

Exploring Biochemical and Hematological Variations in Malaria Cases: A Retrospective Analysis at a Health Center

Abstract

Introduction: In Mumbai, an overpopulated metropolis with tropical conditions, malaria poses a persistent challenge, particularly in damp slum areas. Despite reported declines in death and incidence rates, the city faces a significant burden. Our project focuses on an unusual aspect: a heightened incidence among males, especially during sporadic monsoon outbreaks and in areas undergoing extensive redevelopment. Through an epidemiological survey of infected populations in municipal clinics, supported by microscopic, hematological, and microbiological evidence, we aim to provide insights into this atypical pattern.

Material and method: A cross-sectional, partly retrospective study was designed with participants being sourced from regional slums and chawls, during the immediately preceding three-year period of project completion. Patients attending OPDs were randomly selected from amongst those with febrile symptoms related to malarial disease and tested.

Results:

Out of 1651 participants, 8.18% were malaria-positive, predominantly males (87.41%). *P. vivax* was the main parasite (87.41%), followed by *P. falciparum* (6.67%) and mixed infections (5.93%). ANOVA revealed significant RBC count differences ($F = 6.32$, $p = 0.003$). Tukey's HSD test showed *P. vivax* patients had higher RBC counts than mixed infection ($p = 0.002$), while *P. falciparum* counts were similar to *P. vivax* ($p = 0.089$) but higher than mixed infection ($p = 0.014$). Predominance of *P. vivax* emphasizes its impact on RBC counts and diagnostic complexities. The p values indicate that for all parameters, except serum creatinine in males, there is a statistically significant difference in the mean values between the malaria positive and negative groups.

Conclusion: This study highlights high malaria prevalence in urban India, with males more susceptible, possibly due to increased mosquito exposure. *P. vivax* is predominant, aligning with national data, emphasizing the need for tailored control measures. Surprisingly, *P. vivax* is

associated with higher RBC counts than *P. falciparum* or mixed infection, suggesting differences in erythrocytic cycles. These findings have critical implications for regional malaria management, prompting further research into underlying mechanisms.

{**Keywords**–Gender-specific,malarial,parasite,microscopic,, haematological, biochemical }

1. Introduction

Recent downward trends of malaria cases, especially in the Indian context, are encouraging, to say the least. Nationwide, an 85.1 per cent fall in sheer infected numbers, along with a 83.36 % decline in the associated mortality was seen from 2015 to 2022, as stated by the Union Minister for Health & Family Welfare in March 2023, during the Asia Pacific Leaders' Conclave on Malaria Elimination.¹ Another most heartening and recent piece of news involves the recommendation of the R21/Matrix-MTM malaria vaccine² by the World Health Organization (WHO), which has been jointly developed by the Serum Institute of India (SII) and the University of Oxford. Yet we are probably years away from "zero malaria", part of the World Malaria Day 2023 theme.³ In 2021, the WHO South-East Asia Region accounted for about 2% of the burden of malaria cases globally, India accounting for 79% of active cases and for about 83% of all malaria deaths in this region.⁴ Though about 40% of all cases in the region are due to *P. vivax*⁵, *P. falciparum* was the dominant species in some parts of India, Bangladesh, and Indonesia.⁶

Tropical South-East Asian habitats, with their naturally associated humid environments are veritable hotbeds of diseases, being conducive to a variety of bacterial, viral and parasitic microorganisms, and to their natural zoonotic vectors, including rats, and insects like flies, mosquitoes, ticks, and cockroaches; malaria, leptospirosis, dengue, and cholera featuring prominently. This situation is further accentuated in a congested metropolis like Mumbai, bursting as it is at the seams, and characterized by pockets of dense, damp and squalid slums, existing cheek by jowl. Numerous redevelopment schemes only add to its woes, owing to massive soil excavations and formation of dirty water storage reservoirs. This scenario for vector-borne illnesses worsens noticeably during the monsoons.

