

REVIEW ARTICLE

Urinary biomarkers of renal involvement in *Plasmodium falciparum* malaria: Biological relevance, diagnostic potential, and clinical perspectives

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ABSTRACT

Plasmodium falciparum malaria remains a major cause of severe disease and mortality, with acute kidney injury (AKI) representing an important complication associated with poor clinical outcomes. Although conventional blood-based methods remain essential for malaria diagnosis, they primarily establish parasite infection and provide limited information on evolving host responses and renal injury. Urine represents an attractive complementary biological matrix because it can be collected non-invasively and repeatedly and may contain parasite-derived products, metabolites, inflammatory mediators, hemolysis-associated molecules, and biomarkers of structural kidney injury. This review summarizes current evidence on urinary biomarkers in falciparum malaria, with particular emphasis on their biological relevance, relationship with renal involvement, diagnostic potential, and clinical applicability. Available evidence suggests that urinary biomarkers fall into three interconnected domains: parasite-related signals, host response indicators, and structural renal injury markers. Parasite-derived antigens, particularly histidine-rich protein 2, and malaria-associated urinary metabolites have demonstrated potential for non-invasive detection, although their diagnostic performance and specificity remain variable. Dipstick abnormalities may reflect hemolysis, renal involvement, and hepatobiliary dysfunction but are not specific to malaria. Urinary inflammatory and immune-related mediators have shown preliminary associations with parasitemia and disease severity, whereas evidence supporting their routine clinical application remains limited. Structural tubular injury biomarkers, particularly neutrophil gelatinase-associated lipocalin and kidney injury molecule-1, appear promising for detecting malaria-associated AKI and may provide information beyond conventional creatinine-based assessment. However, the available literature is characterized by small study populations, heterogeneous clinical settings, differences in specimen processing and analytical platforms, inconsistent biomarker thresholds, and limited external validation. Urinary biomarkers therefore represent promising complementary tools rather than replacements for established blood-based malaria diagnostics. Standardized prospective multicenter studies, particularly in malaria-endemic and resource-limited settings, are needed to determine their diagnostic, prognostic, and clinical utility.

Keywords: acute kidney injury, falciparum malaria, kidney injury molecule-1, malaria diagnostics, neutrophil gelatinase-associated lipocalin, renal biomarkers, tubular injury, urinary biomarkers.

INTRODUCTION

Malaria remains one of the most consequential vector-borne infectious diseases worldwide, causing substantial morbidity, mortality, and socioeconomic disruption despite decades of control efforts [1, 2]. Transmitted primarily through bites from infected female *Anopheles* mosquitoes, malaria is a complex One Health challenge shaped by interactions among humans, vectors, parasites, and environmental conditions. Recent estimates indicate more than 260 million cases annually and nearly 600,000 deaths each year, with the vast majority occurring in sub-Saharan Africa [1, 2]. Ongoing transmission is further influenced by climate variability, insecticide resistance, emerging antimalarial drug resistance, population displacement, and inequities in healthcare access [1].

Among human malaria parasites, *Plasmodium falciparum* is responsible for most severe malaria and malaria-related mortality worldwide [3]. Its pathogenicity is closely associated with cytoadherence and sequestration of infected erythrocytes within the microvasculature, leading to endothelial activation, hemolysis, inflammatory dysregulation, tissue hypoperfusion, and subsequent organ injury [4, 5]. These processes contribute to severe complications, including cerebral malaria, severe anemia, acute respiratory distress syndrome, and acute kidney

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injury (AKI) [6, 7]. Recent meta-analytic evidence indicates that malaria-associated AKI occurs in approximately 26.5% of hospitalized patients with malaria and is associated with substantial mortality, the need for renal replacement therapy, and potential progression to chronic kidney disease (CKD) among survivors [8]. Pediatric studies from sub-Saharan Africa have additionally reported an AKI prevalence of approximately 24%–59% in severe malaria cohorts, highlighting important regional and population-specific differences in disease burden [6].

Although microscopy, rapid diagnostic tests (RDTs), and molecular assays remain central to malaria diagnosis, these approaches primarily establish parasite presence and do not fully capture evolving host injury and organ dysfunction in severe falciparum malaria [6, 9]. Urine has therefore emerged as a potentially valuable complementary biological matrix because it can be obtained non-invasively and repeatedly and may contain parasite-derived products, hemolysis-associated molecules, inflammatory mediators, and biomarkers of renal injury [6, 10]. This potential is particularly relevant because malaria-associated renal involvement is increasingly recognized as an important determinant of adverse clinical outcomes.

Despite growing interest in urine-based biomarkers, important knowledge gaps remain. Available evidence is dispersed across distinct biomarker classes, including parasite-derived antigens and metabolites, conventional urinalysis abnormalities, inflammatory mediators, and markers of structural renal injury, and these biomarkers have been investigated using heterogeneous assay platforms, study populations, clinical settings, and outcome definitions. Consequently, their respective contributions to parasite detection, characterization of host responses, identification of renal injury, and assessment of malaria-associated kidney dysfunction remain difficult to interpret within a unified clinical framework. Moreover, the combined clinical utility of these biologically distinct urinary signals has not been prospectively validated, limiting conclusions regarding their complementary diagnostic or prognostic value in falciparum malaria.

Therefore, this review aimed to critically synthesize current evidence on urinary biomarkers in *P. falciparum* malaria using a urine-focused framework comprising three complementary biological domains: parasite-derived signals reflecting parasite-related processes; host response signals reflecting hemolytic, inflammatory, and hepatobiliary disturbances; and structural renal injury biomarkers reflecting tubular epithelial damage. Particular emphasis was placed on parasite-derived urinary markers, dipstick abnormalities, inflammatory mediators, and structural renal injury biomarkers in relation to falciparum malaria severity and AKI. The review further evaluates their biological relevance, diagnostic potential, limitations, and possible clinical applicability. A narrative synthesis was considered appropriate because the available evidence encompasses heterogeneous biomarker classes, assay platforms, study populations, and clinical outcome definitions, limiting meaningful quantitative synthesis across studies.

STANDARD METHODS FOR MALARIA DETECTION

Standard laboratory diagnosis of malaria relies primarily on parasite detection in blood using microscopy, RDTs, and molecular methods [11, 12]. Microscopy of Giemsa-stained blood films remains widely used because it enables direct parasite visualization, parasite density estimation, and differentiation of *Plasmodium* species [11]. However, microscopy is highly operator-dependent and may have reduced sensitivity for low-density or mixed infections [11]. RDTs provide a rapid, operationally practical alternative based on detecting parasite antigens, including histidine-rich protein 2 (HRP2), *Plasmodium* lactate dehydrogenase (pLDH), and aldolase. Molecular methods, including polymerase chain reaction (PCR), provide greater analytical sensitivity for detecting low-density infections [12]. Nevertheless, molecular approaches are relatively expensive and require specialized laboratory infrastructure, trained personnel, and longer turnaround times, which may restrict their routine implementation in many malaria-endemic and resource-limited settings [12]. Conventional blood-based methods and emerging non-invasive approaches, including saliva-, breath-, and urine-based strategies, differ substantially in their detection targets, analytical performance, operational requirements, and potential applications, as summarized in Table 1 [6, 11–25].

REVIEW METHODOLOGY

This narrative review summarizes and critically evaluates current evidence on urinary biomarkers in falciparum malaria, with particular emphasis on their biological relevance, association with renal involvement, diagnostic performance, and potential clinical applicability. Given the heterogeneity of the available evidence, the review was designed as a narrative synthesis rather than a systematic review or meta-analysis.

Table 1: Comparison of conventional and emerging malaria diagnostic approaches relevant to *Plasmodium falciparum* detection and monitoring.

Diagnostic approach	Specimen	Detection target	Advantages	Major limitations	Key findings	Key references
Light microscopy	Peripheral blood smear	Morphological identification of parasites	Reference standard for malaria diagnosis; permits species identification, parasite staging, and parasitemia quantification; relatively inexpensive and widely available	Requires experienced microscopists and quality assurance; reduced sensitivity in low-density infection; substantial methodological and interobserver variability; unable to detect sequestered parasites in severe falciparum malaria	Microscopy remains the reference standard for malaria diagnosis, although considerable variation exists in staining procedures, parasite quantification methods, and slide interpretation across studies. Peripheral parasitemia may underestimate total parasite biomass in severe falciparum malaria because mature sequestered parasites are absent from circulating blood	[11–13]
RDTs	Peripheral blood	HRP2, pLDH, aldolase	Rapid, simple, field-deployable, and suitable for point-of-care testing with minimal equipment requirements	Reduced sensitivity in low-density infection; limited ability to quantify parasite burden; <i>pfhrp2/pfhrp3</i> deletions may produce false-negative HRP2-based RDT results	Blood-based RDTs provide operationally practical malaria diagnosis in endemic settings, although diagnostic performance may decline in low-parasitemia infections and regions with <i>pfhrp2/pfhrp3</i> deletions	[12, 14, 15]
PCR/qPCR	Peripheral blood	<i>Plasmodium</i> nucleic acids	High analytical sensitivity and specificity; capable of detecting submicroscopic infections; useful for epidemiological surveillance and species confirmation	Expensive and laboratory-dependent; requires specialized equipment, trained personnel, and longer turnaround time; limited field applicability in many endemic settings	PCR-based methods provide high analytical sensitivity for detecting low-density infections but remain difficult to implement routinely in many resource-limited settings	[12]
Saliva-based detection	Saliva	HRP2 and pLDH antigens	Non-invasive, simple, and repeatable sampling; potentially suitable for community-based surveillance	Variable sensitivity; influenced by parasite density and occult blood contamination; generally less sensitive than blood-based approaches	In 706 patients with suspected malaria, saliva demonstrated 57.0% sensitivity and 100% specificity relative to corresponding blood-based RDT results; sensitivity decreased to 13.3% in saliva samples without occult blood contamination	[16]
Breath-based analysis	Exhaled breath	Malaria-associated volatile organic compounds	Completely non-invasive; may reflect host–parasite metabolic interactions	Requires specialized analytical instrumentation; findings were derived from small controlled human malaria cohorts and require validation in naturally acquired malaria	Four malaria-associated breath thioethers varied during controlled human <i>P. falciparum</i> infection. After a 24-h phase adjustment, the best-performing thioethers showed strong correlations with parasitemia ($r^2 = 0.92–0.97$). Thioether levels subsequently declined after antimalarial treatment in parallel with parasite clearance	[17]
Urine-based antigen detection	Urine	Primarily HRP2, with limited evaluation of pLDH-containing	Non-invasive and readily repeatable; potentially useful when blood collection is difficult or impractical	Diagnostic sensitivity varies substantially among studies and is strongly influenced by parasite density and	Reported sensitivity ranged from 35.2% to 86.7%, whereas specificity ranged from 84.0% to 100.0%. Direct comparisons showed lower	[16, 18–20]

Diagnostic approach	Specimen	Detection target	Advantages	Major limitations	Key findings	Key references
		platforms		assay characteristics; urine-based testing may have lower sensitivity than corresponding blood-based methods	sensitivity than corresponding blood-based RDTs in some studies, whereas overall diagnostic performance was comparable in others. Performance was strongly influenced by parasite density	
Urine-based molecular detection	Urine	<i>P. falciparum</i> DNA	Non-invasive molecular approach with potential utility for repeated sampling and surveillance	Variable amplification success depending on DNA extraction method, parasite density, and primer set; DNA isolation and amplicon yields require further optimization	PCR detection of <i>P. falciparum</i> DNA in urine demonstrated the feasibility of urine-based molecular detection. Urine-derived amplicons showed parasite genotypes corresponding to those detected in peripheral blood, although amplification success depended on DNA extraction method, parasite density, and primer set. Further optimization and validation are required before large-scale screening or epidemiological surveillance	[21]
Urinary metabolomic profiling	Urine	Parasite-associated metabolites and host metabolic signatures	May capture dynamic host–parasite metabolic interactions; potential for treatment-associated metabolic assessment	Requires advanced metabolomic platforms and bioinformatic analysis; limited validation across populations; biological origin and malaria specificity of individual urinary metabolites remain uncertain	Acetylated polyamines, including N-acetylspermidine and N-acetylputrescine, were significantly elevated in urine during active falciparum malaria, whereas the overall urinary metabolomic profile and candidate metabolite levels shifted toward those of healthy controls following antimalarial treatment	[22, 23]
Emerging host biomarker approaches	Urine	Kidney injury and inflammatory biomarkers (e.g., NGAL, KIM-1, and sTNFR-2)	May provide complementary information on renal involvement, tubular injury, and inflammatory responses beyond parasite detection alone	Clinical validation remains limited; interpretation may be influenced by concurrent renal disease, systemic inflammation, hydration status, and non-malarial causes of tubular injury	Urinary biomarkers may provide complementary information on renal injury and systemic inflammatory responses. NGAL and KIM-1 are recognized markers of tubular injury in broader AKI settings, whereas urinary inflammatory markers such as sTNFR-2 have shown exploratory diagnostic associations in malaria	[6, 24, 25]

AKI = acute kidney injury; DNA = deoxyribonucleic acid; HRP2 = histidine-rich protein 2; KIM-1 = kidney injury molecule-1; NGAL = neutrophil gelatinase-associated lipocalin; PCR = polymerase chain reaction; pLDH = parasite lactate dehydrogenase; qPCR = quantitative polymerase chain reaction; RDT = rapid diagnostic test; sTNFR-2 = soluble tumor necrosis factor receptor 2.

