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

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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

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municipal clinics, supported by microscopic, hematological, and microbiological evidence, we aim to provide insights into this atypical pattern.

Materials and Methods: 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. Symptomatic individuals were tested for malaria microscopically using peripheral blood smear (PBS) and confirmed by employing the malaria antigen test (RMAT). Blood urea by enzymatic method and serum creatinine by Jaffes method.

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; hematological; 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-M™ malaria vaccine [2] 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].

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]. 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 degrees [14], 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].

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 al [10], 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.

1.1 Aims and Objectives

To undertake an epidemiological and demographic study amongst infected populations, based on microscopic evidence, and corroborated with relevant hematological, 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.

2. METHODOLOGY

2.1 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 (up to 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 [18].

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.

2.2 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 [19]. 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/µL or even less) [20].

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 µl 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.

2.3 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) [21,22]. 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.

2.4 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.Mixed infections can also be readily detected using this kit.

2.5 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.

2.6 Statistical Tools 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.

3. 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

Table 1. Biochemical and hematological parameters

Parameters	Gender	Malaria test Negative	Malaria test Positive	P Value
Means of the RBC counts	**Male**	4.77 × 10^6/µL	4.30 × 10^6/µL	0.003
	Female	3.91 × 106/µL	3.75× 10^6/µL	0.021
	Total	4.41 × 10^6/µL	4.23 × 10^6/µL	0.008
Blood Urea in mg/dl (Mean ± SD)	**Male**	18.38±0.34	11.21±0.38	<0.001
	Female	21.32±0.14	13.22±0.33	<0.001
Sr Creatinine in mg/dl (Mean ± SD)	**Male**	0.89±0.21	0.75±0.15	0.012
	Female	1.24±0.13	0.85±0.34	<0.001

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%).

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 × 106/µL) than those with mixed infection (3.55 × 106/µL, p = 0.002), while the patients with P. falciparum had a similar RBC count (3.83 × 106/µL) 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.

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.

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.

4. 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 [23]. 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 [24.]. 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% [25]. However, this may vary depending on the geographical location, climatic conditions, and vector species [26]. 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 [27]. 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 [28]. Another possibility is that P. vivax has a lower parasitemia than P. falciparum, which may reduce the hemolysis and destruction of RBCs [29]. However, these hypotheses need to be verified by further studies.

5. 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.

CONSENT

As per international standards or university standards, Participants' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standards or university standards written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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