Malaria is a typically vector-borne disease, capable of assuming life-threatening proportions but preventable and curable, if treated well in time. It is caused by protozoan parasites of the genus

Plasmodium cycling between a vertebrate and a mosquito host.⁷ Malaria spreads predominantly through the bites of infected female *Anopheles* mosquitoes, most commonly the *A. stephensi* and *A. culicifacies* species. Symptoms (usually fever, headache and chills) are displayed within 10–15 days of getting bitten by an infected mosquito. Because some indications are not specific, getting tested early is important. Out of the parasitic species causing malaria in humans, two – *Plasmodium falciparum* and *Plasmodium vivax* – pose the greatest threat. *P. falciparum* is the deadliest malaria parasite; left untreated, it may cause severe illness and death within 24 hours. Most deaths occur as a result of cerebral malaria caused by *P. falciparum*. Prognosis is dependent upon various factors like infecting *Plasmodium* species, host immunity and efficacy of treatment.⁸ In endemic areas, other important risk factors responsible for the manifestation and severity of disease are known to include the age and gender of the patient^{9,10}, and relapses in resource-limited settings.¹¹

The human phase of the malaria life cycle begins following injection of malarial parasites (MPs) into its hosts during a blood meal. The initial hepatocytic phase is asymptomatic and except for the minor discomfort caused by irritation of the breached skin at the entry point, no morbidity is observed or experienced during hepatocyte infection – marked by schizont development and formation of merozoites, which are then released into the bloodstream to infect erythrocytes.¹² The pathogenesis of the infection is largely attributed to its erythrocytic life cycle stage.¹³ The total life-cycle within each infected RBC lasts for about 48 hours, after which the cell membrane is ruptured and new merozoites are released into the bloodstream. This marks the advent of the clinically symptomatic stage. Malarial parasites are known to specifically invade red blood cells, multiplying *within* them in order to evade the immune response. The haematological profile of most infected patients is altered and typically includes severe anaemia, coagulation disturbances, leukocyte numerical or functional changes and spleen involvement to varying degrees¹⁴, depending upon factors such as malarial endemicity and immunity, background haemoglobinopathy, demographic factors, nutritional status, etc.^{15,16} Abnormalities reported are found to be most pronounced in *P. falciparum* infection, probably as a result of the higher levels of parasitemia found in these patients.¹⁷

In spite of encouraging reports of decline in death and incidence rates in recent times, malaria continues to be a source of considerable distress and morbidity for those affected, posing an omnipresent challenge for civic authorities, and for health departments in particular. Besides

concerted global efforts that constantly evolve for the prevention and treatment of malaria, an understanding of the pathogenetic mechanisms which render resistance to malaria, as seen in the selection of protective genetic variants in malaria endemic areas, such as the thalassaemic¹⁸ and HbS (Haemoglobin S)¹⁹ variants, first reported as far back as the early 1950s. Since then, multiple genetic loci conferring malaria protection have been reported.²⁰ Other lesser known factors pertain to gender-specific roles.^{21,22} Several investigations report an increased susceptibility in males, to parasitic diseases in particular, as regards both frequency and severity.^{23,24} Very few such studies with an emphasis on the demographic aspects with reference to gender specificity have been reported in the Indian context. An earlier study on somewhat similar lines was executed by Pathak et al¹⁰, way back in 2012. Our project attempts to delineate the gender-specific propensity of the disease peculiar to our project, namely the atypically increased incidence on display in males, especially during sporadic outbreaks during the monsoons and within breeding hotbeds characterized by extensive redevelopment and construction work in full swing.

2. Aims and Objectives

To undertake an epidemiological and demographic study amongst infected populations, based on microscopic evidence, and corroborated with relevant haematological, clinical and microbiological profiles.

To examine the association of the disease with gender specificity in the study population, if any, with a discussion of the probable underlying factors.

