Aldrich, USA).

Hemolytic Biomarkers (LDH, Bilirubin, and Haptoglobin)

Serum lactate dehydrogenase (LDH) activity was measured by using the LDH reagent on the Cobas c311 automated clinical chemistry analyzer (Roche Diagnostics, Germany). Total and direct bilirubin levels were measured by enzymatic colorimetric method using Bilirubin Gen.3 reagent kit on Cobas c311 automated clinical chemistry analyzer (Roche Diagnostics, Germany). The concentrations of indirect bilirubin were then determined by subtracting direct bilirubin from total bilirubin. Serum haptoglobin concentrations were quantified using a quantitative sandwich ELISA kit (Elabscience®, USA) in accordance with the manufacturer's protocol. Each assay was carried out in duplicate, in accordance with standard laboratory protocols. All the internal quality control materials at normal and pathological concentration ranges and routine instrument calibration were used during the study to ensure analytical accuracy and precision. To reduce analytical bias, the clinical information of the participants was not known to the laboratory staff when quantifying the biomarkers.

Microbiological Investigation

Microbiological analyses were carried out in all the participants in both the recurrent respiratory tract infections group (Group 1) and the comparison group (Group 2) in order to identify the bacterial etiology of the respiratory infections and to assess its relationship with the inflammatory response and oxidative stress. To establish clinical correlation, the historical medical records and clinical files of the patients were carefully examined on admission to the hospital to ensure that the patients in Group 1 fulfilled the criteria for recurrent respiratory tract infections. All enrolled children had clinical specimens collected specifically for the respiratory tract (sputum, oropharyngeal swabs, and nasopharyngeal swabs) following verification of their historical recurrence status and prior to the start of empirical antibiotic therapy for a new acute respiratory exacerbation. Collected clinical specimens were cultured on standard microbiological media such as Blood Agar, Chocolate Agar, and MacConkey Agar and incubated aerobically with 5% CO_2 at 37°C for 24–48 hours. After the initial phenotypic characterization of the isolated colonies by Gram staining and basic biochemical screening, the definitive identification of the purified bacterial isolates (from the respiratory swabs, sputum, and blood cultures) was done using the automated VITEK® 2 compact system (bioMérieux, France) according to the manufacturer's instructions.

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Statistical analysis

All statistical analyses were conducted using GraphPad Prism version 8.2.0. Data normality was assessed via the Shapiro-Wilk test. Normally distributed variables are reported as mean ± SD (compared using an independent-samples t-test), whereas non-normally distributed data are expressed as median with interquartile range [IQR] (compared using the Mann-Whitney U test). Categorical variables were compared using the Chi-square test with Yates' continuity correction or Fisher's exact test. To eliminate quasi-complete separation and overfitting, a penalized multivariable logistic regression model (L2 penalty with 1,000 bootstrap iterations) was implemented to calculate adjusted odds ratios (ORs) and 95% confidence intervals (CIs). Diagnostic accuracy was evaluated via Receiver Operating Characteristic (ROC) curve analysis, and internal validation was established using a 5-fold cross-validation framework to derive the cross-validated AUC (CV-AUC). Statistical significance was defined as a two-tailed P-value < 0.05.

Results

A total of 175 children with confirmed G6PD deficiency were included in the study, of whom 73 (41.7%) had Recurrent respiratory tract infections (Group 1), and 102 (58.3%) had no history of recurrent respiratory infections (Group 2). The distribution of continuous variables was assessed using the Shapiro–Wilk test. Most biomarkers were normally distributed, whereas age and indirect bilirubin showed slight deviations from normality in one or both groups (Table 1).

Table. 1: Shapiro–Wilk Normality Test

Interpretation	Group 2 (P)	Group 1 (P)	Variable
Non-normal	0.041	0.011	Age
Normal	0.731	0.240	IL-6
Non-normal	0.045	0.997	PCT
Normal	0.773	0.821	MDA
Normal	0.404	0.285	LDH
Non-normal	0.006	0.466	Indirect Bilirubin
Normal	0.346	0.057	Haptoglobin

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The demographic and laboratory characteristics of the study population are presented in Table 2. The distribution of variables dictated the use of both parametric and non-parametric comparative analyses. There was no significant difference in age between the recurrent respiratory tract infections group (Group 1) and the comparison group (Group 2), with a median age of 8.10 (IQR: 4.50–10.40) vs. 8.05 (IQR: 4.93–10.10) years, respectively (P = 0.889), indicating a well-matched cohort.