Literature identification

Relevant literature was identified primarily through PubMed/MEDLINE using iterative, topic-directed searches conducted during the development of the review. Searches focused on urinary biomarkers, urine-based malaria diagnostics, malaria-associated AKI, and renal injury associated with *P. falciparum* infection. Search queries were formulated ad hoc based on the evolving thematic focus of the review rather than from a predefined list of keywords or Boolean combinations; therefore, no prespecified database-specific search string or formal systematic search strategy was used. Additional relevant publications were identified from the reference lists of selected primary studies and review articles. No formal deduplication procedure was applied. When the same publication was encountered more than once during iterative literature searching or reference-list checking, the repeated record was recognized manually and retained only once as a unique source. Only English-language

articles for which the full text was available were included in the final narrative synthesis. This restriction may have introduced language and availability bias by excluding potentially relevant evidence published in other languages or not available in full text. No lower publication-date limit was prespecified; older foundational studies and more recent publications were considered when relevant to the scope of the review. The literature search was last updated on August 28, 2026. No review protocol was prospectively registered or published for this narrative review.

Study selection and prioritization

Potentially relevant publications were selected iteratively according to their relevance to the evolving thematic scope of the review. Because this was a narrative review, no formal sequential screening process based on title/abstract screening followed by full-text assessment was applied. The literature search, selection of relevant publications, extraction of relevant study information, and evidence interpretation were conducted by the sole author. Relevant information was extracted directly from the selected publications as needed for the narrative synthesis, including study characteristics and reported findings relevant to the review scope. No standardized data-extraction form was used, and no independent duplicate screening or duplicate data extraction was performed. Priority was given to human studies evaluating urinary biomarkers, renal involvement, disease severity, or diagnostic performance in falciparum malaria. Studies investigating parasite-derived antigens, urinary metabolites, cytokines, dipstick abnormalities, and structural renal injury biomarkers, including neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1), were included to represent the major biomarker categories addressed in this review. Mechanistic, translational, animal, and *in vitro* studies were additionally considered when they directly addressed mechanisms relevant to urinary biomarker alterations, malaria-associated renal injury, tubular epithelial injury, or the biological interpretation of findings reported in human studies. Studies with only indirect or peripheral relevance to these topics were not considered.

Beyond the stated exclusions and the English-language full-text requirement, no prespecified restrictions were applied with respect to study design, participant age, geographic setting, malaria severity, or publication period. With respect to publication type, conference abstracts and editorials were not considered primary evidence; other full-text publications were considered according to their relevance to the review scope.

Studies focusing exclusively on non-falciparum malaria species without relevant *P. falciparum* data, conference abstracts, editorials, and duplicate publications without substantive primary data were not considered primary evidence in the narrative synthesis. Animal and *in vitro* studies meeting the supplementary inclusion criteria described above were used to provide mechanistic context and were not considered primary clinical evidence.

Narrative synthesis and evidence interpretation

Evidence was synthesized narratively according to biomarker category and pathophysiological relevance, including urinary metabolites, cytokines, HRP2, dipstick abnormalities, and structural renal injury biomarkers. The synthesis emphasized biological plausibility, diagnostic performance, associations with disease severity and renal involvement, and potential translational applicability in malaria-endemic settings. When quantitative diagnostic data were available, including sensitivity, specificity, area under the receiver operating characteristic curve (AUROC), and predictive values, these findings were summarized descriptively. Diagnostic performance measures, including sensitivity, specificity, AUROC, and predictive values, were extracted directly as reported in the original studies; no recalculation or re-estimation was performed by the author.

Given the narrative scope of the review and heterogeneity in study designs, biomarker methodologies, patient populations, assay platforms, and clinical outcome definitions, quantitative pooling was not performed. Formal risk-of-bias assessment tools, including the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) and Newcastle–Ottawa Scale, were not applied. No formal or standardized qualitative appraisal was performed. Instead, methodological features relevant to the interpretation of individual studies were considered narratively, where applicable, including study design and sample size, participant characteristics and clinical setting, diagnostic or reference standards, biomarker measurement methods and thresholds, outcome definitions, and generalizability. These features were used to contextualize study findings rather than to generate formal quality ratings or scores.

When findings differed among studies, potential differences in study populations, clinical severity, assay platforms, diagnostic reference standards, biomarker thresholds, and outcome definitions were considered in interpreting the evidence. Methodological strengths and limitations of individual studies were also considered

where appropriate. No formal study-quality weighting scheme was applied. Where findings from heterogeneous studies were discussed together, they were summarized descriptively and were not interpreted as direct comparative estimates.

URINE AS AN ALTERNATIVE DIAGNOSTIC MATRIX FOR MALARIA DETECTION

Urine has emerged as a promising alternative matrix for malaria diagnosis and monitoring because it is non-invasive, readily obtainable, and suitable for repeated sampling, thereby avoiding venipuncture and potentially reducing the procedural burden, biohazard exposure, and infrastructure requirements associated with blood-based diagnostics [10]. These characteristics may be particularly advantageous when repeated sampling is required and in resource-limited settings where simple and scalable diagnostic approaches are needed. Nevertheless, the diagnostic performance of urine-based approaches should be interpreted in conjunction with established blood-based methods [10]. The non-invasive nature of urine collection may also improve acceptability and willingness to participate in repeated malaria testing, although evidence from implementation studies remains limited [26].

The biological plausibility of urine as a diagnostic matrix is supported by the systemic pathophysiology of *P. falciparum* infection. Following hepatic replication, merozoites invade erythrocytes and initiate the intraerythrocytic cycle. Cytoadherence and sequestration of infected erythrocytes within the microvasculature contribute to endothelial activation, impaired microvascular perfusion, and subsequent organ injury, as illustrated in Figure 1 [27, 28]. Concurrently, extensive intravascular hemolysis releases hemoglobin, free heme, parasite-derived antigens, and inflammatory mediators into the circulation [29, 30]. In severe falciparum malaria, cell-free hemoglobin generated during intravascular hemolysis has been associated with oxidative stress and AKI, supporting a potential contribution of hemolysis to renal tubular injury [31]. Excessive filtered loads of circulating molecules may exceed proximal tubular reabsorptive capacity, resulting in increased urinary excretion through overflow and impaired tubular reabsorption [32]. In parallel, soluble antigens and other low-molecular-weight components may cross the glomerular filtration barrier and become detectable in urine, particularly in the presence of malaria-associated renal injury or altered tubular handling [10, 33, 34].

Endothelial dysfunction and inflammation may further increase glomerular permeability, facilitating the passage of plasma constituents into the urinary space, whereas impaired tubular reabsorption may prolong their persistence. Severe hemolysis can also result in hemoglobinuria and contribute to malaria-associated AKI, in which cell-free hemoglobin and heme promote oxidative and cytotoxic injury to renal tubular epithelial cells [6, 27, 35]. Sequestration of infected erythrocytes within the renal microvasculature may further impair perfusion and exacerbate ischemic injury, thereby contributing to renal dysfunction in severe disease, as illustrated in Figure 2 [34].

Pre-analytical and biological factors can substantially influence urinary biomarker measurements and their interpretation. These factors include the timing of urine collection, hydration status, urine concentration or dilution, urine output, pH, bacterial contamination, storage temperature, specimen preservation, delayed processing, and coexisting urinary tract abnormalities (Table 2) [36–41]. Storage temperature and specimen preservation are important pre-analytical considerations for quantitative urinary protein measurement [36].

Variations in urinary concentration may also affect the interpretation of quantitative biomarkers, particularly in patients with oliguria, severe dehydration, or evolving kidney injury. Therefore, standardized procedures for collection timing, specimen processing, storage, and assessment of urine concentration are important for improving comparability among samples and studies [36–40]. Variations in urinary dilution should also be considered during interpretation. Normalization to urinary creatinine or adjustment for urine specific gravity may account for such differences; however, both approaches have limitations, and the selected normalization method may influence biomarker interpretation according to the biomarker and characteristics of the study population [37, 38]. Key pre-analytical and biological considerations affecting urine-based malaria biomarker analysis are summarized in Table 2 [36–41].

Collectively, these interconnected mechanisms, including solute overload, altered glomerular permeability, and impaired tubular handling, provide a physiological basis for the appearance of malaria-associated biomarkers in urine and support the potential utility of urine as a complementary non-invasive diagnostic matrix. Urinary biomarkers investigated in malaria broadly encompass parasite-derived metabolites, host immune mediators, circulating parasite antigens, and markers of organ dysfunction. The subsequent sections examine these biomarker categories in falciparum malaria, including urinary metabolites, urinary cytokines, HRP2, dipstick-based chemical abnormalities, and renal injury biomarkers such as NGAL and KIM-1 in relation to malaria-associated

kidney dysfunction.

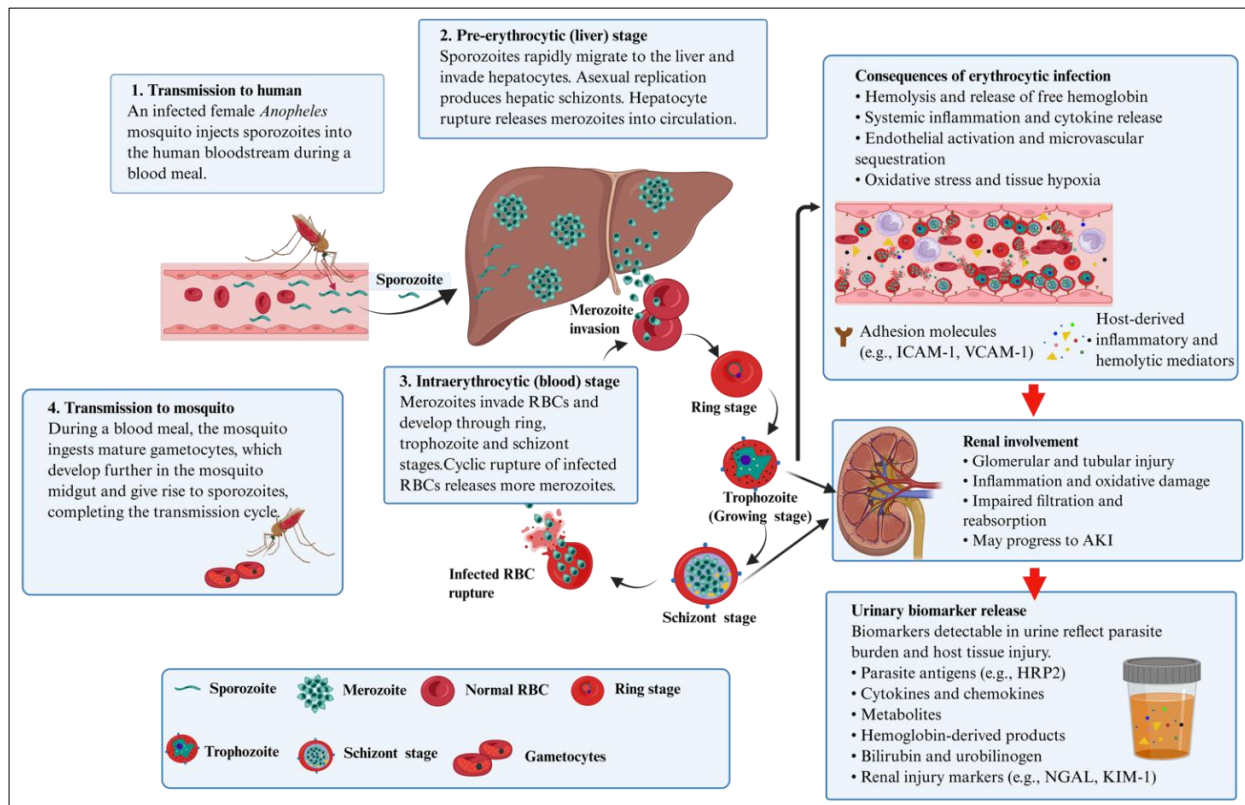


Figure 1: Life cycle of *Plasmodium falciparum* and its relationship to renal involvement and urinary biomarker release. Following inoculation by an infected female *Anopheles* mosquito, sporozoites migrate to the liver and undergo pre-erythrocytic development. Merozoites released into the circulation subsequently invade red blood cells (RBCs) and progress through ring, trophozoite, and schizont stages. Rupture of infected RBCs releases merozoites that continue the erythrocytic cycle, whereas a subset of parasites differentiates into gametocytes that are taken up by mosquitoes during a subsequent blood meal. During the erythrocytic stage, hemolysis, systemic inflammation, endothelial activation, oxidative stress, and microvascular sequestration may contribute to renal involvement, including glomerular and tubular injury. These parasite- and host-derived processes may generate urinary signals, including parasite antigens, inflammatory mediators, metabolites, hemoglobin-derived products, bilirubin-related products, and structural renal injury biomarkers such as NGAL and KIM-1. The figure was created by the author as a conceptual synthesis of the malaria life cycle and pathways linking erythrocytic infection to renal involvement and urinary biomarker release [6, 10, 22, 24, 25, 27–35].

