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Gender Variation of Plasmodium Infection by Different Malarial Testing Techniques in District Hyderabad

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Abstract

Background: This study aimed to assess gender-based variation in Plasmodium infection among individuals in the Hyderabad district, using blood smear microscopy, immunochromatographic test (ICT), and real-time PCR (RT-PCR).

Methodology: A cross-sectional study was conducted in Hyderabad, Sindh, from June to July 2024. Finger-prick blood samples were collected from males and females of all age groups. Symptomatic individuals were initially screened using microscopy. ICT was employed for samples testing negative by microscopy, and RT-PCR targeting the Plasmodium-specific 18S rRNA gene (SSUrRNA) was performed for further confirmation. Data were analyzed using SPSS version 23 and presented as frequencies, percentages, and means.

Results: Blood samples were analyzed from 74 males (mean age: 28.94±16.9) and 53 females (mean age: 30.89±11.97). Microscopy revealed 21 (28.38%) male and 14 (26.42%) female positive cases. Of 92 microscopy-negative samples, ICT identified 12 (13.05%) positive cases. Among 80 ICT-negative samples, RT-PCR detected 10 (12.5%) additional positives. These findings suggest a slightly higher prevalence of Plasmodium in males.

Conclusion: The infection rate was marginally higher in males across all diagnostic modalities. Microscopy, supplemented by ICT, effectively identified both symptomatic and asymptomatic cases. However, RT-PCR remains the most sensitive method, capable of detecting infections missed by conventional tests.

Keywords: Malaria; Plasmodium; ICT; RT-PCR; blood smear

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Introduction

Malaria is one of the most harmful hazards of infection for humans, but it is still one of the most challenging tasks not only for Pakistan but for the rest of the globe (1, 2). It is a burden, especially for developing or less developed countries, to be considered as the third leading cause of death in the population. Until recently, only five Plasmodium species, including Plasmodium falciparum, P. vivax, P. ovule, and P. malaria, were identified as the only etiological agents (3). Among these species, P. Falciparum and P. vivax caused a significant threat in 2018 (4). P. falciparum is considered for 99.7% of determined plasmodium parasite cases in the WHO African region, 50% of cases in the WHO Southeast Asia region, 71% of cases in the eastern Mediterranean, and 65% in the western Pacific. P. vivax is the predominant parasite in the WHO region of the Americas, causing 75% of malaria cases (5).

According to the WHO, malaria is caused by parasites of the Plasmodium genus, transmitted to humans through the bites of infected female Anopheles mosquitoes, known as "malaria vectors"(6). Common symptoms of malaria include fever, fatigue, nausea, and headache, with more severe cases potentially leading to jaundice, coma, or death. Without careful treatment, there is a risk of recurring infections months later. Malaria is prevalent in tropical and subtropical regions (7). particularly around the equator, encompassing much of sub-Saharan Africa, Asia, and the Americas.

In 2023, Pakistan reported 2.7 million confirmed malaria cases. The most affected regions are Sindh and Balochistan, with Sindh Province alone accounting for 49.4% of cases (8). According to data from the health department, December 2023 saw 417,213 reported cases in Sindh, with Hyderabad accounting for 40,061 of these cases (9).

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Understanding gender differences in malaria burden is crucial for enhancing comprehension of factors influencing susceptibility to malaria, strategies for case management, and targeted interventions. Various factors, including social, cultural, and behavioral differences, may impact exposure to mosquito vectors, perception of illness, health-seeking behavior, and approaches to managing cases (10,12). Additionally, specific to gender, aspects like the interplay between malaria and pregnancy are well-documented (13,15). Moreover, biological differences between sexes could affect susceptibility to malaria infection, the progression from infection to clinical disease, and the ability to clear infections (16). Given these intricate relationships, the associations between gender and malaria are likely shaped by local epidemiological factors, demographics (such as age), and the specific malaria outcomes being studied (such as infection versus clinical disease).

Considering all these factors, his current study was designed to determine if there is any variation of plasmodium infection among male and female subjects in the Hyderabad district by blood smear, ICT device test, and RT-PCR.

Methodology

This cross-sectional study was conducted in the rural area of the Wheel Pak colony of the district of Hyderabad Sindh from June to July 2024 after getting approval from the institutional ethical review committee.. This period was selected explicitly as it represents the post-monsoon season in Sindh when standing water creates optimal breeding conditions for Anopheles mosquitoes, resulting in higher malaria transmission rates. The study included all the patients with malarial symptoms after informed consent. Participants were recruited using consecutive sampling from those presenting with malarial symptoms at local healthcare facilities. To minimize selection bias, standardized inclusion criteria were applied regardless of gender, age, or socioeconomic status. Detailed demographic information, including occupation, education level, and living conditions, was collected to control for potential confounding factors in the analysis.