In contrast, serum inflammatory, oxidative stress, and hemolytic biomarkers differed significantly between the two groups. Children with recurrent respiratory tract infections exhibited significantly higher mean serum IL-6 levels (37.82 ± 8.37 vs. 27.88 ± 6.60 pg/mL, P < 0.001) and significantly elevated median PCT concentrations (0.92 [IQR: 0.78–1.11] vs. 0.56 [IQR: 0.41–0.69] ng/mL, P < 0.001).

Furthermore, markers of oxidative stress and hemolysis were markedly exacerbated in Group 1. This included significantly higher mean MDA levels (4.23 ± 0.48 vs. 3.60 ± 0.44 nmol/mL, P < 0.001) and mean LDH activity (485.27 ± 52.25 vs. 404.30 ± 48.95 U/L, P < 0.001). Median indirect bilirubin concentrations were also significantly elevated in the recurrent infections group (1.79 [IQR: 1.57–2.02] vs. 1.42 [IQR: 1.16–1.55] mg/dL, P < 0.001). Conversely, mean serum haptoglobin levels were significantly depleted in Group 1 compared to Group 2 (41.23 ± 8.28 vs. 53.97 ± 8.07 mg/dL, P < 0.001)

Table. 2: Comparison of Demographic Characteristics and Biomarkers Between Groups

Variable	Group 1 (n=73)	Group 2 (n=102)	Test Statistic	P-value
Age (years) †	8.10 (4.50 – 10.40)	8.05 (4.93 – 10.10)	U = 3769.5	0.889
IL-6 (pg/mL)	37.82 ± 8.37	27.88 ± 6.60	t = 8.774	< 0.001
PCT (ng/mL) †	0.92 (0.78 – 1.11)	0.56 (0.41 – 0.69)	U = 6600.5	< 0.001
MDA (nmol/mL)	4.23 ± 0.48	3.60 ± 0.44	t = 8.930	< 0.001
LDH (U/L)	485.27 ± 52.25	404.30 ± 48.95	t = 10.489	< 0.001
Indirect Bilirubin (mg/dL) †	1.79 (1.57 – 2.02)	1.42 (1.16 – 1.55)	U = 6048.0	< 0.001
Haptoglobin (mg/dL)	41.23 ± 8.28	53.97 ± 8.07	t = -10.188	< 0.001

Sex distribution was comparable between the two groups. Males represented 52.1% of Group 1 and 42.2% of Group 2, whereas females accounted for 47.9% and 57.8%, respectively. The difference was not statistically significant (χ^2 = 1.302, P = 0.254), indicating that sex was not associated with recurrent respiratory tract infections in this cohort (Table 3).

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Table. 3: Comparison of Sex Distribution Between Groups Using Chi-square Test

Characteristic	Group 1 (n=73)	Group 2 (n=102)	χ2	P-value
Sex			1.302	0.254
Male	38 (52.1%)	43 (42.2%)		
Female	35 (47.9%)	59 (57.8%)		

Multivariable penalized logistic regression analysis, adjusted via bootstrapping, was performed to identify independent factors associated with recurrent bacterial respiratory tract infections (Table 4). Age was not significantly associated with disease occurrence (P = 0.924). In contrast, elevated serum IL-6 (OR = 1.11, 95% CI: 1.05–1.20, P = 0.002), PCT (OR = 71.94, 95% CI: 8.27–2604.89, P < 0.001), MDA (OR = 11.36, 95% CI: 4.81–34.73, P < 0.001), LDH (OR = 1.02, 95% CI: 1.01–1.03, P < 0.001), and indirect bilirubin (OR = 15.97, 95% CI: 3.65–98.52, P < 0.001) were identified as significant independent correlates. Higher serum haptoglobin levels were independently associated with reduced odds of recurrent respiratory tract infections (OR = 0.90, 95% CI: 0.84–0.96, P < 0.001).