AKI = acute kidney injury; HRP2 = histidine-rich protein 2; ICAM-1 = intercellular adhesion molecule 1; KIM-1 = kidney injury molecule-1; NGAL = neutrophil gelatinase-associated lipocalin; RBC = red blood cell; VCAM-1 = vascular cell adhesion molecule 1.

For clinical interpretation, the potential applications of urinary biomarkers should be distinguished according to their intended purpose. In this review, diagnostic utility for malaria refers to the detection of *P. falciparum* infection, whereas detection of malaria-associated AKI refers to the identification of renal injury in patients with established malaria. Prognostic utility refers to the ability of a biomarker to predict subsequent clinical outcomes, including progression of kidney injury, renal recovery, or mortality. These applications are considered separately where supported by the available evidence.

Conceptually, urinary biomarkers in falciparum malaria can be organized into three complementary pathophysiological domains: markers of parasite-related processes, indicators of host inflammatory and hemolytic responses, and biomarkers of structural renal injury. Parasite-derived antigens and metabolites primarily represent the parasite-related domain; dipstick abnormalities and inflammatory mediators reflect components of the host response; and tubular injury biomarkers such as NGAL and KIM-1 provide information on evolving renal injury. This framework emphasizes that these biomarker classes capture distinct but biologically interconnected aspects of falciparum malaria rather than representing interchangeable diagnostic tests.

URINARY METABOLITES OF *P. FALCIPARUM*

Blood-stage *P. falciparum* infection is associated with broad metabolic alterations involving multiple

pathways related to parasite development and host–parasite interactions [23, 42, 43]. Metabolomic analysis of the intraerythrocytic developmental cycle has demonstrated marked stage-dependent changes in metabolite abundance, with many periodic metabolites reaching peak levels during the late trophozoite and early schizont stages, when energy metabolism and macromolecular biosynthesis are particularly active [43]. These metabolic alterations reflect contributions from parasite metabolism, infected erythrocytes, and host responses, resulting in complex malaria-associated metabolic profiles that can be detected in biological fluids [23, 42].

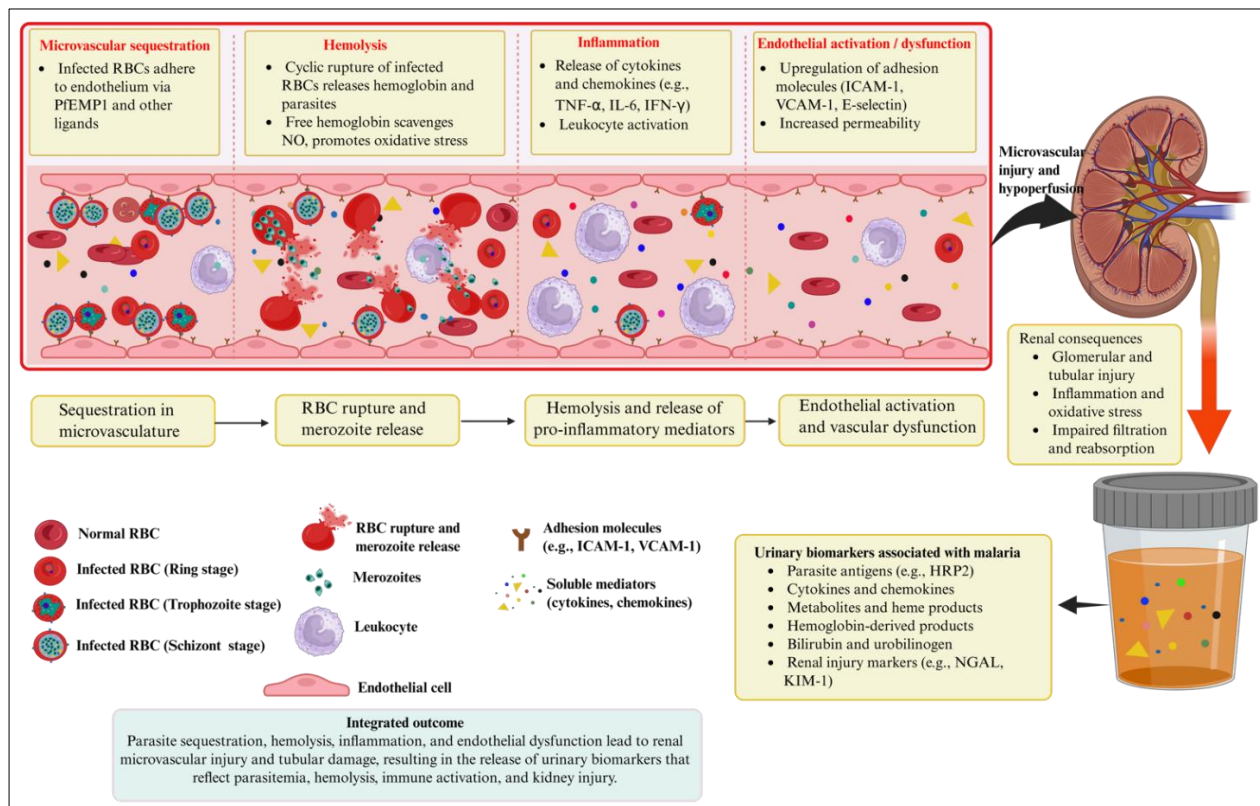


Figure 2: Proposed pathophysiological basis for the appearance of malaria-associated urinary biomarkers during *Plasmodium falciparum* infection. Intraerythrocytic parasite development, cytoadherence, sequestration, and erythrocyte rupture contribute to hemolysis, endothelial and microvascular dysfunction, inflammation, and the release of parasite- and host-derived molecules into the circulation. Concurrent renal involvement may alter glomerular permeability and tubular handling, which may contribute to the urinary appearance of parasite antigens, inflammatory mediators, metabolites, hemoglobin-derived products, bilirubin, urobilinogen, and renal injury biomarkers. Urinary detection is influenced by parasite and circulating antigen burden, renal filtration and tubular handling, and the extent of malaria-associated renal involvement. The figure was created by the author as a conceptual synthesis of the proposed pathways linking parasite sequestration, hemolysis, inflammation, endothelial dysfunction, renal involvement, and urinary biomarker release [6, 10, 22, 24, 25, 27–35]. HRP2 = histidine-rich protein 2; ICAM-1 = intercellular adhesion molecule 1; IFN- γ = interferon-gamma; IL-6 = interleukin-6; KIM-1 = kidney injury molecule-1; NGAL = neutrophil gelatinase-associated lipocalin; NO = nitric oxide; PfEMP1 = *Plasmodium falciparum* erythrocyte membrane protein 1; RBC = red blood cell; TNF- α = tumor necrosis factor-alpha; VCAM-1 = vascular cell adhesion molecule 1.

Table 2: Important pre-analytical and biological variables affecting urine-based malaria biomarker analysis.

Pre-analytical or biological factor	Potential impact on urinary biomarkers	Examples of affected analytes	Practical considerations	Representative references
Timing of urine collection	Urinary biomarker concentrations and their clinical interpretation may vary according to biomarker kinetics and the timing of sampling relative to the evolution of kidney injury	NGAL, KIM-1	The timing of urine collection relative to clinical presentation and renal injury assessment should be documented, particularly when serial or longitudinal measurements are performed	[39, 40]
Urine dilution/urinary flow	Variation in urinary flow can substantially affect absolute urinary biomarker concentrations and between-sample comparability	NGAL, KIM-1, and other quantitative urinary biomarkers	Quantitative studies should report whether biomarker concentrations are expressed as absolute values or normalized for urine dilution. Urinary creatinine or osmolality may be used, although each normalization approach has limitations	[40]

Pre-analytical or biological factor	Potential impact on urinary biomarkers	Examples of affected analytes	Practical considerations	Representative references
Oliguria/reduced urine output	Reduced or variable urine output may affect the interpretation of absolute urinary biomarker concentrations	Urinary NGAL and other quantitative kidney injury biomarkers	Urine output should be considered when interpreting quantitative urinary biomarker measurements, particularly in the setting of AKI	[39, 40]
Coexisting urinary tract pathology or hematuria	Hematuria may arise from malaria or coexisting conditions such as urogenital schistosomiasis, complicating attribution of heme-positive urinary findings to malaria alone	Heme-positive dipstick, hematuria, proteinuria	Coexisting causes of urinary abnormalities should be considered when interpreting urinalysis findings in malaria-endemic settings	[41]
Storage temperature and specimen preservation	Storage temperature, duration, and preservatives may influence quantitative urinary protein measurement	24-h urinary protein	Storage temperature, duration, and use of preservatives should be documented and standardized within studies	[36]

AKI = acute kidney injury; KIM-1 = kidney injury molecule-1; NGAL = neutrophil gelatinase-associated lipocalin.

Human metabolomic studies have identified several urinary metabolites associated with falciparum malaria, including the amino acid-related metabolites alanine, N-acetylglutamine, and N-acetylglutamine; the organic acid succinate; and other low-molecular-weight compounds such as taurine and pipecolic acid [22]. Among these candidates, the acetylated polyamines N-acetylspermidine, N-acetylputrescine, and 1,3-diacetylpropane were significantly elevated in patients with active malaria compared with healthy controls [22]. Following antimalarial treatment, the overall urinary metabolomic profile of patients with malaria shifted toward that of healthy controls, accompanied by decreases in candidate metabolite levels [22]. The prominence of acetylated polyamines is biologically plausible because polyamines are important for the growth and proliferation of *P. falciparum* [44]. However, the relationship between parasite polyamine metabolism and the urinary appearance of specific acetylated polyamine derivatives remains to be established. Collectively, these findings indicate that urinary metabolic profiles change during active falciparum malaria and following antimalarial treatment, although the biological origins and malaria specificity of individual urinary metabolites require further clarification. The principal metabolites identified in human urine and experimental studies, together with their reported associations, analytical platforms, and major limitations, are summarized in Table 3 [22, 44-46].

Evidence from controlled human malaria infection (CHMI) studies further suggests that urinary metabolic signatures may vary according to prior malaria exposure and subsequent parasitemia status. In a longitudinal metabolomic study involving malaria-naïve European and malaria-exposed African volunteers undergoing *P. falciparum* sporozoite challenge, urinary metabolites, including formate, urea, trimethylamine, threonine, choline, acetate, pyruvate, dimethylglycine, and myo-inositol, differed between thick blood smear (TBS)-positive and TBS-negative African participants at specific time points and/or in their temporal patterns [45]. Baseline phenylacetylglutamine (PAG) concentrations were also higher among African participants who subsequently remained TBS-negative, suggesting a potential association with subsequent parasitemia status in this CHMI cohort [45].

A metabolomic study using synchronized *P. falciparum*-infected erythrocyte cultures further demonstrated marked stage-associated increases in extracellular pipecolic acid, gamma-aminobutyric acid (GABA), and alpha-ketoglutarate during trophozoite and schizont development, whereas extracellular homocysteine increased at the schizont stage [46]. These findings provide experimental evidence of stage-associated metabolic changes during intraerythrocytic parasite development; however, their direct relevance to metabolites detected in human urine has not been established [46].