3. Methodology

3.1. Study Design and Settings

A cross-sectional, partly retrospective study was designed with participants from local chawls and slums and (in Worli, Upper Worli, and Lower Parel, all roughly situated within a mile's radius from the BDD Municipal Dispensary), ranging from toddlers (> 1 year) to nonagenarians (upto 92 years of age). Patients were recruited from amongst the vast majority of individuals

with febrile symptoms similar to those manifested in malarial disease. These included varying combinations of fever and flu-like illness, including rigors, headache, muscle aches, and tiredness. The study was conducted only on those symptomatic individuals attending municipal OPD clinics from June 2021 to August 2023, limited to the monsoon period (June–September) of each year, and from whom prior informed consents were duly received. Utmost care was taken to ensure privacy and dignity of all participants. Thus the population under study was restricted to those obtained by passive surveillance, as this method was best for obtaining informed consents willingly and with ease. Newby et al consider passive case detection (PCD) to be the foundation of malaria surveillance and the primary mechanism to detect and treat malaria.²⁵

A total of 1651 participants irrespective of class, creed, age and gender, were finally included as an integral part of the study. They were screened for malaria by the standard diagnostic techniques of microscopy and RMAT, following which a complete blood count was also executed.

3.2 Standard Diagnostic Procedures Related to the Scope of the Project

Symptomatic individuals were tested for malaria microscopically using peripheral blood smear (PBS) and confirmed by employing the malaria antigen test (RMAT), as per guidelines issued by the Malaria Surveillance Division under the aegis of the MCGM (Municipal Corporation of Greater Mumbai) Health Department and conforming to WHO protocols. WHO recommends prompt parasite-based diagnosis in the pre-treatment stage.²⁶ The method of choice and convenience is either by microscopy or rapid diagnostic tests (RDTs) for all suspected patients. Light microscopy is the standard against which other diagnostic methods have traditionally been compared. It entails visualization of the malaria parasites in a peripheral blood smear of the patient. Blood for both microscopic and rapid diagnostic testing is commonly obtained from a finger-prick. Microscopy, by far, the most commonly employed, is a laborious and lengthy procedure; diagnosis requires examination of both thin and thick films from the same patient. RDTs, on the other hand, detect specific antigens produced by malaria parasites that are present in the blood of infected individuals. They are relatively simple to perform and interpret, they rapidly provide results, require limited training; advanced versions allow for species-specific diagnosis of malaria at the community level.

A complete blood count was executed on those found positive to ascertain the ensuing haematological changes, as an outcome of malarial infection. Though indirect, it provides a fairly good correlation with the above-mentioned, more precise diagnostic tools. Low density malarial infections, however, can be detected only by nucleic acid amplification tests (NAATs) (to sensitivity levels of 1 parasite/ μ L or even less).

Briefly, the suspected individual, after being properly identified, is initially subjected to a finger prick using a lancet, and the oozing drops of blood spread onto a clean, appropriately labelled slide for thick and thin smears. Simultaneously, 5 μ l of blood is quickly taken and processed further for RMAT analysis. The slide is then allowed to air dry. Phlebotomy is executed separately for the same patient, specifically for haematology procedures using 2 ml EDTA tubes.

3.4 Standard Technique for Malaria Microscopy:

After air drying, only the thick smeared part of the slides thus prepared were then completely dehaemoglobinised using distilled water and stained (using a mixture of Field A and B solutions according to WHO guidelines) within a maximum of 48 hours of receipt in the laboratory. Other routinely used stains include Giemsa, Leishman, JSB1, and JSB2 (Jaswant Singh and Bhattacharya).^{27,28} The slides thus stained were then air-dried and observed under an oil immersion lens (100x). The microscope utilized for this purpose was the Labomed CXR3 infinity corrected research model. Approximately 100 fields of thick smears need to be screened for accurate reporting. Specific stages of *falciparum* and *vivax* are represented by distinct structural patterns and are stained specifically. Gradation of detected species (very scanty, scanty, moderate and heavy) is done depending upon the number of structures found per field and per 100 fields and reported.

3.3 Rapid malaria antigen test

Malaria was detected in the laboratory using the Malarigen Bivalent Ag (*P.f/P.v*) Rapid Diagnostic Test Kit (Aspen Laboratories Pvt. Ltd.), based on immunochromatography. Briefly, qualitative identification was done by employing the antigens HRP-II (Histidine rich protein II) and pLDH (plasmodium Lactate dehydrogenase) for *P.f* (*P.falciparum*) and *P.v* (*P.vivax*) respectively.²⁹ Mixed infections can also be readily detected using this kit.

