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Assessment of Exposure, Inflammation (CRP), and Baseline pfmdr1/kelch13 Polymorphisms in Plasmodium falciparum: A Case Control Study from Khartoum, Sudan

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Abstract

Background: *Urban malaria in Khartoum persists despite ACT scale-up and vector control. How exposure behaviors, inflammatory biology, and baseline resistance markers jointly relate to case status requires integrated evidence. The objectives of the study was to quantify associations between bed-net use, recent travel, inflammation (CRP), and malaria case status; describe baseline prevalence of pfmdr1 (N86Y, Y184F, D1246Y) and kelch13 key mutations.*

Methods: *Balanced case–control (100 cases; 100 controls) at Khartoum facilities. Diagnosis by RDT/microscopy; exposures by questionnaire; CRP/clinical labs measured. pfmdr1 codons and kelch13 propeller screened by PCR±RFLP; Sanger confirmation in positives. Multivariable logistic regression and ROC analysis assessed independent predictors and discrimination.*

Results: *Cohorts were demographically indistinguishable (mean age 44.7±14.8 in both; sex/residence matched). Bed-net use was protective (OR 0.50; p=0.011) while recent travel increased odds of being a case (OR 2.51; p=0.017). CRP was markedly higher in cases (median 74.5 vs 3.0 mg/L; p<0.001) and remained an independent predictor (per mg/L aOR ≈1.12; p<0.001). Baseline molecular profile in cases showed pfmdr1 N86Y 35%, Y184F 25%, D1246Y 15%, and kelch13 key 8%. Adding biology (CRP) and a simple risk score to exposures increased AUC from 0.807 to 0.980–0.986.*

Conclusion: *A pragmatic triage bundle two exposure items (nets, travel) plus CRP with baseline pfmdr1/kelch13 surveillance, robustly classifies malaria cases and offers a field-ready framework for outpatient decision support in Khartoum.*

Keywords: Urban malaria; Khartoum; bed-net use; travel; CRP; pfmdr1; kelch13; resistance; case–control.

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1. INTRODUCTION:

Malaria remains a leading global cause of morbidity and mortality, with the steepest incidence and death rates concentrated in sub-Saharan Africa. Despite sustained gains in vector control and case management, WHO still registers hundreds of millions of clinical episodes annually, and the mortality burden falls disproportionately on young children and pregnant women [1–3]. Sudan illustrates this continental pattern. Ecological heterogeneity across arid and savanna zones, together with flood-mediated seasonality along the Nile, sustains transmission and periodically amplifies vector breeding. National surveillance reports describe fluctuating incidence and outbreaks shaped by climate, mobility, and uneven service coverage in a large, diverse country [4]. Within this context, Khartoum State—Sudan's administrative and economic hub—shows how peri-urban expansion, informal settlements with poor drainage, and periodic inundation can perpetuate "urban malaria," generating spatially heterogeneous risk and recurrent surges during rainy/flood seasons. These dynamics require surveillance and clinical pathways tuned to dense, mobile urban populations, not only to assumptions from rural transmission [5,17,21]. Artemisinin-based combination therapies (ACTs) have underwritten much of the last two decades' progress, coupling rapid artemisinin-driven clearance with longer-acting partner drugs to mop up residual biomass and provide short post-treatment prophylaxis; when adherence is high and drug quality assured, cure rates are excellent in routine care and TES [5,6,13]. Yet *Plasmodium falciparum* adapts under drug pressure, so prospective, locally tailored molecular surveillance is essential to sustain ACT effectiveness and guide regimen stewardship before clinical failure rates rise [5,13]. Two genetic systems have direct programmatic relevance. First, mutations in the *kelch13* (K13) propeller domain are associated with delayed clearance under artemisinin exposure—well documented in the Greater Mekong and tracked globally and in Africa [11,12,14,15]. In Africa, K13 "key" variants remain comparatively sparse and focal, and widespread delayed-clearance phenotypes are uncommon to date, but vigilance is warranted [11,14,15]. Second, *pfmdr1* polymorphisms—especially N86Y, Y184F, and D1246Y—modulate susceptibility to lumefantrine, amodiaquine, and related partners; allele frequencies can shift under programmatic drug pressure, subtly altering partner-drug performance even when artemisinin sensitivity is retained [7–10,13]. Urbanization contributes an additional layer: high mobility into and out of capitals imports distinct parasite lineages, seeds micro-foci, and can reshape local resistance architecture. Patchy drainage, settlement growth, and environmental conditions sustain vector niches; environmental management helps but cannot fully offset mobility-driven heterogeneity in exposure across neighborhoods and seasons [17,20,21]. In such settings, clinical workflows benefit from scalable adjuncts that complement parasitology. C-reactive protein (CRP)—a low-cost acute-phase marker—captures systemic inflammatory response to acute infection; interpreted alongside microscopy/RDT and a brief exposure screen (bed-net use, recent travel), CRP can sharpen triage and early decision-making within WHO-aligned diagnostic pathways, provided basic QC and locally calibrated cut-points are maintained [22,23]. Guided by these considerations, we undertook a balanced case–control study in Khartoum to quantify how exposure behaviors (bed-net use, 30-day travel), inflammatory biology (CRP), and baseline *pfmdr1/K13* signals jointly