Despite these findings, validation of urinary metabolite biomarkers remains limited. Most available evidence is derived from relatively small exploratory cohorts, CHMI models, or experimental systems, and independent validation across geographically distinct malaria-endemic populations remains insufficient [22, 45, 46]. Moreover, many reported metabolites are endogenous host-associated compounds, and their specificity for malaria has not been established in available clinical studies. Current analytical approaches also rely predominantly on advanced metabolomic platforms, including liquid chromatography–mass spectrometry (LC-MS), gas chromatography–mass spectrometry (GC-MS), hydrophilic interaction liquid chromatography–mass spectrometry (HILIC-MS), and nuclear magnetic resonance (NMR) spectroscopy, which are technically demanding and may limit implementation in many malaria-endemic settings.

Accordingly, currently reported urinary metabolite signatures should be regarded as exploratory and are not yet validated for routine clinical use in malaria diagnosis or management. Prospective longitudinal studies

incorporating paired blood–urine metabolomic profiling are needed to characterize temporal metabolite kinetics and determine their relationships with disease severity, treatment response, and malaria-associated organ dysfunction. As summarized in Table 3, the available evidence also varies substantially in biological source and evidentiary strength, ranging from human urinary studies to CHMI cohorts and *in vitro* infected-erythrocyte experiments [22, 44-46]. The detection of malaria-associated urinary metabolites nevertheless indicates that parasite- and host-associated metabolic processes during infection can generate measurable urinary signals, providing a rationale for investigating urinary immune mediators in malaria.

Table 3: Candidate urinary metabolites associated with falciparum malaria.

Candidate metabolite	Biological significance	Reported direction or association	Analytical platform	Key observations	Evidence type	Major limitations	Reference
N-acetylspermidine; N-acetylputrescine; 1,3-diacetylpropane	Acetylated polyamines/polyamine-related metabolites	Increased during active infection	UHPLC-HRMS	Significantly higher in patients with malaria than in healthy controls; levels decreased following antimalarial treatment	Human urine case–control study	Biological origin and malaria specificity remain uncertain; limited external validation	[22, 44]
Pipecolic acid	Lysine degradation-related metabolite	Increased during active infection	UHPLC-HRMS	Elevated in urine of patients with <i>P. falciparum</i> malaria compared with healthy controls	Human urine case–control study	Biological origin and malaria specificity remain uncertain; limited external validation	[22]
Taurine	Amino acid-related metabolite	Increased during active infection	UHPLC-HRMS	Elevated in urine of patients with <i>P. falciparum</i> malaria compared with healthy controls	Human urine case–control study	Biological origin and malaria specificity remain uncertain; limited external validation	[22]
Alanine; N-acetylglutamine; N-acetylasparagine	Amino acid-related metabolites	Increased during active infection	UHPLC-HRMS	Higher levels were observed in urine of patients with malaria than in healthy controls	Human urine case–control study	Biological significance and malaria specificity remain uncertain; limited external validation	[22]
Succinate	TCA cycle-related metabolite	Increased during active infection	UHPLC-HRMS	Higher urinary levels were observed in patients with malaria than in healthy controls	Human urine case–control study	Biological origin and malaria specificity remain uncertain; limited external validation	[22]
Formate; urea; trimethylamine; threonine; choline; myo-inositol; acetate	Urinary metabolites differing according to post-challenge parasitemia status	Higher in TBS-positive African participants	NMR spectroscopy and HILIC-MS	Significantly higher levels were reported in TBS-positive than in TBS-negative malaria-exposed African participants following <i>P. falciparum</i> sporozoite challenge	CHMI cohort	Small exploratory cohort; biological significance and generalizability remain uncertain	[45]
Pyruvate; 3-methylhistidine; dimethylglycine	Urinary metabolites differing according to post-challenge parasitemia status	Lower in TBS-negative than in TBS-positive African participants	NMR spectroscopy and HILIC-MS	Significantly lower levels were reported in African participants who remained TBS-negative	CHMI cohort	Small exploratory cohort; biological significance and generalizability remain uncertain	[45]
PAG	Baseline urinary metabolite associated with subsequent parasitemia status	Higher baseline concentration in participants who subsequently remained TBS-negative	NMR spectroscopy and HILIC-MS	Higher before challenge among malaria-exposed African participants who subsequently remained TBS-negative	CHMI cohort	Exploratory association from a small cohort; predictive performance and external validity have not been established	[45]

Candidate metabolite	Biological significance	Reported direction or association	Analytical platform	Key observations	Evidence type	Major limitations	Reference
Pipecolic acid; GABA; α -ketoglutarate	Stage-associated extracellular metabolites in <i>P. falciparum</i> -infected erythrocyte cultures	Increased during trophozoite and schizont development	GC-MS and LC-MS/MS	Marked stage-associated increases were observed in infected erythrocyte culture supernatants during trophozoite and schizont development	<i>In vitro</i> infected-erythrocyte study	Experimental rather than human urinary evidence; clinical urinary relevance has not been established	[46]
Homocysteine	Extracellular metabolite altered during intraerythrocytic development	Increased at the schizont stage	GC-MS and LC-MS/MS	Extracellular homocysteine increased at the schizont stage in infected erythrocyte cultures	<i>In vitro</i> infected-erythrocyte study	Experimental rather than human urinary evidence; clinical urinary relevance has not been established	[46]

CHMI = controlled human malaria infection; GABA = gamma-aminobutyric acid; GC-MS = gas chromatography–mass spectrometry; HILIC-MS = hydrophilic interaction liquid chromatography–mass spectrometry; HRMS = high-resolution mass spectrometry; LC-MS/MS = liquid chromatography–tandem mass spectrometry; NMR = nuclear magnetic resonance; PAG = phenylacetylglutamine; TBS = thick blood smear; TCA = tricarboxylic acid; UHPLC-HRMS = ultra-high-performance liquid chromatography–high-resolution mass spectrometry.

URINARY CYTOKINES IN MALARIA: BIOLOGICAL CONSTRAINTS, ANALYTICAL CHALLENGES, AND EMERGING OPPORTUNITIES FOR NON-INVASIVE IMMUNE MONITORING

Blood-stage malaria induces complex inflammatory responses involving cytokines and chemokines that contribute to both parasite control and the pathophysiology of severe disease [27, 47]. However, associations between individual circulating cytokines and malaria severity are heterogeneous, and most cytokines have shown weak or inconsistent relationships across studies [48]. Despite extensive characterization of circulating cytokine responses, urinary immune signals in malaria remain poorly understood. This knowledge gap is clinically relevant because renal involvement is increasingly recognized as an important contributor to adverse outcomes in severe *falciparum* malaria [9].

Under physiological conditions, cytokines are medium-to-large hydrophilic proteins with limited urinary excretion because glomerular filtration is restricted by molecular size and charge selectivity, whereas filtered proteins are efficiently reabsorbed and degraded by proximal tubular cells [32, 33]. In severe malaria, systemic inflammation, endothelial dysfunction, hemolysis-associated renal stress, and microvascular injury may disrupt glomerular and tubular barrier integrity, thereby increasing the likelihood of urinary detection of inflammatory mediators [27, 35]. Urinary cytokines and related immune markers may therefore arise through transglomerular passage of circulating mediators, impaired proximal tubular reabsorption, or local intrarenal production by activated epithelial and endothelial cells [24, 49].

Direct evidence for urinary cytokines and immune-related biomarkers in malaria remains limited, but available studies provide important proof-of-concept observations. In malaria during pregnancy, urinary soluble tumor necrosis factor receptor 1 (sTNFR-1) and soluble tumor necrosis factor receptor 2 (sTNFR-2) were significantly higher in malaria-positive pregnant women than in malaria-negative pregnant women, and both markers correlated strongly with peripheral parasitemia [25]. In that study, urinary sTNFR-2 showed an area under the receiver operating characteristic (ROC) curve of 0.892 (95% confidence interval, 0.787–0.998) for discriminating malaria-positive from malaria-negative pregnant women. ROC curve-derived cutoffs of 9.8 and 13.6 ng/mL yielded sensitivity/specificity values of 82.6%/87.0% and 78.3%/95.7%, respectively [25]. However, these estimates were derived from a small cohort of 46 pregnant women using peripheral blood microscopy as the reference standard, and the proposed thresholds have not been externally validated.

In children with cerebral malaria, urinary bicyclo-prostaglandin E2/creatinine was lower than in children with asymptomatic parasitemia and was positively correlated with interleukin-10 (IL-10) [50]. More recently, paired plasma–urine biomarker profiling in children with cerebral malaria showed that fatal outcomes were associated in univariate analyses with increased plasma inflammatory markers, including tumor necrosis factor, interleukin-1 β (IL-1 β), IL-10, interleukin-8 (IL-8), C-X-C motif chemokine ligand 9, granzyme B, and angiopoietin-2, together with lower urinary prostaglandin E2 metabolite (PGEM) [51]. Among survivors, longitudinal profiling at days 3 and 30 showed increasing urinary 15-F2t-isoprostane concentrations from day 0 to day 30 [51]. Collectively, these studies indicate that urine may capture selected inflammatory and oxidative-response signals during malaria. However, the strongest urinary evidence currently concerns soluble receptors and lipid-derived immune

mediators rather than canonical urinary cytokines such as interleukin-6 (IL-6), IL-8, tumor necrosis factor-alpha (TNF- α), or IL-10. Representative findings are summarized in Table 4 [25, 50, 51].

Several biological and analytical factors may account for the limited and inconsistent evidence. Urinary cytokines are often present at low concentrations and are influenced by biological variability, pre-analytical handling, urine dilution, and matrix effects that may reduce assay sensitivity and reproducibility [52]. These challenges may be particularly relevant in malaria because hemolysis-associated products, including free hemoglobin, free heme, bilirubin-related compounds, oxidative metabolites, and parasite-derived hemozoin, may interfere with optical or antibody-based assays, alter protein stability, or contribute to nonspecific inflammatory signals. Urine dilution, oliguria, dehydration, acidic pH, bacterial contamination, and delayed processing may further reduce comparability among studies.

Systemic cytokine studies also demonstrate substantial heterogeneity across populations, age groups, clinical phenotypes, and malaria-endemic settings, indicating that individual cytokines are unlikely to function as reliable standalone biomarkers without standardized sampling, paired plasma–urine profiling, and external validation [29, 48, 53]. Furthermore, it remains unclear whether canonical cytokines such as IL-6, IL-8, TNF- α , and IL-10 are consistently detectable in urine or are reliably associated with disease severity and renal involvement in falciparum malaria. Further studies incorporating paired plasma–urine profiling, longitudinal sampling, and standardized normalization strategies are therefore needed to clarify the diagnostic and prognostic utility of urinary cytokines and related immune markers.

Importantly, reported associations between selected urinary immune markers and parasitemia, disease severity, or clinical outcomes should not be interpreted as evidence of established diagnostic or prognostic utility. As summarized in Table 4, the available evidence is based on a small number of heterogeneous studies involving distinct populations, biomarker panels, and clinical outcomes [25, 50, 51]. Thus, urinary inflammatory and immune-related mediators should currently be regarded as investigational biomarkers rather than clinically validated tools for malaria assessment, while their potential as non-invasive indicators of inflammation, oxidative stress, and organ involvement warrants further investigation.