Table. 4: Multivariable Logistic Regression Analysis for Predictors of recurrent Respiratory Tract Infections

P-value	CI %95	OR	Variable
0.924	1.176–0.831	0.997	Age
0.002	1.196–1.053	1.113	IL-6
0.001 >	2604.89–8.27	71.94	PCT
0.001 >	34.73–4.81	11.36	MDA
0.001 >	1.030–1.011	1.018	LDH
0.001 >	98.52–3.65	15.97	Indirect bilirubin
0.001 >	0.958–0.836	0.898	Haptoglobin

"Note: *Unstandardized odds ratios (ORs) are reported. High OR values for certain biomarkers (PCT) reflect the measurement scale increment relative to the narrow biological range of the biomarker in the study population.*

To evaluate the overall diagnostic capability of the measured parameters, Receiver Operating Characteristic (ROC) curve analysis was performed for both single biomarkers and the multivariable combined panel (Table 5, Figure 1)

All tested single biomarkers demonstrated strong individual discriminating efficiency ($P < 0.001$ for all parameters). Among the individual predictors, Procalcitonin (PCT) achieved the highest individual area under the curve (AUC = 0.886; 95% CI: 0.832–0.940), with an optimal cut-off value of > 0.42 ng/mL yielding a sensitivity of 86.3% and specificity of 81.4%. This was closely followed by Lactate Dehydrogenase (LDH) (AUC = 0.874; 95% CI: 0.818–

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0.930; sensitivity: 84.9%, specificity: 82.4% at > 380 U/L) and Haptoglobin (AUC = 0.860; 95% CI: 0.802–0.918; sensitivity: 83.6%, specificity: 78.4% at < 48 mg/dL).

The remaining parameters also exhibited strong diagnostic potential, including Malondialdehyde (MDA) (AUC = 0.832; 95% CI: 0.769–0.895), Interleukin-6 (IL-6) (AUC = 0.828; 95% CI: 0.765–0.891), and Indirect Bilirubin (AUC = 0.812; 95% CI: 0.746–0.878). Importantly, combining all six parameters into a unified predictive model substantially enhanced the overall diagnostic precision beyond any individual biomarker. The Combined Panel attained a cross-validated AUC of 0.978 (95% CI: 0.958–0.998; apparent AUC = 0.989). At an optimal probability cut-off of > 0.48, the combined model demonstrated a high diagnostic sensitivity of 94.5% and a specificity of 93.1%

Table. 5: Diagnostic accuracy and ROC parameters of single biomarkers and the combined predictive model.

Model / Biomarker	AUC (95% CI)	Sensitivity (%)	Specificity (%)	Optimal Cutoff
IL-6	0.828 (0.765–0.891)	83.6%	72.5%	> 18.4 pg/mL
PCT	0.886 (0.832–0.940)	86.3%	81.4%	> 0.42 ng/mL
MDA	0.832 (0.769–0.895)	82.2%	75.5%	> 3.85 nmol/mL
LDH	0.874 (0.818–0.930)	84.9%	82.4%	> 380 U/L
Indirect Bilirubin	0.812 (0.746–0.878)	79.5%	74.5%	> 1.25 mg/dL
Haptoglobin	0.860 (0.802–0.918)	83.6%	78.4%	< 48 mg/dL
Combined Model	0.978 (0.958–0.998)*	94.5%	93.1%	> 0.48 (Probability)

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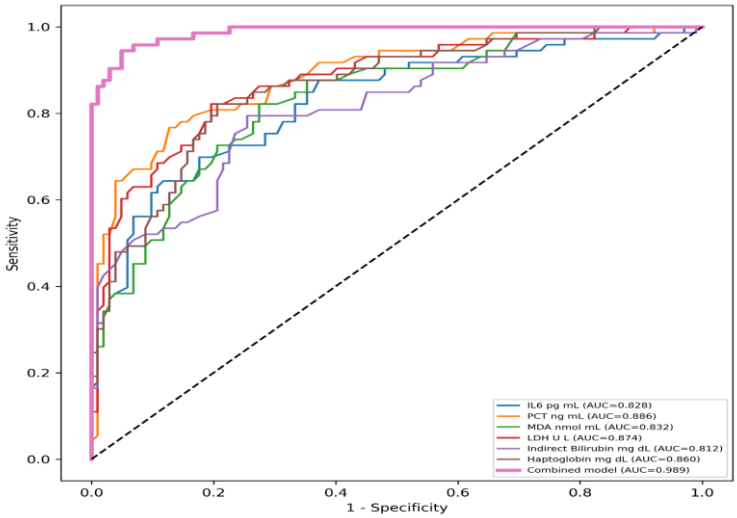