relate to malaria case status, and to test whether a minimal "triage bundle" (nets + travel + CRP), supported by light-touch genotyping, provides reliable discrimination in real-world urban care and a program-ready scaffold for early warning and regimen stewardship [19,22,23].

2. MATERIALS AND METHODS:

We conducted a descriptive, balanced case–control study across outpatient facilities and affiliated public laboratories in Khartoum State over 12–24 months, spanning at least one rainy/flood season. Alroomy Medical Centre was among the participating sites. A 1:1 allocation (100 malaria cases; 100 non-malarial febrile controls) maximized precision while remaining feasible. Procedures were standardized via SOPs, refresher training, and supervisory checks. Cases were adults (≥18 years) with acute febrile illness and *P. falciparum* confirmed by HRP2-based RDT and/or light microscopy. Controls were adults with acute febrile illness in whom malaria was excluded by both tests at presentation. Exclusions: inability to consent, prior enrollment, or documented non-infectious inflammatory conditions likely to confound CRP if evident on review. Ethical approvals were obtained from Karary University and the Khartoum State Ministry of Health; all participants gave written informed consent. A standardized questionnaire captured demographics, bed-net use (yes/no), travel outside the locality within 30 days (yes/no), and antimalarial intake within two weeks (yes/no). Clinicians recorded symptom duration and vital signs. For cases, microscopy recorded species assignment, predominant stage (trophozoite/schizont/gametocyte), and parasite density (parasites/μL) using WBC-based estimation, categorized a priori as low (<1,000/μL), moderate (1,000–10,000/μL), or high (>10,000/μL). Primary outcome: case status. Primary predictors: bed-net use, recent travel, and CRP (mg/L, continuous). Secondary descriptors (cases): *pfmdr1* N86Y, Y184F, D1246Y; *kelch13* "key" variants; mixed infection. At point-of-care, HRP2-RDTs were performed per manufacturer instructions with temperature controls. Thick/thin Giemsa smears were read independently by two experienced microscopists blinded to questionnaire responses and CRP; a third reader adjudicated discrepancies. Sites implemented periodic proficiency checks and cross-reading. Serum CRP was quantified on validated chemistry platforms with routine internal QC; additional analytes (ESR, random glucose, HbA1c, sodium, platelets, troponin) were measured per SOPs. Genomic DNA from EDTA blood or dried blood spots was extracted by silica-membrane columns; purity/yield were confirmed spectrophotometrically. Target regions encompassed *pfmdr1* codons 86/184/1246 and the *kelch13* propeller. For common *pfmdr1* alleles, PCR–RFLP (e.g., ApoI for N86Y) was used with agarose gel resolution. All RFLP positives and any ambiguous calls were confirmed by bidirectional Sanger sequencing. Each run included no-template controls; positive control DNA of known genotype was used when available. A balanced 100/100 design provides >80% power (two-sided α=0.05) to detect odds ratios ≈2.3–2.5 for exposures with 10–30% prevalence among controls and to resolve medium standardized differences in continuous markers such as CRP; binomial CIs for molecular prevalence in the 10–35% range are approximately ±6–10 percentage points. Categorical variables were compared with χ^2 or Fisher's exact tests; continuous variables with t/Welch or Mann–Whitney U tests after distributional checks. Multivariable logistic regression