Table 4: Representative urinary inflammatory and immune-related biomarker findings in falciparum malaria

Study	Population and clinical context	Specimen	Biomarkers measured	Key findings	Correlation or clinical outcome	Interpretation and current relevance
Tassi Yunga <i>et al.</i> [25]	Pregnant and nonpregnant Cameroonian women with and without malaria	Urine	sTNFR-1, sTNFR-2, HRP2 comparator	Urinary sTNFR-1 and sTNFR-2 were significantly higher in malaria-positive pregnant women than in malaria-negative pregnant women	Urinary sTNFR-1 and sTNFR-2 correlated strongly with parasitemia. Among 46 pregnant women, urinary sTNFR-2 showed an AUC of 0.892 (95% CI, 0.787–0.998); ROC-derived cutoffs of 9.8 and 13.6 ng/mL yielded sensitivity/specificity values of 82.6%/87.0% and 78.3%/95.7%, respectively	Provides direct evidence that urinary soluble TNF receptors are associated with malaria and parasitemia during pregnancy; however, diagnostic performance was derived from a small pregnancy-specific cohort, and the proposed thresholds have not been externally validated
Perkins <i>et al.</i> [50]	Tanzanian children with asymptomatic parasitemia, noncerebral malaria, cerebral malaria with complete recovery, and cerebral malaria with death or neurological sequelae	Urine and plasma	Urinary bicyclo-PGE2/creatinine; plasma TNF- α and IL-10	Children with asymptomatic parasitemia had the highest urinary bicyclo-PGE2/creatinine, whereas children with cerebral malaria had significantly lower urinary bicyclo-PGE2/creatinine	Urinary bicyclo-PGE2/creatinine was positively correlated with IL-10 but was not significantly associated with parasitemia, hemoglobin, or TNF- α	Provides evidence that altered urinary PGE2-related metabolite levels are associated with cerebral malaria phenotype and host immune responses; clinical diagnostic or prognostic utility has not been established
Royo <i>et al.</i> [51]	Children aged 2–6 years with cerebral malaria in Benin (n = 70; 50 survivors and 20 deaths)	Plasma and urine	17 plasma inflammatory/oxidative biomarkers and 7 urinary biomarkers	Fatal cerebral malaria was associated in univariate analysis with higher plasma TNF, IL-1 β , IL-10, IL-8, CXCL9, granzyme B, and angiopoietin-2, together with lower urinary PGEM	Elevated plasma IL-8 was the main independent risk factor for death identified in multivariable analysis. Among survivors, urinary 15-F2t-isoprostane increased from day 0 to day 30 during longitudinal follow-up	Provides paired plasma–urine evidence of inflammatory and oxidative changes associated with mortality and recovery but does not establish urinary biomarkers as validated outcome-monitoring tools

AUC = area under the receiver operating characteristic curve; CI = confidence interval; CXCL9 = C-X-C motif chemokine ligand 9; HRP2 = histidine-rich protein 2; IL = interleukin; PGE2 = prostaglandin E2; PGEM = prostaglandin E2 metabolite; ROC = receiver operating characteristic; sTNFR = soluble tumor necrosis factor receptor; TNF- α = tumor necrosis factor-alpha.

HRP2 IN URINE: DIAGNOSTIC POTENTIAL AND CHALLENGES

HRP2 is a water-soluble antigen released by *P. falciparum* during asexual blood-stage replication and early gametocytogenesis and remains one of the most widely used biomarkers for malaria diagnosis [10, 54]. In blood, HRP2-based RDTs are widely used because of their high sensitivity, operational simplicity, thermal stability, and reduced dependence on microscopy expertise [1, 12, 55]. Interest in urine-based HRP2 detection has increased because urine provides a non-invasive and repeatable sampling matrix that may be advantageous in settings where blood collection is difficult or impractical [10, 19, 56].

Urinary HRP2 is generally regarded as a transrenal biomarker whose detectability is influenced by circulating antigen burden, parasite biomass, and renal filtration processes [19]. Plasma HRP2 concentrations correlate with total parasite biomass, including sequestered parasites that are not represented by peripheral blood parasite counts [13, 57]. Because mature sequestered parasites are absent from the peripheral circulation, plasma HRP2 may better reflect total parasite biomass and disease severity than peripheral parasitemia alone [57]. In severe malaria, endothelial dysfunction, microvascular injury, hemolysis-associated renal stress, and altered glomerular permeability may facilitate the urinary appearance of circulating HRP2 or its degradation products [27, 35]. Experimental studies have further demonstrated that HRP2 is actively secreted from infected erythrocytes and can persist extracellularly in the host circulation, supporting its detectability in peripheral biological fluids [58].

Clinical studies have reported variable diagnostic performance of urine-based HRP2 assays across different populations and study settings [18-20, 56]. In a comparative study, urine HRP2 testing showed lower diagnostic performance than paired blood HRP2 testing [19]. In a Nigerian field evaluation of the urine malaria test (UMT), overall sensitivity was 83.75%; however, sensitivity decreased to 50% at parasite densities ≤ 200 parasites/ μL and increased to 89.71% at densities ≥ 201 parasites/ μL [56]. Another Nigerian study reported a sensitivity of 65.0% and specificity of 90.7% for the UMT [18], whereas a Ghanaian evaluation of a modified HRP2/pLDH RDT applied to urine reported an overall sensitivity of 35.2% and specificity of 100% [16]. These findings indicate that urine-based HRP2 detection is strongly influenced by parasite density, assay characteristics, and study population. The diagnostic performance and methodological characteristics of the principal urine-based antigen and molecular detection studies are summarized in Table 5 [16, 18–21, 56, 59].

Table 5: Diagnostic performance and methodological characteristics of urine-based antigen and molecular detection approaches for falciparum malaria.

Study	Population/sample size	Urine-based approach/target	Reference standard	Assay/threshold	Diagnostic performance	Key limitations
Oguonu <i>et al.</i> [56]	Nigeria; febrile patients, 203 enrolled (195 analyzed)	UMT; HRP2	Blood smear microscopy	UMT; qualitative urine HRP2 detection; LLD 120 parasites/ μL	Sensitivity = 83.75%; specificity = 83.48%; PPV = 77.91%; NPV = 88.07%. Sensitivity = 50% at ≤ 200 parasites/ μL and 89.71% at ≥ 201 parasites/ μL	Reduced sensitivity at low parasite densities; further improvement in sensitivity required before field deployment
Oyibo <i>et al.</i> [20]	Nigeria; febrile participants, n = 566 (from 1,691 enrolled participants)	UMT; HRP2	Blood smear microscopy	UMT; qualitative urine HRP2 detection	Sensitivity = 85% (95% CI = 79–89); specificity = 84% (95% CI = 80–88); pooled AUC = 0.84 (95% CI = 0.82–0.87)	Sensitivity varied with fever status and parasite density, with lower sensitivity at lower parasite densities
Samal <i>et al.</i> [19]	India; febrile patients, n = 111 (60 smear-positive <i>P. falciparum</i> cases and 51 smear-negative controls)	Urine HRP2-based RDT	Blood smear microscopy	HRP2-based RDT applied to urine	Urine RDT: sensitivity = 86.67% (95% CI = 75.83–93.09); specificity = 94.12% (95% CI = 84.08–97.98). Paired blood RDT: sensitivity = 91.67% (95% CI = 81.93–96.39); specificity = 98.04% (95% CI = 89.70–99.65)	Reduced sensitivity at low parasite density; all five cases with <200 parasites/ μL were negative by urine RDT
Godwin <i>et al.</i> [18]	Nigeria; febrile patients, n = 199	UMT; HRP2	Giemsa-stained blood smear microscopy	UMT; reported detection limit corresponding to 125 parasites/ μL of blood	Sensitivity = 65.0%; specificity = 90.7%; PPV = 93.5%; NPV = 55.6%; accuracy = 73.36%; AUROC not reported	Moderate overall sensitivity and poor detection at low parasite density; no detection at ≤ 100 parasites/ μL in this cohort

Study	Population/sample size	Urine-based approach/target	Reference standard	Assay/threshold	Diagnostic performance	Key limitations
Aninagyei <i>et al.</i> [16]	Ghana; patients with suspected malaria, n = 706 with complete blood, urine, and saliva specimens	Modified HRP2/pLDH RDT using urine	Corresponding blood HRP2/pLDH RDT results	SD Bioline HRP2/pLDH RDT; 30 µL urine	Overall sensitivity = 35.2% (95% CI = 30.38–40.20); specificity = 100% (95% CI = 98.87–100); sensitivity decreased to 7.6% in urine without blood contamination; AUC not reported	Detection varied with hematuria and parasite density; antigenuria was not detected below 60,000 parasites/µL
Castro-Sesquen <i>et al.</i> [59]	Experimental proof-of-concept using HRP2-spiked human urine; no clinical diagnostic cohort	MB-QD assay; HRP2	Not applicable	MB-QD HRP2 assay; analytical LOD = 0.5 ng/mL in urine; evaluated range = 33–2,000 ng/mL	Clinical sensitivity, specificity, and AUC were not evaluated	Experimental analytical proof-of-concept; clinical diagnostic performance and external validation have not been established
Mharakurwa <i>et al.</i> [21]	Malaria-infected individuals; 47 thick-film-positive participants and 4 negative controls; proof-of-concept molecular study	Urine-based molecular detection; <i>P. falciparum</i> DNA	Blood-based parasite detection/molecular assessment	PCR-based detection of <i>P. falciparum</i> DNA in urine; no formal diagnostic threshold established	Demonstrated feasibility of detecting <i>P. falciparum</i> DNA in urine; formal diagnostic accuracy metrics and thresholds were not established	Variable amplification success depending on DNA extraction method, parasite density, and primer set; DNA isolation techniques and amplicon yields require further optimization

AUC = area under the receiver operating characteristic curve; AUROC = area under the receiver operating characteristic curve; CI = confidence interval; DNA = deoxyribonucleic acid; HRP2 = histidine-rich protein 2; LLD = lower limit of detection; LOD = limit of detection; MB-QD = magnetic bead–quantum dot; NPV = negative predictive value; PCR = polymerase chain reaction; pLDH = *Plasmodium* lactate dehydrogenase; PPV = positive predictive value; RDT = rapid diagnostic test; UMT = Urine Malaria Test.

Head-to-head comparisons of blood- and urine-based HRP2 assays further demonstrate clinically relevant discordance. Urine-based assays generally show lower sensitivity than paired blood HRP2 testing, particularly in low-density infections [19, 56]. Conversely, HRP2 may remain detectable after parasite clearance because of antigen persistence, similar to that observed with blood-based HRP2 assays [56]. Such persistence may limit the usefulness of urinary HRP2 for test-of-cure applications and complicate interpretation following recent antimalarial treatment.

An additional challenge is the increasing prevalence of *pfhrp2* and *pfhrp3* gene deletions in some malaria-endemic regions. Parasites lacking these genes may evade detection by HRP2-based assays, resulting in false-negative findings despite active *P. falciparum* infection [14, 15, 55]. The World Health Organization recommends consideration of alternative non-HRP2-based diagnostic strategies when the prevalence of deletion-associated false-negative HRP2 results exceeds 5% within a region [55]. Recent surveillance studies have demonstrated marked geographic variation in deletion prevalence. In Tanzania, *pfhrp2* deletions accounted for only 0.24% of false-negative HRP2-RDT results, supporting continued use of HRP2-based diagnostics in that setting [15]. In contrast, a 2025 Ethiopian study reported a substantially higher regional prevalence of *pfhrp2/pfhrp3* deletions, with most discordant HRP2-negative but microscopy-positive infections attributable to gene deletions [14]. Although this evidence is derived primarily from blood-based HRP2 testing, it has important implications for urine-based HRP2 assays because urinary detection also depends on parasite HRP2 expression.

Biological and analytical factors may further influence urinary HRP2 performance. Formation of endogenous HRP2–antibody immune complexes has been demonstrated in patients with symptomatic malaria and may reduce freely detectable antigen concentrations in some individuals [60]. Environmental temperature, urine concentration, hematuria, renal dysfunction, and assay sensitivity may also contribute to variability among studies and inconsistent diagnostic performance [56, 60]. Emerging nanoparticle-enhanced assays have demonstrated improved analytical sensitivity for urinary HRP2 detection. A magnetic bead–quantum dot assay achieved an analytical limit of detection of 0.5 ng/mL in HRP2-spiked human urine under experimental conditions [59]. However, this approach remains an analytical proof-of-concept, and its clinical diagnostic performance has not been established [60].

Overall, urinary HRP2 represents a promising adjunctive approach for non-invasive malaria detection, but its diagnostic performance remains strongly influenced by parasite density, renal and urinary factors, assay characteristics, and parasite genetic variation affecting HRP2 expression [15, 19, 56]. As summarized in Table 5,

available studies demonstrate substantial heterogeneity in sensitivity, specificity, reference standards, analytical thresholds, and study populations [16, 18-21, 56, 59]. In a predominantly pediatric cohort from a malaria-endemic setting, the urine-based HRP2 assay showed potential as a non-invasive diagnostic approach, although sensitivity improvements were needed before broader field deployment [56]. Given the lower and more variable sensitivity of urine-based HRP2 testing compared with blood-based HRP2 testing in direct comparative evaluations, urinary HRP2 assays should currently be considered complementary rather than replacement diagnostic approaches [19].

CHEMICAL SCREENING OF URINE BY DIPSTICK: INDICATORS OF HEMOLYSIS AND DISEASE SEVERITY IN FALCIPARUM MALARIA

Urine dipstick abnormalities are common in malaria, particularly in *P. falciparum* infection, and primarily reflect downstream host injury rather than the direct presence of parasites [61, 62]. Unlike microscopy, RDTs, or molecular assays, which establish parasite infection, dipstick urinalysis provides a rapid and inexpensive assessment of malaria-associated hemolytic, renal, and hepatobiliary abnormalities [61, 62]. This distinction is clinically important because dipstick abnormalities predominantly reflect host tissue injury and systemic complications rather than parasite burden alone [27]. Routine urine chemistry may therefore provide a practical adjunct for recognizing malaria-associated complications, particularly in resource-limited settings where access to advanced biochemical testing is limited [63].