3.5 Haematology

Venous whole blood collected in a 2 ml BD EDTA (purple top) Vacutainer® sufficed as the preferred sample for haematological testing. Transportation, if required, was done on ice. The samples were processed within 6 hours to avoid sample degradation. Haemolyzed or clotted blood was discarded, and fresh samples procured again at the earliest. A comprehensively complete blood cell count (CBC) was effected by means of the state-of-the-art Elite 580 Erba Mannheim Haematology Analyser (Transasia Bio-Medicals), capable of achieving a throughput of 80 samples per hour. This fully automated analyser utilizes the principle of electrical impedance, wherein the change in electrical resistance produced by a blood cell suspended in conductive diluent as it passes through an aperture. Just 20 µl of blood volume is required using this method.



Fig .1 Assessment of *Haematology*

3.6 Statistical tool employed

This was reported by papers with similar parameters

{Categorical variables were described as frequencies and continuous variables were expressed as mean, standard deviation (SD), and range. The comparisons of proportions between males and females were performed using the chi-square test, with a threshold of <0.05 for rejecting the null hypothesis in two-tailed tests.

Results

Out of the total of 1651 participants selected over a three-year period, 135 (about 8.18%) were diagnosed with malaria, both by the classic microscopy techniques and the WHO-recommended RMAT. A gender-wise analysis of the participants chosen randomly on a “first come, first

served” basis revealed that 996 (60.33%) were males, the remainder (655, i.e., 39.67%) being females. Amongst the population testing negative, 638 (42.08%) were females, but the corresponding figure for those diagnosed malaria-positive, was significantly lower, only 17 (12.59%) being females, the vast majority (118, i.e., 87.41%) being infected males.

Further laboratory investigations of the positive population revealed *P. vivax* to be the predominant parasite, comprising ~87.41% of the total, followed by *P. falciparum* (6.67%), the remainder being accounted for by mixed infections (5.93%).

Table 1. Result of malaria test analysis

Population	Malaria test Negative	Malaria test Positive
Total Participants (1651)	1516	135
Gender Distribution		
Male	996	118
Females	520	17

Table.2.RBC counts

	P vivax	P falciparum	Mixed infection	
Means of the RBC counts	$4.31 \times 10^6/\mu\text{L}$,	$3.83 \times 10^6/\mu\text{L}$,	$3.55 \times 10^6/\mu\text{L}$	<p>We found a significant difference in the mean RBC counts among the three groups ($F = 6.32$, $p = 0.003$).</p> <p>Post hoc tests revealed that the mean RBC count of <i>P. vivax</i> was significantly higher than that of mixed infection ($p = 0.002$), but not significantly different from that of <i>P. falciparum</i> ($p = 0.089$).</p> <p>The mean RBC count of <i>P. falciparum</i> was also significantly higher than that of mixed infection ($p = 0.014$).</p>

The RBC counts of the patients with different types of malaria infection were compared using ANOVA. The results showed that there was a significant difference among the three groups (*P.*

vivax, *P. falciparum*, and mixed infection) with $F = 6.32$ and $p = 0.003$. Further analysis using Tukey's HSD test indicated that the patients with *P. vivax* had a significantly higher RBC count ($4.31 \times 10^6/\mu\text{L}$) than those with mixed infection ($3.55 \times 10^6/\mu\text{L}$, $p = 0.002$), while the patients with *P. falciparum* had a similar RBC count ($3.83 \times 10^6/\mu\text{L}$) to those with *P. vivax* ($p = 0.089$). However, the patients with *P. falciparum* also had a significantly higher RBC count than those with mixed infection ($p = 0.014$). These findings suggest that the type of malaria parasite may affect the RBC count of the infected patients. **Table.3.**

Table.3.Parasite Distribution in positive patients

		Parasite Distribution in positive patients	
		P vivax	118(~87.41%)
		P falciparum	9(6.67%)
		Mixed infection	8 (5.93%)

The prevalence of different species of malaria parasites among the positive patients was analyzed in this study. The majority of the patients (118 out of 135, approximately 87.41%) were infected with *Plasmodium vivax*, which is the most common and widely distributed human malaria parasite. Only nine patients (6.67%) had *Plasmodium falciparum* infection, which is the most lethal and responsible for most malaria deaths. Eight patients (5.93%) had mixed infection of both *P. vivax* and *P. falciparum*, which can complicate the diagnosis and treatment of malaria.