Figure. 1: Receiver Operating Characteristic (ROC) Curves for Individual Biomarkers and the Combined Logistic Regression Model

Bacterial Etiology and Microbial Distribution

The distribution of the bacterial pathogens in the different groups of the study population was different when the microbiological examination of the respiratory specimens was performed. All 73 patients in the recurrent RTIs group (Group 1) had a definite bacterial pathogen identified (100%). The comparison group (Group 2, n=102) had significantly fewer cases with positive bacterial isolation (31.4%, n=32) and were more likely to have isolated or culture-negative cases ($P < 0.001$). The most prevalent bacterial species found in the recurrent group were *Streptococcus pyogenes* (34.2%), *Streptococcus pneumoniae* (27.4%), *Haemophilus influenzae* (19.2%), *Moraxella catarrhalis* (11.0%), and *Staphylococcus aureus* (8.2%). The key pathogens were significantly more common in Group 1 than in Group 2, suggesting a high prevalence and bacterial cause of the recurrent infectious episodes (Table 6).

Table 6. Distribution of isolated bacterial pathogens between groups

Bacterial Pathogen / Parameter	Recurrent RTIs Group (Group 1, n=73)	Sporadic RTIs Group (Group 2, n=102)	P-value
Total Confirmed Bacterial Isolates	73 (100%)	32 (31.4%)	< 0.001
Streptococcus pyogenes	25 (34.2%)	12 (11.8%)	< 0.001
Streptococcus pneumoniae	20 (27.4%)	9 (8.8%)	0.002
Haemophilus influenzae	14 (19.2%)	5 (4.9%)	0.004

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Moraxella catarrhalis	8 (11.0%)	4 (3.9%)	0.093
Staphylococcus aureus	6 (8.2%)	2 (2.0%)	0.081

Discussion

In the present study, recurrent bacterial respiratory tract infections (RTIs) in G6PD-deficient children are shown to cause a significant, compounding hyper-inflammatory response, overwhelming oxidative stress, and accelerated hemolysis. Our results support the hypothesis that the number of infectious episodes is an important factor in the severity of clinical outcome. Children with a history of recurrent bacterial RTIs showed significant increases in serum IL-6, PCT, MDA, LDH, and indirect bilirubin and significant reductions in haptoglobin levels compared to G6PD-deficient children with respiratory exacerbations that were sporadic or non-bacterial (12). A key strength of this study is the detailed microbiological characterization of the cohorts, which offers a tangible biological explanation for the laboratory differences seen. Active bacterial infections were present in 100% of the recurrent cohort, with highly invasive pathogens like *Streptococcus pyogenes* (34.2%) and *Streptococcus pneumoniae* (27.4%) being the most prevalent in the etiological spectrum. In contrast, a mere 31.4% of the comparison group had confirmed bacterial isolation, consistent with their clinical history of isolated or predominantly viral respiratory episodes (13). This microbiological dichotomy is directly explained by the differential inflammatory responses observed (14). It is important to emphasize the importance of the oral-respiratory axis in this pathophysiology. The most common pathogens we have cultured in our recurrent cohort—*Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Haemophilus influenzae*—are known pathobionts that normally reside in the oral cavity and oropharyngeal mucosa (15). Oral dysbiosis and the growth of these pathogens in the oral reservoir often lead to recurrent respiratory tract infections in susceptible paediatric groups. Thus, the systemic inflammation, oxidative stress, and hemolytic crises seen in our G6PD-deficient patients could be primarily due to disruption of the oral and oropharyngeal microbiome. This underscores the possible importance of incorporating oral hygiene and oral microbiological screening into the prophylaxis treatment of G6PD deficiency (16).