Among dipstick abnormalities, heme-positive reactions appear particularly informative. Destruction of both parasitized and non-parasitized erythrocytes during falciparum malaria releases cell-free hemoglobin and free heme, which contribute to oxidative stress and endothelial injury [35]. Excess circulating hemoglobin may subsequently appear in urine following substantial intravascular hemolysis, resulting in hemoglobinuria and heme-positive dipstick findings [61, 62]. Consequently, dipstick “blood” positivity in malaria may reflect hemoglobinuria rather than true hematuria because the reagent pad detects the pseudoperoxidase activity of heme pigments [64]. Reported frequencies of heme-positive dipstick findings are approximately 30%–40%, with higher frequencies observed in *P. falciparum* infection [61, 62]. In one study of adults with falciparum malaria, dipstick-detected urinary blood that was microscopically confirmed at ≥ 25 RBCs/ μ L showed a sensitivity of 94.1% and specificity of 90.8% for renal impairment [63]. However, these findings originated from a single study and should not be interpreted as establishing heme-positive dipstick findings as a validated screening marker for malaria-associated renal injury.

Proteinuria is also frequently observed in falciparum malaria but appears less specific for renal involvement than heme-positive urinary findings. In children with *P. falciparum* infection, proteinuria was detected significantly more often than in healthy controls (81.0% vs. 19.0%, $p < 0.001$), whereas its correlation with parasite density was weak and non-significant ($r = 0.155$, $p = 0.247$) [62]. This pattern suggests that proteinuria may reflect host renal and endothelial responses to infection rather than parasite burden itself. Increased urinary protein excretion may result from transient disruption of glomerular barrier integrity associated with endothelial activation, inflammatory injury, and oxidative stress [32, 35]. In one adult cohort, proteinuria showed lower specificity for renal impairment than hematuria, limiting its utility as an isolated urinary marker [63]. Nevertheless, proteinuria occurring with other dipstick abnormalities may contribute to recognition of malaria-associated renal involvement. In malaria-associated AKI, proteinuria may reflect combined glomerular and tubular injury, as experimental models have demonstrated increased albuminuria, proximal tubular epithelial injury, and glomerular capillary tuft collapse during infection [65].

Markers of bilirubin metabolism provide complementary information regarding hemolysis and hepatobiliary involvement during falciparum malaria and may be reflected by bilirubin-related dipstick abnormalities [27, 62]. Bilirubinuria may result from increased hemoglobin breakdown together with hepatocellular dysfunction or cholestatic disturbance, permitting conjugated bilirubin to appear in urine [27]. Among routinely reported dipstick abnormalities, bilirubinuria has demonstrated one of the stronger associations with parasite density and clinical severity [62]. In children with *P. falciparum* infection, bilirubinuria showed a moderate positive correlation with parasite density ($r = 0.438$, $p = 0.001$), whereas proteinuria and hematuria showed weaker and non-significant correlations [62]. In that study of 58 children with falciparum malaria, the mean parasite density was $110,912.21 \pm 18,146.60$ parasites/ μ L, and 50 children (86.2%) were classified by the investigators as having severe infection based on a parasite density $> 7,000$ parasites/ μ L [62]. Although this significant correlation suggests that increasing parasite burden may contribute to bilirubinuria, its moderate magnitude indicates that parasite density alone does not explain the observed variation. Bilirubinuria in falciparum malaria is therefore more likely to reflect the

combined effects of parasite burden, intravascular hemolysis, and hepatobiliary dysfunction than to serve as a direct surrogate for parasitemia.

Similarly, bilirubinuria and urobilinogenuria have been reported more frequently in patients with higher parasitemia [61]. These findings suggest that bilirubin-related urinary abnormalities may provide simple indicators of hemolytic and hepatobiliary stress during falciparum malaria. However, associations between individual dipstick abnormalities, parasite density, and clinical severity are inconsistent and should be interpreted cautiously [61, 62].

Dark urine has also been associated with renal and hepatic dysfunction in malaria [66]. Interpretation of dipstick abnormalities nevertheless requires careful clinical context because coexisting conditions common in malaria-endemic settings, including bacterial urinary tract infection, dehydration, schistosomiasis, CKD, and pregnancy-associated urinary abnormalities, may independently contribute to hematuria, leukocyturia, or proteinuria and thereby reduce specificity for malaria-associated organ injury. Evidence regarding dipstick performance in adults, pregnancy-associated malaria, mixed *Plasmodium* infections, and malaria with bacterial co-infection also remains limited.

Overall, among routinely reported dipstick abnormalities, heme-positive findings appear most closely associated with intravascular hemolysis and renal stress, whereas proteinuria may reflect glomerular barrier injury and bilirubin-related abnormalities may indicate combined hemolytic and hepatobiliary involvement. Although these abnormalities are insufficiently specific for parasitological diagnosis, dipstick urinalysis may still provide a rapid and inexpensive adjunct for identifying abnormalities associated with severe disease, particularly in malaria-endemic and resource-limited settings [61-63]. Reported dipstick abnormalities and their associations with parasite density and clinical outcomes in falciparum malaria are summarized in Table 6 [61-63, 66].

Table 6: Prevalence and clinical relevance of urine dipstick abnormalities reported in falciparum malaria.

Study	Country/ population	Dipstick abnormality	Reported prevalence/ frequency	Association with parasite density or clinical outcome	Predictive performance	Key interpretation/limitations
Pati and Mishra [63]	India; 77 hospitalized adults with smear-positive <i>P. falciparum</i> malaria	Hematuria (dipstick-detected and microscopically confirmed)	23/77 patients (29.8%)	Positively correlated with plasma urea ($r = 0.56$, $p < 0.001$) and creatinine ($r = 0.46$, $p < 0.01$); evaluated for detection of renal impairment defined as plasma creatinine ≥ 1.2 mg/dL	Sensitivity = 94.1%; specificity = 90.8% at ≥ 25 RBCs/ μ L for detecting renal impairment	Demonstrated high sensitivity and specificity for detecting renal impairment in this small, single-center hospitalized adult cohort; one patient with leukocytes and nitrites was excluded from the diagnostic analysis; external validation was not reported
Pati and Mishra [63]	India; 77 hospitalized adults with smear-positive <i>P. falciparum</i> malaria	Proteinuria ≥ 300 mg/L	52/77 patients (67.5%)	Not significantly different between patients with and without renal impairment and not correlated with plasma urea or creatinine	Sensitivity = 88.2%; specificity = 62.7% for detecting renal impairment	High sensitivity but limited specificity for renal impairment in this cohort; proteinuria was not significantly associated with renal impairment
Ephraim et al. [62]	Ghana; 58 children aged ≤ 12 years with confirmed <i>P. falciparum</i> malaria and 58 age- and sex-matched healthy controls	Proteinuria	81.0% in infected children vs. 19.0% in controls	Weak, non-significant positive correlation with parasite density ($r = 0.155$, $p = 0.247$)	Not reported	Proteinuria was significantly more frequent in malaria cases than in controls, but its correlation with parasite density was weak and non-significant; renal impairment was not directly assessed
Ephraim et al. [62]	Ghana; children with <i>P. falciparum</i> infection	Bilirubinuria	84.5% in malaria cases vs. 36.8% in controls ($p < 0.001$)	Moderate positive correlation with parasite density ($r = 0.438$, $p = 0.001$); the only evaluated urinary abnormality showing a statistically significant correlation with parasite density	Not reported	May be associated with hepatobiliary dysfunction or hemolysis, but the underlying mechanism was not directly assessed; plasma bilirubin was not measured. Small sample size and short study duration limit interpretation

Study	Country/ population	Dipstick abnormality	Reported prevalence/ frequency	Association with parasite density or clinical outcome	Predictive performance	Key interpretation/limitations
Ugwuja and Ugwu [61]	Nigeria; 365 malaria-positive outpatients, including 74 (20.3%) with <i>P. falciparum</i> , and 81 healthy controls	Heme-positive dipstick/blood	Urinary blood abnormalities were reported in patients with malaria; the study did not provide a separate exact prevalence for heme-positive findings specifically in the <i>P. falciparum</i> subgroup	No significant increase with higher <i>P. falciparum</i> parasitemia was reported	Not reported	Heme-positive findings were more frequent in <i>P. falciparum</i> than in other malaria species; renal function was not assessed, limiting interpretation of their relationship with renal impairment
Ugwuja and Ugwu [61]	Nigeria; malaria outpatients	Bilirubinuria/urobilinogenuria	Significantly more frequent in patients with malaria than in controls and in <i>P. falciparum</i> than in other malaria species ($p < 0.05$)	Significantly more frequent at higher <i>P. falciparum</i> parasitemia ($p < 0.05$)	Not reported	May be associated with hepatobiliary dysfunction or hemolysis, but these mechanisms were not directly assessed; plasma bilirubin and liver enzymes were not measured, and clinical utility requires confirmation
Tobón-Castaño <i>et al.</i> [66]	Colombia; 620 patients with malaria, predominantly <i>P. falciparum</i> and <i>Plasmodium vivax</i> infections	Urobilinuria	184/602 patients (30.6%)	Associated with thrombocytopenia (OR = 2.6), neurological complications (OR = 7.3), and hepatic complications (OR = 2.7)	Not reported	Associated with thrombocytopenia and neurological and hepatic complications; findings were derived from a mixed-species malaria cohort
Tobón-Castaño <i>et al.</i> [66]	Colombia; 620 patients with <i>P. vivax</i> or <i>P. falciparum</i> malaria	Ketonuria	48/559 patients (8.6%)	Associated with severe neurological dysfunction (OR = 1.1, 95% CI = 1.0–1.2)	Not reported	Interpretation is limited by the small number of patients with neurological complications and available glucose/ketone measurements

CI = confidence interval; OR = odds ratio; RBC = red blood cell.

URINARY BIOMARKERS OF RENAL INJURY IN MALARIA: FOCUS ON NGAL AND KIM-1

Malaria-associated AKI is increasingly recognized as an important complication of severe *P. falciparum* infection and contributes substantially to malaria-related morbidity and mortality [6]. Because structural tubular injury may precede overt reductions in glomerular filtration, conventional functional markers such as serum creatinine may underestimate early renal damage [24]. This limitation has prompted increasing interest in urinary biomarkers that more directly reflect renal tubular epithelial injury. Among these, NGAL and KIM-1 are among the most extensively investigated and biologically informative biomarkers of structural tubular injury [24].

NGAL

NGAL, also known as lipocalin-2 (LCN2), is one of the most extensively investigated early biomarkers of acute tubular injury and is particularly relevant to malaria-associated AKI, in which conventional functional markers may underestimate evolving renal damage [24, 67]. NGAL is a low-molecular-weight (~25 kDa) iron-binding glycoprotein produced by activated neutrophils and several epithelial tissues and is markedly upregulated in renal tubular cells following ischemic, toxic, or inflammatory injury [68]. Once induced, NGAL is rapidly released into plasma and urine, frequently preceding measurable increases in serum creatinine by several hours and thereby providing a potential window for earlier recognition of kidney injury [24, 67]. In nonmalarial AKI cohorts, urinary NGAL may increase within approximately 2–6 h after tubular injury, substantially earlier than creatinine-based evidence of functional decline [67, 68]. Although malaria-specific temporal kinetics remain incompletely characterized, available malaria evidence similarly suggests that NGAL elevation may precede overt creatinine-defined AKI in some patients [39]. This potential temporal advantage is particularly relevant in severe malaria, in which AKI may develop rapidly and delayed recognition is associated with increased mortality [6].

Renal injury in *P. falciparum* infection is multifactorial and reflects the combined effects of intravascular hemolysis, sequestration-related microvascular dysfunction, endothelial activation, oxidative heme toxicity, volume depletion, and dysregulated host inflammation [6]. These processes may particularly affect the tubular compartment, where ischemic and hemolytic-inflammatory stress can induce NGAL expression before overt

reductions in glomerular filtration become clinically apparent [6, 68]. Consequently, NGAL may identify subclinical tubular stress earlier than conventional creatinine-based markers [67]. More recent evidence from adults and children with severe falciparum malaria further showed that elevated plasma NGAL concentrations were strongly associated with AKI severity and magnetic resonance imaging (MRI)-defined cytotoxic brain edema, supporting an association between NGAL and broader organ injury pathways in severe malaria [69].