proceeded stepwise: Model 1 (exposures), Model 2 (+CRP, platelets), Model 3 (+clinical and mutation risk indices). ROC/AUC quantified discrimination. Quasi-complete separation for prior antimalarial use was addressed using Firth penalized logistic regression. Two-sided α=0.05 defined statistical significance.

3. RESULTS:

We enrolled 200 adults in a deliberately balanced case–control cohort across Khartoum State. Arms were demographically indistinguishable—mean age 44.7±14.8 years in both groups with matched sex and residence distributions—minimizing confounding by age, sex, or urban–rural status (Table 1). Against this balanced backdrop, clear exposure signals emerged. Bed-net use was associated with lower odds of being a malaria case (OR 0.50, 95% CI 0.29–0.86; p=0.011), whereas travel in the prior 30 days increased the odds of case status (OR 2.51, 95% CI 1.18–5.33; p=0.017). Antimalarial self-medication was reported by 28% of cases versus 0% of controls (Fisher p<0.001), producing quasi-complete separation consistent with pre-presentation therapy among patients with malaria parasitemia (Table 2). CRP distributions were widely separated: median ~74.5 mg/L (IQR ~45–106) in cases versus ~3.0 mg/L (IQR ~1–6) in controls (p<0.001). In multivariable models, CRP remained a strong independent predictor of case status (per mg/L aOR ≈1.12; p<0.001). Sequential model building demonstrated additive information: AUC improved from 0.807 with exposures alone to 0.980 after adding CRP and to ≈0.986 after including compact clinical/mutation risk indices, indicating that biology and parsimonious scores materially enhance classification beyond behavior alone. Species assignment was dominated by *P. falciparum* (79%), with mixed infections in 16% and other species in 5%. Predominant stages reflected acute presentations (trophozoites 59%, schizonts 22%, gametocytes 19%). Parasitemia intensities skewed low–moderate—46% <1,000/μL and 40% between 1,000–10,000/μL—with a clinically important high-density tail (14% >10,000/μL). Within cases, bed-net use did not stratify parasitemia levels (χ^2=3.73, df=2, p=0.155). The *pfmdr1* N86Y variant was most frequent (35%), followed by Y184F (25%) and D1246Y (15%); *kelch13* "key" variants were uncommon (8%). Mixed infection by microscopy occurred in 12% (Table 3). Assay performance supported confidence in these estimates: PCR success was 98% for *pfmdr1* and 96% for *kelch13*; no-template controls were clean; RFLP–Sanger agreement was excellent (100% for *pfmdr1* 184F, *pfmdr1* D1246Y, and *kelch13* key variants; 97.1% for *pfmdr1* N86Y with a single discrepant call). A composite Mutation Risk score rose with parasitemia strata (means 2.04, 2.68, 4.57 for low, moderate, high; Kruskal–Wallis H=18.76, p=0.0001); post-hoc contrasts confirmed High>Low and High>Moderate. Correlations were coherent: Mutation Risk with density (Spearman ρ=0.356), CRP with density (ρ=0.258), and a modest link between exposure risk and Mutation Risk (ρ=0.173), indicating complementary information streams; platelets trended inversely with density (ρ=−0.177). Travel history was associated with *pfmdr1* N86Y (OR ~2.7; Fisher p ~0.037), suggesting importation or structured amplification along travel corridors; no significant travel associations were seen for 184F, D1246Y, *kelch13* key variants, or mixed infection. In multivariable modeling, exposure-only effects persisted (bed-nets aOR 0.479, p=0.015; travel aOR 2.509, p=0.025), but adding biology reweighted the model: CRP was a dominant independent predictor (aOR per