Table 4.Biochemical and hematological parameters

Parameters	Gender	Malaria test Negative	Malaria test Positive	P Value
Means of the RBC counts	Male	$4.77 \times 10^6/\mu\text{L}$	$4.30 \times 10^6/\mu\text{L}$	0.003
	Female	$3.91 \times 10^6/\mu\text{L}$	$3.75 \times 10^6/\mu\text{L}$	0.021
	Total	$4.41 \times 10^6/\mu\text{L}$	$4.23 \times 10^6/\mu\text{L}$	0.008
Blood Urea in mg/dl (Mean \pm SD)	Male	18.38 \pm 0.34	11.21 \pm 0.38	<0.001
	Female	21.32 \pm 0.14	13.22 \pm 0.33	<0.001
Sr Creatinine in mg/dl (Mean \pm SD)	Male	0.89 \pm 0.21	0.75 \pm 0.15	0.012
	Female	1.24 \pm 0.13	0.85 \pm 0.34	<0.001

The p values indicate that for all parameters, except serum creatinine in males, there is a statistically significant difference in the mean values between the malaria positive and negative groups. This means that we can reject the null hypothesis and accept the alternative hypothesis for these parameters. For serum creatinine in males, the p value is greater than 0.05, which means that we cannot reject the null hypothesis and we cannot conclude that there is a difference in the mean values between the malaria positive and negative groups.

The table also shows that for all parameters, except RBC count in females, the mean values are lower in the malaria positive group than in the malaria negative group. This suggests that malaria infection may have a negative impact on these parameters, which are related to kidney function and oxygen transport in the blood.

Discussion

The results of this study indicate that malaria is a prevalent disease among the participants selected from the urban areas of India, and that there is a significant gender difference in the infection rate, with males being more susceptible than females. The study also reveals that *P. vivax* is the most common parasite causing malaria, and that it has a different impact on the RBC count of the patients than *P. falciparum* or mixed infection. These findings have important implications for the diagnosis, treatment, and prevention of malaria in this region.

One possible explanation for the higher infection rate among males is that they are more exposed to the mosquito vectors than females, due to their outdoor activities or occupations. This is consistent with previous studies that have reported a male predominance in malaria cases in India [30].

Another factor that may contribute to the gender difference is the hormonal or immunological differences between males and females, which may affect their susceptibility or resistance to

malaria infection [31,32]. Further research is needed to elucidate the underlying mechanisms of this gender disparity.

The predominance of *P. vivax* over *P. falciparum* or mixed infection in this study is in agreement with the national data on malaria epidemiology in India, which shows that *P. vivax* accounts for about 60% of the total malaria cases, while *P. falciparum* accounts for about 40%, and mixed infection accounts for less than 1% [33]. However, this may vary depending on the geographical location, climatic conditions, and vector species [34]. Therefore, it is important to monitor the malaria situation in different regions and to implement appropriate control measures accordingly. The finding that *P. vivax* causes a higher RBC count than *P. falciparum* or mixed infection is interesting and somewhat unexpected, as *P. vivax* is usually associated with a lower degree of anemia than *P. falciparum* [35]. One possible reason for this discrepancy is that *P. vivax* has a shorter erythrocytic cycle than *P. falciparum* (48 hours vs. 72 hours), which may result in a faster recovery of RBC production after infection [36]. Another possibility is that *P. vivax* has a lower parasitemia than *P. falciparum*, which may reduce the hemolysis and destruction of RBCs [37]. However, these hypotheses need to be verified by further studies.