Urinary NGAL may provide greater anatomical specificity for renal injury than circulating NGAL because it reflects both intrarenal production and impaired proximal tubular reabsorption following epithelial injury [24, 67]. In contrast, plasma NGAL concentrations may increase during systemic infection through release from activated neutrophils and extrarenal tissues independently of intrinsic kidney injury [24]. This distinction is particularly relevant in severe malaria, in which intense systemic inflammation may amplify circulating NGAL concentrations and thereby reduce the renal specificity of plasma measurements [6, 24].

Available clinical evidence is consistent with this framework. In adults with imported falciparum malaria, urinary NGAL measured at presentation using a commercial enzyme-linked immunosorbent assay (ELISA) showed an AUROC of 1.00 for the presence or subsequent development of AKI, with a data-derived optimal cutoff of 308 ng/mL in a pilot cohort [39]. In the same study, serum NGAL also showed strong diagnostic performance, with an AUROC of 0.98 at a cutoff of approximately 168 ng/mL, although urinary NGAL appeared to provide greater renal specificity and a higher positive predictive value (PPV) than circulating measurements [39]. Several patients also had elevated urinary NGAL despite preserved serum creatinine concentrations, suggesting subclinical tubular injury that was not captured by serum creatinine [39].

The potential clinical relevance of this biomarker-positive, creatinine-negative phenotype is supported by evidence from broader AKI populations, in which NGAL-positive patients without diagnostic increases in serum creatinine had higher rates of renal replacement therapy and mortality [70]. However, whether this phenotype has comparable prognostic significance in *P. falciparum* malaria remains unknown. The reported diagnostic performance should also be interpreted cautiously. The study was a pilot investigation involving 39 travelers with imported falciparum malaria, only six of whom developed AKI [39]. AKI was defined according to Kidney Disease: Improving Global Outcomes (KDIGO) criteria; when measured baseline serum creatinine was unavailable, baseline renal function was estimated assuming normal premorbid renal function [39]. Biomarkers were measured in samples obtained at initial presentation, and optimal cutoffs were derived from the study dataset using the Youden index rather than being prespecified [39].

The restricted clinical spectrum and small number of AKI events in this imported malaria cohort may have contributed to the apparently high discriminatory performance, particularly the AUROC of 1.00 reported for urinary NGAL. Travelers with imported malaria may present earlier during infection than patients with severe malaria in endemic settings, potentially limiting the generalizability of these biomarker estimates to other clinical populations [39]. The small number of AKI events also limited statistical power, and urinary NGAL did not show a statistically significant improvement in AUROC compared with serum creatinine [39]. Moreover, the study lacked external validation; therefore, the reported performance estimates and thresholds require confirmation in larger and epidemiologically distinct populations, particularly adult and pediatric cohorts from malaria-endemic regions.

The non-invasive, repeatable nature of urine sampling may be advantageous in children, critically ill patients, and resource-limited settings where repeated phlebotomy is impractical. However, longitudinal studies are needed to establish the utility of serial urinary NGAL measurements for monitoring renal recovery or persistent tubular injury after antimalarial treatment.

Several limitations should nevertheless be considered. Although highly sensitive, NGAL is not kidney-specific and may increase during systemic inflammation even in the absence of intrinsic renal injury [67]. Urinary concentrations may also be influenced by oliguria and urine dilution, and clinically validated thresholds for malaria-associated AKI remain insufficiently standardized across populations and assay platforms [52, 68]. Additional potential confounders in malaria-endemic settings include age-related physiological variation, malnutrition, systemic bacterial co-infection, neutrophil activation, and severe intravascular hemolysis, all of which may independently influence NGAL concentrations. Plasma NGAL may be particularly susceptible to amplification by systemic inflammation, whereas urinary NGAL appears to reflect tubular epithelial stress more directly [24]. Evidence regarding sex-specific NGAL performance and pregnancy-associated malaria also remains limited. Accordingly, NGAL is best interpreted as an early indicator of tubular epithelial stress rather than as an isolated diagnostic marker of malaria-associated AKI.

Evidence from endemic pediatric populations is also available for circulating NGAL. In a prospective Ugandan

cohort of children with severe malaria, plasma NGAL measured using a Luminex-based assay was evaluated using an established high-risk threshold of ≥ 300 ng/mL and was associated with unresolved AKI at 24 h and in-hospital mortality [71]. High-risk plasma NGAL was also associated with acute kidney disease (AKD) at 1-month follow-up [71]. However, these findings relate to plasma rather than urinary NGAL and therefore do not establish the diagnostic or prognostic performance of urinary NGAL in endemic pediatric populations.

KIM-1

KIM-1, also known as T-cell immunoglobulin and mucin domain-containing protein-1 (TIM-1; HAVCR1), is a type I transmembrane glycoprotein that is minimally expressed in healthy kidneys but undergoes marked upregulation following proximal tubular injury [72, 73]. Under physiological conditions, renal KIM-1 expression is negligible; however, ischemic, toxic, or inflammatory injury induces marked expression in dedifferentiated proximal tubular epithelial cells, making KIM-1 one of the most anatomically specific biomarkers of structural tubular injury currently available [24]. Unlike serum creatinine, which primarily reflects reduced glomerular filtration, KIM-1 directly reflects epithelial injury within the proximal nephron. Its utility as a urinary biomarker largely stems from ectodomain shedding, in which the extracellular domain of KIM-1 is cleaved and released into the tubular lumen after epithelial injury, enabling noninvasive urinary detection as a soluble fragment [24, 74]. Urinary KIM-1 therefore more directly reflects proximal tubular epithelial injury than alterations in glomerular permeability, distinguishing it conceptually from proteinuria and other nonspecific urinary abnormalities [24, 74].

This distinction is particularly relevant in malaria-associated AKI, in which structural tubular damage is increasingly recognized as an important pathological feature [6, 75]. Renal injury in severe falciparum malaria is multifactorial and may result from intravascular hemolysis, sequestration-related microvascular obstruction, endothelial dysfunction, oxidative heme toxicity, hypovolemia, and dysregulated inflammatory responses [6]. These processes promote tubular hypoxia, oxidative stress, and epithelial cell death, particularly within metabolically vulnerable proximal tubular segments [6]. In this context, KIM-1 reflects direct tubular epithelial injury rather than secondary functional decline alone [24, 74].

Malaria-specific evidence further supports the role of KIM-1 as a marker of tubular epithelial injury in malaria-associated AKI. A histopathological study demonstrated significantly increased renal KIM-1 expression in patients with falciparum malaria-associated AKI compared with patients with malaria without AKI and nonmalarial controls. KIM-1 expression was also correlated with serum creatinine, blood urea nitrogen (BUN), and the severity of proximal tubular necrosis [75]. These findings support the concept that tubular epithelial injury represents a major component of malaria-associated nephropathy [6, 75].

Clinical biomarker studies suggest that urinary KIM-1 has moderate diagnostic performance for AKI detection, although its overall discriminatory performance appears lower than that of urinary NGAL in the currently available malaria cohort [39]. In the same pilot cohort of adults with imported falciparum malaria, urinary KIM-1 measured at presentation using a commercial ELISA showed an AUROC of 0.87 (95% CI = 0.75–0.99) for the presence or subsequent development of AKI, with a data-derived optimal cutoff of 1.83 ng/mL [39]. However, only one of the six patients who developed AKI had a urinary KIM-1 concentration above the reported upper limit of normal (5.33 ng/mL), underscoring the uncertainty around clinically applicable thresholds in malaria-associated AKI. Moreover, although the data-derived cutoff of 1.83 ng/mL yielded high sensitivity, specificity was 0.72 and the PPV was only 0.40 [39].

As with urinary NGAL, these estimates were derived from only six AKI events in a small imported malaria cohort and have not undergone external validation. The reported performance characteristics should therefore be interpreted cautiously and require confirmation in larger and epidemiologically distinct malaria populations [39]. Compared with *P. falciparum* malaria without AKI, *P. falciparum* malaria with AKI showed substantially greater renal tubular KIM-1 expression, supporting an association between tissue KIM-1 expression and the severity of structural tubular injury [75]. However, studies directly comparing urinary KIM-1 concentrations between uncomplicated and severe malaria remain limited, and standardized malaria-specific thresholds have not been established.

The potential value of urinary KIM-1 lies in its association with structural proximal tubular injury rather than delayed functional decline alone. Urinary KIM-1 may therefore provide complementary information on proximal tubular damage when interpreted alongside conventional renal indices and other structural injury biomarkers such as NGAL. Although NGAL and KIM-1 are currently the most extensively investigated structural biomarkers in malaria-associated AKI, several additional tubular injury biomarkers have emerged from the broader AKI

literature. These include tissue inhibitor of metalloproteinases-2 (TIMP-2), insulin-like growth factor-binding protein 7 (IGFBP7), liver-type fatty acid-binding protein (L-FABP), and interleukin-18 (IL-18), which reflect complementary mechanisms including cell-cycle arrest, oxidative stress, and inflammatory tubular injury [24]. However, direct malaria-specific clinical evidence for these biomarkers remains limited, whereas the available biological and clinical evidence in falciparum malaria is substantially stronger for NGAL and KIM-1 [6, 39]. Accordingly, NGAL and KIM-1 remain the best-characterized urinary biomarkers of structural renal injury in malaria-associated AKI.

In contrast to their potential role in early detection of renal injury, the long-term prognostic significance of early tubular injury biomarker elevation during malaria-associated AKI remains unresolved. Persistent tubular injury and maladaptive repair following severe AKI may contribute to CKD progression, interstitial fibrosis, and tubular atrophy [76, 77]. Experimental malaria-associated AKI models have additionally demonstrated sustained proximal tubular injury responses, increased KIM-1 expression, albuminuria, and glomerular structural abnormalities during acute infection [65]. However, longitudinal human studies evaluating whether early urinary NGAL or KIM-1 elevation predicts CKD progression, incomplete renal recovery, or persistent tubular dysfunction after malaria-associated AKI remain lacking.

Longitudinal studies nevertheless demonstrate that malaria-associated AKI may have important post-discharge consequences. In Ugandan children with severe malaria, AKI was associated with CKD at 1 year and post-discharge mortality, whereas AKD was reported in 15.6% of children at 1-month follow-up [71, 78]. Longer-term follow-up further showed associations between prior AKI and major adverse kidney events and mortality, although the adjusted association with CKD was not statistically significant [79]. These studies establish the importance of long-term renal surveillance after malaria-associated AKI but do not determine whether acute-phase urinary NGAL or KIM-1 concentrations predict these outcomes. Prospective longitudinal studies incorporating serial urinary biomarker profiling are therefore needed to clarify the long-term prognostic significance of structural renal injury biomarkers in malaria survivors.

Key comparative features, diagnostic performance, and clinical relevance of renal injury biomarkers in malaria-associated AKI are summarized in Table 7 [6, 24, 39, 69, 71, 75, 78, 79]. From an implementation perspective, most malaria-specific evidence for urinary NGAL and KIM-1 has been generated using quantitative laboratory-based immunoassays, including commercially available ELISA platforms [39]. Translation into routine malaria care will therefore require validation of assay performance, standardized measurement procedures, and clinically relevant thresholds in the intended populations and healthcare settings.

Table 7: Comparative performance and clinical relevance of renal injury biomarkers in malaria-associated acute kidney injury.