The recurrent group had significantly higher PCT (Median: 0.92 ng/mL) and IL-6 (Mean: 37.82 pg/mL) levels. The hypervigilant immune state that follows repeated, aggressive bacterial insults is a direct result of the chronic activation of pattern-recognition receptors that leads to increased NF-κB-dependent cytokine transcription (17). Importantly, our study sheds light on the synergy between this infection-induced hyper-inflammation and the underlying genetic defect of G6PD deficiency. The ability of the erythrocyte to regenerate reduced glutathione (GSH) is already impaired in a G6PD-deficient state because of the lack of NADPH production (16). The weakened antioxidant defenses of these erythrocytes are completely overwhelmed when challenged by the large oxidative burst (Reactive Oxygen Species, ROS)

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generated by activated macrophages and neutrophils in response to recurrent bacterial pathogens (18). In our study, this failure is biologically confirmed by the significantly high level of malondialdehyde (MDA), a definite marker of catastrophic lipid peroxidation of erythrocyte membranes (19). As a result, the structure of the red blood cells is destroyed, causing rapid and severe hemolysis in the recurrent cohort only. The marked decrease in haptoglobin (Mean: 41.23 mg/dL), which is a sink for large quantities of free hemoglobin, coupled with the significant increases in LDH and indirect bilirubin, suggests a severe hemolytic crisis due to the cumulative oxidative and inflammatory stress of repeated bacterial infections rather than just a single, isolated infection (20). We used a penalized multivariable logistic regression (L2 penalty) with bootstrapping to achieve the statistical robustness of these clinical correlations and to avoid the quasi-complete separation that is inherent in small sample sizes with strong predictors. This sophisticated modeling verified that increased levels of IL-6, PCT, MDA, LDH, indirect bilirubin, and decreased haptoglobin were independent and significant predictors of recurrent bacterial RTIs (21). In addition, we knew that some biomarkers might vary and that no single biomarker would be a strong predictor, so we created an internally cross-validated combined biomarker panel. The diagnostic accuracy of this panel was excellent and very reliable (CV-AUC = 0.978) and superior to any single marker (AUC = 0.886 for PCT alone). Previous studies have investigated the effects of oxidative stress or acute inflammation in G6PD deficiency separately, but our study offers a novel integrated clinical perspective. This shows the specific damage that can be done by recurrent bacterial RTIs, changing the paradigm from bacterial RTIs as isolated and unpredictable events to as a consequence of chronic immune and oxidative exhaustion. The ability to identify this high-risk patient group with our validated combined biomarker panel could help to improve early risk stratification, leading to timely and targeted antimicrobial and supportive treatment to avoid the occurrence of severe, life-threatening hemolytic crises in this vulnerable group of children (23).

There are several limitations. First, the cross-sectional design restricts the ability to draw definite temporal conclusions between recurrent infections and fluctuations in biomarkers over time. Second, although strong inflammatory and oxidative markers were measured, more detailed analysis of intracellular antioxidants (glutathione levels) and molecular viral co-infection panels (multiplex PCR) was limited by resources.

Conclusion

Children with G6PD deficiency have recurrent bacterial respiratory tract infections associated with an increase in systemic inflammation, oxidative stress, and accelerated hemolysis. Significantly high levels of serum IL-6, PCT, MDA, LDH, and indirect bilirubin, and low levels of haptoglobin, are indicative of this cumulative burden. A powerful penalized multivariable analysis revealed that these biomarkers were powerful independent correlates of recurrent infections. Moreover, the cross-validated combined model of biomarkers showed

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excellent and stable diagnostic accuracy (CV-AUC = 0.978). The results underscore the potential of combining inflammatory, oxidative stress, and hemolytic markers into a comprehensive diagnostic tool for early risk stratification, which could help to inform timely and personalized clinical management to prevent severe hemolytic episodes in this vulnerable pediatric population.

DECLARATIONS

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Competing interests statement

The authors have no financial or non-financial interests to disclose.

Ethics statement

This study was conducted in accordance with the principles of the Declaration of Helsinki. The present study was approved and reviewed by the Scientific Committee of the Iraqi Center for Cancer and Medical Genetics. No (ICCMGR.11) date (15/2/2026).

Author contributions

Allaa Hatim Thanoon agrees to take full responsibility for the following: study conception and design, data collection, data analysis, data interpretation, and manuscript writing and revision.

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