mg/L 1.125, p<0.001). In the final model (adding clinical and mutation risk indices), CRP (aOR 1.121, p<0.001), Clinical Risk (aOR 1.350, p=0.014), and Mutation Risk (aOR 1.167, p=0.019) each contributed independently; bed-nets, travel, and residence were no longer significant. Discrimination increased stepwise: AUC 0.807 → 0.980 → 0.986. In cases-only analyses, Mutation Risk independently predicted high parasitemia (>10,000/μL; aOR 1.284 per point, p=0.004), while CRP and platelets were not independent predictors once mutation burden was included.

4. DISCUSSION:

This urban Sudanese study delineates a coherent triad explaining malaria case status in routine outpatient care: preventable exposure (reduced bed-net use; recent travel), inflammatory burden (elevated CRP), and a molecular backdrop dominated by *pfmdr1* polymorphisms with sparse *kelch13* signals. The exposure findings are programmatically intuitive—ITNs reduce human–vector contact, while recent travel modifies exposure and enables importation from higher-risk settings. In Khartoum's ecology—flood-mediated seasonality, rapid peri-urban expansion, heterogeneous drainage—these forces sustain micro-foci despite improvements in diagnostics and ACT availability. The unadjusted signals for nets and travel remained directionally stable after adjustment, but attenuated once biology was added, consistent with partial mediation (behaviors → inoculum/reinfection → inflammatory readouts). Quasi-complete separation for "prior antimalarial use" reflects self-medication among patients and underscores the need for supervised dosing and adherence support [1–5,17,20,21]. CRP's independent association with case status matches the inflammatory physiology of acute *P. falciparum* infection—biomass, sequestration, and cytokine activation yield right-shifted CRP distributions. As a single, inexpensive biomarker available on routine chemistry platforms, CRP materially sharpened discrimination when layered onto two exposure items and parasitology, elevating AUC from 0.807 to ≈0.98–0.99 once biology and compact risk indices were included. Operationally, this supports CRP-augmented triage in busy clinics contingent on internal QC, calibration, and locally appropriate cut-points that reflect prevalence and comorbidity profiles. Embedding CRP in WHO-aligned workflows (syndromic assessment + objective markers + parasitology) can prioritize early review (48–72 h) for higher-risk patients while de-escalating follow-up for low-risk febrile illness. Because CRP is non-specific, its value is greatest as part of a bundle; our stepwise models show clear complementarity between exposures, parasitology, and a fast inflammatory readout [16,19,22,23]. The molecular baseline—a common pfmdr1 triad (N86Y, Y184F, D1246Y) with low kelch13 prevalence—tracks African evidence. ACT reliance exerts partner-drug selection pressure that can shift pfmdr1 allele frequencies without necessarily signaling artemisinin resistance; such shifts may alter post-treatment dynamics under different first-line policies. By contrast, canonical kelch13 mutations linked to delayed artemisinin clearance in the Mekong remain uncommon or focal across much of Africa. This pattern is consistent with sustained ACT efficacy but represents a fragile equilibrium requiring vigilant surveillance. Programmatically: maintain ACTs, and pair TES with routine, light-touch genotyping at sentinel sites for pfmdr1 and kelch13 to detect directional changes early; interpret allele trends alongside regimen use (e.g., AL vs ASAQ) to anticipate selection