Conclusion

This study reveals a significant prevalence of malaria in urban India, with males showing higher susceptibility than females, possibly due to increased mosquito exposure. *P. vivax* emerges as the predominant parasite, consistent with national data, emphasizing the need for region-specific control measures. Surprisingly, *P. vivax* is associated with higher RBC counts compared to *P. falciparum* or mixed infection, suggesting potential differences in erythrocytic cycles and parasitemia. These findings have crucial implications for malaria management in the region, warranting further research to validate and explore underlying mechanisms.

References:

1. PressReleaseDetails, April 24, 2023. Press Information Bureau.
<https://pib.gov.in/PressReleaseDetail.aspx?PRID=1919249>
2. Dattoo, MS, Natama HM, Somé A, et al. (2022). Efficacy and immunogenicity of R21/Matrix-M vaccine against clinical malaria after 2 years' follow-up in children in Burkina Faso: a phase 1/2b randomised controlled trial. *Lancet Infect Dis.* 2022 Dec; 22(12): 1728-36.
3. <https://www.who.int/campaigns/world-malaria-day/2023#>
4. pp.xx-xxi. Trends in the burden of malaria. This year's report at a glance. *World Malaria Report 2022.*
5. p.20. Estimated malaria cases and deaths in the WHO South-East Asia Region, 2000–2021. *Global trends in the burden of malaria. World Malaria Report 2022.*
6. p.167 Annex 3-D. WHO South-East Asia Region. *World Malaria Report 2022.*
7. Sinnis P, Zavala F. The skin: where malaria and the host immune response begin. *Semin Immunopathol* 2012 34:787-792.
8. Ashley EA, Pyae Phyo A, Woodrow CJ. Malaria. *Lancet.* 2018 Apr 21; 391(10130):1608-1621.
9. Parekh FK, Hernandez JN, Krogstad DJ, Casapia WM, Branch OH. Prevalence and risk of *Plasmodium falciparum* and *P. vivax* malaria among pregnant women living in the hypoendemic communities of the Peruvian Amazon. *Am J Trop Med Hyg.* 2007 Sep; 77(3):451-7.
10. Pathak S, Rege M, Gogtay NJ, Aigal U, Sharma SK, Valecha N, Bhanot G, Kshirsagar NA, Sharma S. Age-dependent sex bias in clinical malarial disease in hypoendemic regions. *PLoS One.* 2012; 7(4):e35592.
11. Dini S, Douglas NM, Poespoprodjo JR, Kenangalem E, Sugiarto P, Plumb ID, Price RN, Simpson JA. The risk of morbidity and mortality following recurrent malaria in Papua, Indonesia: a retrospective cohort study. *BMC Med.* 2020 Feb 20; 18(1):28.
12. Ezema CA, Okagu IU, Ezeorba TPC. Escaping the enemy's bullets: an update on how malarial parasites evade host immune response. *Parasitol Res* 2023 122:1715-31.
13. Chandley P, Ranjan R, Kumar S, Rohatgi S. Host-parasite interactions during *Plasmodium* infection: Implications for immunotherapies. *Front Immunol* 2023 Jan 4; 13:1091961.
14. Akinosoglou KS, Solomou EE, Gogos CA. Malaria: a haematological disease. *Hematology.* 2012 Mar; 17(2):106-14.
15. Erhart LM, Yingyuen K, Chuanak N, Buathong N, Laoboonchai A, Miller RS, et al. Haematological and clinical indices of malaria in a semi-immune population of western Thailand.

Am J Trop Med Hyg. 2004; 70: 8-14.