Blood was drawn from the study participants and was examined for malarial parasites under a microscope. For quality control in microscopy, all positive and a random 10% of opposing slides were reexamined by a second microscopist blinded to the initial results. A third senior microscopist resolved any discrepancies. Standard operating procedures for slide preparation, staining, and examination were followed according to WHO guidelines. All the positive cases on slide microscopy were then tested for malarial antigen via slide test method using a malarial kit (SD Bio line Malaria Ag P. v test kit). Malarial parasite DNA was then again checked in all malarial positive cases of slide test using the PCR Method. The ChelexTM technique was used to extract DNA from peripheral blood collected in EDTA (BioRad, USA). For PCR quality assurance, positive and negative controls were included in each run, and 5% of samples were randomly selected for repeat testing to ensure reproducibility. The PCR laboratory participated in an external quality assessment program to validate the accuracy of the results. Real-time PCR targeted the 18S rRNA gene unique to the genus Plasmodium (SSUrRNA gene).

All the data were entered in SPSS version 23 for analysis, and they were presented as frequencies and percentages.

Results

Table 1 shows the age characteristics of the studied population. The overall 127 subjects were included in this survey. Among them, 74 were male subjects, and 53 of them were females. The mean age for the male subjects was 28.94±16.9 years with a minimum age of 8 years and 71 years maximum age. For the females, the mean age was 30.89±11.97 with a minimum age of 12 years and 56 years maximum age.

Table 1: The age characteristics of the studied population Age (years)

Age (years)			
	Mean±SD	Minimum	Maximum
Male(n=74)	28.94±16.9	8	71
Female(n=53)	30.89±11.97	12	56
Total(n=127)	29.75±15.02	8	71

The blood slides were prepared for all of the samples collected from the subjects with malaria symptoms and microscopically examined. The results of the microscopic examination are illustrated in Table 2. The slide examination was positive for 21 (28.38%) male subjects and 14 (26.42%) female subjects.

Table 2: Microscopy results for the blood slides stained for material parasites

Microscopy		
	Negative	Positive
Male(n=74)	53 (71.63%)	21 (28.38%)
Female(n=53)	39 (73.59%)	14 (26.42%)
Total(n=127)	92 (72.45%)	35 (27.56%)

A total of 92 (72.45%) subjects who were negative with the slide examination were tested using the ICT malaria (P.f. /P.v.) malaria device, the results are shown in table 3. The ICT device test was positive for 7 (13.21%) male subjects, while it was negative for the remaining 46 (86.8%) male subjects. The same test results were positive for 5 (12.83%) female subjects, while 34 (87.18%) were negative.

Table 3: ICT malaria (p.f/p.v) device test for the subjects remained negative with microscopy and ICT malaria device (p.f/p.v) test

ICT malaria (p.f/p.v)		
	Negative	Positive
Male(n=53)	46 (86.8%)	7 (13.21%)
Female(n=39)	34 (87.18%)	5 (12.83%)
Total(n=92)	80 (86.96%)	12 (13.05%)

The subjects remained negative with blood smear observation, and ICT devices were tested for malaria positivity using a PCR test. Samples from a total of 80 (86.96%) subjects were amplified using the PCR test for malaria; among these, 46 were males, and 34 were females. (Table 4). Figure 1 shows the relative contribution of the three assays performed for the diagnosis of malaria.

Table 4: The PCR malaria assay test results for the subjects remained negative with microscopy and ICT malaria device (p.f/p.v)

	PCR	
	Negative	**Positive**
Male(n=46)	40 (86.96%)	6 (13.05%)
Female(n=34)	30 (88.24%)	4 (11.77%)
Total(n=80)	70 (87.5%)	10 (12.5%)

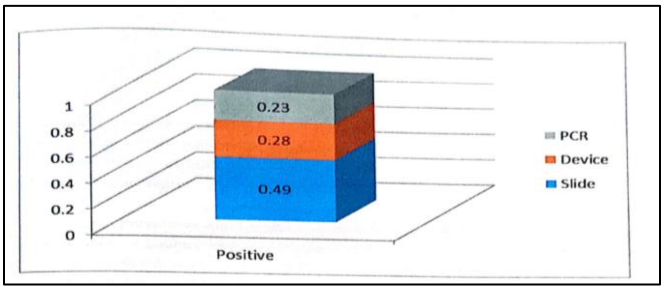

Figure 1: Contribution of each test in malarial diagnosis among study participants

The mean values for WBC count for the total subjects were 6269-884; no significant differences in the mean values were found among different groups based on the sex of the subjects. No significant difference was observed in the mean values of parasite load for both sex groups. The mean values (* SD) for parasitic load are given in Table 5. A significant (0.002) negative correlation (-0.276) was observed between parasitic load and WBCs count. (Table 5)

Table 5: Values for white blood cells (WBC) count and parasite densities in subjects positive with microscopy