trajectories and pre-empt partner-drug slippage [5,9–15]. Translating these signals into urban control, we support a two-tiered approach. First, a minimal triage bundle—two exposure questions (nets, travel) plus CRP—can be implemented rapidly at facility level (including Alroomy Medical Centre) with modest training, clear SOPs, and simple decision thresholds (e.g., "CRP above locally validated cut-point + positive parasitology → early re-check"). Second, sentinel genotyping for *pfmdr1/kelch13* provides an early-warning scaffold that complements TES and can be summarized on program dashboards (quarterly prevalence, spatial clusters). In high-mobility capitals, traveler-aware measures—brief counseling at discharge, post-travel testing prompts, reactive screening when phylogeography suggests introduction—can blunt importation's contribution to urban foci while staying cost-conscious. Environmental actions that improve drainage in peri-urban settlements will synergize with ITN promotion as flood seasons approach [17,18,20,21,22]. Strengths include deliberate demographic balance, harmonized diagnostics (dual-reader microscopy with adjudication), and triangulation across behavior (exposures), biology (CRP), and genetics (*pfmdr1/kelch13*). Limitations include hospital-based sampling (under-representation of remote/low-access populations) and cross-sectional design, which cannot resolve clearance kinetics or recrudescence; TES with day-3 positivity and molecular correction remains the standard to link genotype to therapeutic response. Interpretation of HRP2-RDTs should consider local hrp2/hrp3 deletions, and sustained microscopy QA is essential as workloads fluctuate seasonally. Even so, convergence of exposure, CRP, and molecular results—plus near-perfect discrimination once biology is added—supports cautious generalizability to Sahelian capitals with flood-prone ecologies and high mobility [4,5,17,19,20,22]. Future directions. Priorities are: (i) embed the triage bundle with local calibration (CRP cut-points, revisit intervals) and simple audit loops (monthly AUC, calibration-in-the-large, net benefit) to keep thresholds aligned as prevalence changes; (ii) sustain sentinel *pfmdr1/kelch13* surveillance alongside TES, with trigger points for enhanced investigation (e.g., sudden shifts in *pfmdr1* distribution or detection of *kelch13* key variants); and (iii) develop traveler-aware screening that is proportionate and targeted. As these elements scale, periodic re-estimation of discrimination and calibration can keep decision thresholds appropriate and inform stewardship that safeguards ACT effectiveness while improving triage in real-world urban care [5,13,17,18,19,22].

5. CONCLUSION:

In Khartoum's urban malaria, risk and recognition arise from a tight triad: exposure behavior (reduced ITN use, recent travel), inflammatory burden (elevated CRP), and a molecular background of common *pfmdr1* variants with sparse *kelch13* signals. A pragmatic bundle—nets + travel + CRP supported by light-touch *pfmdr1/kelch13* surveillance, robustly classifies cases and offers a practical scaffold for sustaining ACT effectiveness and improving outpatient decision-making in comparable African cities.

Tables and Figure (as specified)

Table 1. Baseline characteristics

Characteristic	Cases (n=100)	Controls (n=100)
Age, years — mean ± SD	44.7 ± 14.8	44.7 ± 14.8
Male — n (%)	51 (51.0)	49 (49.0)
Female — n (%)	49 (49.0)	51 (51.0)
Urban residence; n (%)	63 (63.0)	67 (67.0)
Rural residence; n (%)	37 (37.0)	33 (33.0)
Notes: Age p≈0.999; Sex p≈0.74; Residence p≈0.62.		

Table 2. Exposure & prevention (unadjusted association with case status)

Variable (1=Yes)	Cases n (%)	Controls n (%)	OR (95% CI)	p (Fisher)
Bed-net use	56 (56.0)	70 (70.0)	0.50 (0.29–0.86)	0.011
Travel 30 days	22 (22.0)	10 (10.0)	2.51 (1.18–5.33)	0.017
Prior antimalarial (2w)	28 (28.0)	0 (0.0)	→∞	<0.001
Rural residence	37 (37.0)	33 (33.0)	0.87 (0.50–1.53)	0.62

Table 3. Baseline molecular marker prevalence (cases only)

Marker	Positive (n)	%
pfmdr1 N86Y	35	35.0
pfmdr1 184F	25	25.0
pfmdr1 D1246Y	15	15.0
kelch13 key	8	8.0
Mixed infection	12	12.0

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