Biomarker	Population/setting	Specimen	Clinical utility	Key malaria-specific findings	Diagnostic performance/quantitative data	Major limitations	Key references
Serum creatinine	Adults with imported <i>P. falciparum</i> malaria; severe malaria cohorts	Blood	Conventional functional marker for AKI classification	Some patients who later developed AKI had normal serum creatinine at presentation despite elevated structural injury biomarkers	Routinely used for AKI staging but less sensitive for early structural tubular injury	Delayed increase after injury; influenced by hydration, muscle mass, age, baseline renal function, and unavailable baseline creatinine in many endemic settings	[24, 39]
BUN	Severe <i>P. falciparum</i> malaria with AKI	Blood	Functional marker of renal dysfunction and perfusion status	Renal KIM-1 expression in <i>P. falciparum</i> malaria-associated AKI correlated with BUN and serum creatinine	No malaria-specific cutoff for early AKI prediction	Nonspecific; affected by dehydration, catabolism, gastrointestinal bleeding, volume depletion, and renal perfusion	[24, 75]
Urinary NGAL	Adults with imported <i>P. falciparum</i> malaria; n = 39 (AKI, n = 6)	Urine	Early structural marker of tubular epithelial stress; potential adjunct for early AKI detection	Urinary NGAL was elevated at presentation in all six patients who developed AKI and showed excellent discrimination for the presence or subsequent development of AKI	Youden-derived cutoff ≥ 308 ng/mL; AUROC = 1.00 (95% CI = 1.00–1.00); sensitivity = 100%; specificity = 100%; PPV = 100%; NPV = 100%. AKI was defined according to KDIGO criteria	Very small pilot cohort with only six AKI events; data-derived cutoff; no external validation; AUROC was not significantly superior to serum creatinine; generalizability to	[39]

Biomarker	Population/setting	Specimen	Clinical utility	Key malaria-specific findings	Diagnostic performance/ quantitative data	Major limitations	Key references
Circulating NGAL (serum/plasma)	Adults with imported <i>P. falciparum</i> malaria (n = 39; AKI, n = 6); adults and children with severe <i>P. falciparum</i> malaria in India (n = 59; 45 adults and 14 children); children with severe malaria in Uganda (n = 598)	Serum or plasma	Structural kidney injury marker; potential adjunct for assessing evolving AKI and organ injury	In imported malaria, serum NGAL showed high discrimination for the presence or subsequent development of AKI. In severe malaria in India, plasma NGAL increased with AKI severity and was associated with cerebral edema. In Ugandan children with severe malaria, high-risk plasma NGAL was associated with unresolved AKI at 24 h, in-hospital mortality, and AKD at 1 month	Imported malaria: cutoff ≥ 168 ng/mL; AUROC = 0.98 (95% CI = 0.93–1.00); sensitivity = 100%; specificity = 88%; PPV = 60%; NPV = 100%. Severe malaria in India: NGAL was significantly higher in KDIGO stage 3 AKI (p < 0.0001); >300 ng/mL was associated with a higher prevalence of cytotoxic cerebral edema (PRR = 5.5, 95% CI = 2.3–13.2; p < 0.001). Ugandan pediatric severe malaria: ≥ 300 ng/mL was associated with unresolved AKI at 24 h (OR = 7.00, 95% CI = 4.16–11.76) and in-hospital mortality (OR = 6.02, 95% CI = 2.83–12.81)	endemic adult and pediatric populations remains uncertain Performance varies by specimen type, assay platform, population, and clinical context. Circulating NGAL may be influenced by systemic inflammation and neutrophil activation. The ≥ 300 ng/mL threshold in the Ugandan pediatric cohort was an established risk threshold rather than a malaria-specific data-derived optimal cutoff. Pediatric endemic evidence relates to plasma NGAL and does not establish urinary NGAL performance	[39, 69, 71]
Urinary KIM-1	Adults with imported <i>P. falciparum</i> malaria; n = 39 (AKI, n = 6)	Urine	Marker of tubular injury and potential adjunct for AKI assessment	Urinary KIM-1 showed discrimination for AKI, although its AUROC was lower than that of urinary NGAL in the same cohort	Youden-derived cutoff ≥ 1.83 ng/mL; AUROC = 0.87 (95% CI = 0.75–0.99); sensitivity = 100%; specificity = 72%; PPV = 40%; NPV = 100%	Small cohort with only six AKI events; data-derived threshold; no external validation; AUROC was not significantly superior to serum creatinine; temporal kinetics in malaria-associated AKI remain uncertain	[39]
Renal tissue KIM-1 expression	Fatal/severe <i>P. falciparum</i> malaria with AKI; non-AKI malaria and nonmalarial controls	Kidney tissue	Histopathological validation of proximal tubular injury	KIM-1 expression was significantly increased in proximal tubules in <i>P. falciparum</i> -associated AKI and correlated with serum creatinine, BUN, and proximal tubular necrosis	Quantitative diagnostic performance not applicable because this is tissue-based evidence	Strong biological evidence but not clinically practical for routine diagnosis or triage	[75]
NGAL + KIM-1 combined interpretation	Malaria-associated AKI evidence synthesized from available clinical and mechanistic studies	Urine/blood	Complementary structural biomarker panel	NGAL and KIM-1 may provide complementary information, with NGAL reflecting early tubular stress and KIM-1 reflecting proximal tubular epithelial injury	Incremental value has not been formally validated in large malaria cohorts	Currently conceptual; incremental value of combined NGAL and KIM-1 assessment has not been validated in malaria and requires prospective comparison of single-marker and multimarker models	[6, 24, 39]
TIMP-2, IGFBP7, L-FABP, IL-18, cystatin C	Broader AKI literature; limited malaria-specific evidence	Urine or blood	Candidate biomarkers reflecting cell-cycle arrest, oxidative stress, inflammatory tubular injury, or renal function	Direct malaria-specific evidence remains limited compared with NGAL and KIM-1	No validated malaria-specific cutoffs or diagnostic performance metrics	Not sufficiently characterized in falciparum malaria; added value over NGAL and KIM-1 remains unclear	[6, 24]
Long-term renal outcome	Children with severe malaria in Uganda; survivors	Blood/urine	Post-AKI renal recovery and	AKI was associated with CKD at 1 year and post-discharge mortality; AKD	CKD at 1 year = 7.6% vs. 2.8%; OR = 2.81 (95% CI = 1.02–7.73). AKD at 1	Longitudinal studies establish adverse post-AKI outcomes but do	[71, 78, 79]

Biomarker	Population/setting	Specimen	Clinical utility	Key malaria-specific findings	Diagnostic performance/ quantitative data	Major limitations	Key references
after malaria-associated AKI	with or without AKI		long-term kidney outcome assessment	was present in 15.6% of children at 1 month. During longer-term follow-up, prior AKI was associated with major adverse kidney events and mortality, although the adjusted association with CKD was not significant	month = 15.6%. Long-term major adverse kidney events = aOR 3.14 (95% CI = 2.23–4.43); mortality = aHR 3.36 (95% CI = 2.22–5.10); CKD = aOR 1.47 (95% CI = 0.84–2.56)	not determine whether acute-phase urinary structural injury biomarkers predict these outcomes. The adjusted association between prior AKI and CKD was not significant in the longest follow-up study	

AKD = acute kidney disease; AKI = acute kidney injury; aHR = adjusted hazard ratio; aOR = adjusted odds ratio; AUROC = area under the receiver operating characteristic curve; BUN = blood urea nitrogen; CI = confidence interval; CKD = chronic kidney disease; ELISA = enzyme-linked immunosorbent assay; HAVCR1 = hepatitis A virus cellular receptor 1; IGFBP7 = insulin-like growth factor-binding protein 7; IL-18 = interleukin-18; KDIGO = Kidney Disease: Improving Global Outcomes; KIM-1 = kidney injury molecule-1; L-FABP = liver-type fatty acid-binding protein; NGAL = neutrophil gelatinase-associated lipocalin; NPV = negative predictive value; OR = odds ratio; PPV = positive predictive value; PRR = prevalence risk ratio; TIM-1 = T-cell immunoglobulin and mucin domain-containing protein-1; TIMP-2 = tissue inhibitor of metalloproteinases-2.

CONCLUSION

Urinary biomarkers may provide biologically informative complementary measures for evaluating falciparum malaria beyond parasite detection alone. As a non-invasive matrix, urine may capture integrated signals related to hemolysis, immune activation, hepatobiliary dysfunction, and evolving renal injury during infection. Current evidence supports complementary roles for parasite-derived markers, dipstick abnormalities, and structural tubular injury biomarkers such as NGAL and KIM-1 in reflecting distinct aspects of malaria pathophysiology and disease severity. Available studies also suggest that tubular injury may precede overt creatinine-defined AKI in some patients with falciparum malaria, highlighting the potential value of urinary biomarkers for earlier recognition of occult renal involvement.

Despite this biological rationale and emerging clinical evidence, important limitations currently restrict clinical translation. These include methodological heterogeneity, small study populations, inconsistent sampling and normalization procedures, variable assay platforms, limited external validation, and a lack of standardized biomarker thresholds. Urine-based biomarkers should therefore be considered complementary to established malaria diagnostics rather than replacements for conventional blood-based methods. Their potential value may be greatest in assessing renal and other organ involvement, particularly in malaria-endemic and resource-limited settings where non-invasive, repeatable sampling may offer practical advantages.

An immediate research priority is prospective validation of urinary NGAL and KIM-1 in pediatric and adult populations from malaria-endemic regions using standardized AKI definitions, sampling protocols, assay procedures, and normalization methods. Large multicenter studies should establish reproducible biomarker thresholds across clinically relevant populations and disease severities and determine whether these biomarkers provide incremental value beyond serum creatinine and routine clinical assessment. Following initial validation, longitudinal studies should assess whether early urinary biomarker abnormalities are associated with renal recovery, persistent kidney dysfunction, and long-term renal outcomes after malaria-associated AKI.

The importance of post-malaria renal follow-up is supported by longitudinal pediatric evidence. Severe malaria-associated AKI has been associated with a higher frequency of CKD at 1 year and increased post-discharge mortality [78], whereas subsequent studies have documented AKD at 1 month after severe malaria [71] and sustained excess mortality following malaria-associated AKI during longer-term follow-up [79]. However, whether early urinary NGAL or KIM-1 concentrations can identify patients at increased risk of incomplete renal recovery or adverse long-term kidney outcomes remains unknown. These biomarkers may also warrant evaluation as exploratory endpoints in future malaria intervention studies, where they could complement serum creatinine by capturing changes in structural tubular injury and renal recovery. However, using them as validated surrogate endpoints would require prospective evidence that biomarker changes are reproducibly associated with clinically meaningful renal outcomes.

The potential clinical roles of urinary biomarkers may span different stages of care, including initial assessment and triage, monitoring for evolving renal involvement, and longitudinal follow-up after AKI. Current evidence, however, is insufficient to support biomarker-guided clinical decision algorithms. The biomarker classes reviewed here also differ substantially in biological specificity, strength of evidence, technical feasibility, and readiness for implementation. Conventional dipstick abnormalities are inexpensive and readily deployable but

have limited biological specificity and should be interpreted as nonspecific indicators of systemic, hemolytic, hepatobiliary, or renal involvement. Urine-based parasite antigen assays provide greater biological specificity for infection but currently show variable sensitivity and remain less reliable than established blood-based diagnostics. Structural renal injury biomarkers, particularly NGAL and KIM-1, may provide more direct information on tubular injury and complement serum creatinine for earlier recognition of renal involvement; however, malaria-specific evidence remains insufficient for routine clinical use. Urinary inflammatory markers and metabolomic signatures may provide additional biological information but currently have limited clinical readiness because of restricted validation, assay complexity, and lack of standardized thresholds.

Taken together, these differences support a staged rather than interchangeable role for urinary biomarkers. Routine urinalysis currently represents the most practical and accessible approach, whereas emerging molecular, inflammatory, metabolomic, and structural renal biomarkers require further validation before integration into routine care. Future translational efforts should prioritize the development and evaluation of affordable point-of-care urinary assays suitable for decentralized and low-resource healthcare environments. Multiplex urinary platforms integrating parasite-derived antigens, dipstick chemistry, and structural renal injury biomarkers warrant investigation as potential approaches for capturing complementary dimensions of malaria-associated abnormalities. However, implementation will depend not only on analytical performance but also on cost-effectiveness, supply-chain reliability, workforce training, quality assurance, and integration into existing malaria triage and clinical workflows.

Harmonized protocols for urine collection, specimen processing, storage, normalization, assay performance, and interpretation will be essential to improve reproducibility among studies and healthcare systems. Future development should also prioritize affordability, scalability, and accessibility for underserved populations, particularly children and rural communities in high-burden malaria-endemic regions.

Overall, urinary biomarkers remain an emerging complementary approach for investigating malaria-associated host responses and organ injury. Current evidence does not support their use as replacements for conventional blood-based malaria diagnostics or as validated tools for biomarker-guided clinical decision-making. Nevertheless, they warrant further investigation as potential indicators of renal involvement, host injury, and disease severity in falciparum malaria. Well-designed multicenter validation studies, longitudinal outcome studies, and implementation-focused research will be essential to determine whether these biomarkers can ultimately provide clinically meaningful, reproducible, and feasible tools for malaria assessment.

DATA AVAILABILITY

No new datasets were generated or analyzed for this narrative review. All information discussed in this article was derived from publicly available published literature cited in the manuscript.

GENERATIVE AI DECLARATION

During the preparation and revision of this manuscript, the author used ChatGPT (OpenAI) to assist with language refinement, manuscript organization, and improvement of clarity and readability. All AI-assisted outputs were critically reviewed, verified against the relevant literature where appropriate, and revised by the author. The author takes full responsibility for the accuracy, interpretation, and final content of the manuscript.

AUTHOR'S CONTRIBUTIONS

SK: Conceptualized the review, conducted the literature review, interpreted the evidence, drafted and critically revised the manuscript, and approved the final version.

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