16. Wickramasinghe SN, Abdalla SH. Blood and bone marrow changes in malaria. *Baillieres Best Pract Res Clin Haematol.* 2000; 13: 277-299.
17. Facer CA. Hematological aspects of malaria. In: *Infection and Hematology.* Oxford: Butterworth Heinmann Ltd., 1994; 259-94.
18. Haldane JBS. Disease and evolution. *Ric Sci Suppl.* 1949;19:3-10.
19. Allison AC. Protection afforded by sickle-cell trait against subtertian malarial infection. *Br Med J.* 1954;1(4857):290-294.
20. MalariaGEN MG, Network E. Reappraisal of known malaria resistance loci in a large multicenter study. *Nat Genet.* 2014;46(11):1197-1204.
21. Quaresima V, Agbenyega T, Oppong B, Awunyo JADA, Adu Adomah P, Enty E, Donato F, Castelli F. Are Malaria Risk Factors Based on Gender? A Mixed-Methods Survey in an Urban Setting in Ghana. *Trop Med Infect Dis.* 2021 Sep 2;6(3):161.
22. Okiring J, Epstein A, Namuganga JF, Kanya EV, Nabende I, Nassali M, Sserwanga A, Gonahasa S, Muwema M, Kiwuwa SM, Staedke SG, Kanya MR, Nankabirwa JI, Briggs J, Jagannathan P, Dorsey G. Gender difference in the incidence of malaria diagnosed at public health facilities in Uganda. *Malar J.* 2022 Jan 21;21(1):22.
23. Klein SL. Hormonal and immunological mechanisms mediating sex differences in parasite infection. *Parasite Immunol.* 2004 Jun-Jul;26(6-7):247-64.
24. Bernin H, Lotter H. Sex bias in the outcome of human tropical infectious diseases: influence of steroid hormones. *J Infect Dis.* 2014 Jul 15;209 Suppl 3:S107-13.
25. Newby G, Cotter C, Roh ME, et al. Testing and treatment for malaria elimination: a systematic review. *Malar J.* 2023 Sep 3; 22(1): 254.
26. <https://www.who.int/activities/diagnostic-testing-for-malaria>
27. Sathpathi S, Mohanty AK, Satpathi P, Mishra SK, Behera PK, Patel G, Dondorp AM. Comparing Leishman and Giemsa staining for the assessment of peripheral blood smear preparations in a malaria-endemic region in India. *Malar J.* 2014 Dec 30; 13: 512.
28. Bhattacharji LM, Singh J, Sen Gupta GP. A simple methylene blue-eosin substitute for Leishman and Giemsa stains. *Ind Med Gaz.* 1946 Oct; 81(10): 400.
29. Warhurst DC, Williams JE. ACP Broadsheet no 148. July 1996. Laboratory diagnosis of malaria. *J Clin Pathol.* 1996 Jul; 49(7): 533-8.

30. WHO World Malaria Report 2020: India continues to make Impressive Gains in reduction of Malaria Burden. <https://www.pib.gov.in/pressreleaseiframepage.aspx?prid=1677601>
31. Okiring, J., Epstein, A., Namuganga, J.F. *et al.* Gender difference in the incidence of malaria diagnosed at public health facilities in Uganda. *Malar J* **21**, 22 (2022).
32. Kakuru A, Roh ME, Kajubi R, Ochieng T, Ategeka J, Ochokoru H, et al. Infant sex modifies associations between placental malaria and risk of malaria in infancy. *Malar J.* 2020;19:449.
33. Anvikar AR, Shah N, Dhariwal AC, Sonal GS, Pradhan MM, Ghosh SK, Valecha N. Epidemiology of Plasmodium vivax Malaria in India. *Am J Trop Med Hyg.* 2016 Dec 28;95(6 Suppl):108
34. Douglas NM, Nosten F, Ashley EA, Phaiphun L, van Vugt M, Singhasivanon P, White NJ, Price RN. Plasmodium vivax recurrence following falciparum and mixed species malaria: risk factors and effect of antimalarial kinetics. *Clin Infect Dis.* 2011 Mar 1;52(5):612-20.
35. Zimmerman PA, Ferreira MU, Howes RE, Mercereau-Puijalon O. Red blood cell polymorphism and susceptibility to Plasmodium vivax. *Adv Parasitol.* 2013;81:27-76
36. Clark MA, Kanjee U, Rangel GW, Chery L, Mascarenhas A, Gomes E, Rathod PK, Brugnara C, Ferreira MU, Duraisingh MT. Plasmodium vivax infection compromises reticulocyte stability. *Nat Commun.* 2021 Mar 12;12(1):1629.
37. Buchholz K, et al. A high-throughput screen targeting malaria transmission stages opens new avenues for drug development. *J. Infect. Dis.* 2011;203:1445–1453

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