WBCs				Parasites		
Mean		**Min**	**Max**	**Mean**	**Min**	**Max**
Male n=21	6338 ±995	5097	8977	5554 ±4428	941	21580
Female n=14	6165 ±706	4940	7516	4119 ±2845	561	12300
Total n=35	6269 ±884	4940	8977	4980 ±3890	561	21580

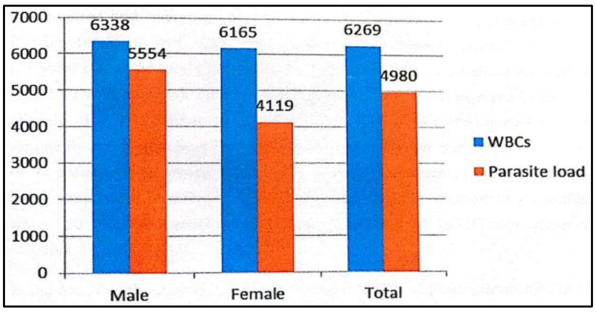

Figure 2: Correlation between parasite load and WBC count among study participants

Discussion

Malaria is an alarming health problem in Pakistan. It has been difficult to evacuate the malaria infection in Pakistan because the diversity in species and distribution of malaria-causing parasites in various components of the country has not been clearly described.

Malaria infection rates were higher in males than females, consistent with findings from a previous study [10]. Our descriptive analysis showed a slightly higher prevalence of malaria infection in males than females across all testing methods, although the current study design and sample size did not allow for multivariate analysis to control for potential confounding factors.

Several factors may explain the higher malaria prevalence among males in our study. First, occupational exposure plays a critical role, as men in rural Hyderabad are likelier to work outdoors in agriculture and construction, particularly during dawn and dusk when mosquito activity peaks (24). A study by Finda et al. (10) demonstrated that outdoor occupations increased exposure to Anopheles mosquitoes by 5.3 times compared to indoor activities. Second, behavioral differences contribute significantly - males in our region typically wear clothing that leaves more skin exposed and are less likely to use preventive measures such as insect repellents or bed nets (25). Third, immunological factors may be involved, as research by Klein and Flanagan (26) has shown that females generally mount more potent immune responses to parasitic infections due to hormonal influences, particularly estrogen, which enhances both innate and adaptive immunity against Plasmodium parasites.

Additionally, healthcare-seeking behavior differs between genders in our study population. Males often delay seeking treatment until symptoms become severe, resulting in higher parasite densities upon presentation, while social norms and family responsibilities may compel females to seek earlier treatment (27). Fischer et al. (16) have documented that sex-based immunological differences affect both susceptibility to infection and parasite clearance rates. This aligns with our observation of higher parasite densities in male patients despite comparable WBC counts between genders.

Several hypotheses suggest reasons for this disparity: males may have increased exposure due to physical labor and travel to areas where Plasmodium infections are prevalent, while females may face barriers to accessing healthcare and may delay seeking treatment compared to males. Studies have indicated a potentially higher prevalence of Plasmodium parasites in males compared to females (18--19). Once patients with suspected malaria were presented at health facilities, there were no observed gender differences in the likelihood of undergoing malaria testing or the type of diagnostic tests administered.

While one study was conducted in Uganda to determine gender differences in malaria diagnosis, they found results opposite to ours. In their study, they found a higher prevalence of malaria among females as compared to males (20). In another study examining the variation of Plasmodium about asymptomatic malaria in a tribal population in a forested village in India, it was found that females were more affected by Plasmodium (21). In contrast, our study showed that males were more affected while females were less impacted. This difference can be attributed to the different seasons and locations of the studies. The Indian study occurred in a hilly village during the monsoon seasons of 2013 and 2014, whereas our study was conducted near lakes and ponds. Consequently, males were more affected than females in our research.

In Hyderabad, Sindh, where our study was conducted, males were particularly challenged by malaria infection. Blood samples were tested using ICT devices, which are reliable for diagnosing symptomatic and asymptomatic patients. Although PCR testing is highly sensitive for detecting malaria in asymptomatic and negative blood smear cases, it is expensive and thus primarily used for symptomatic cases in adults, children, and chronically ill patients. In our study, we used PCR testing only for slide and device-negative cases due to its high cost.

Different methods were tested: if a slide was positive, we did not perform the device test or PCR; if a slide was negative, we proceeded with the device test. This approach contrasts with the burden of asymptomatic malaria among the tribal population in a forested village in India. Similarly, other studies have noted that children can act as reservoirs for parasites, aiding in the transmission of malaria.

Slide microscopy is more cost-effective than other methods. However, it is limited by potential human error and requires expertise from the observer (22).

Coleman and colleagues conducted an extensive surveillance study in Thailand, comparing PCR and microscopy for detecting asymptomatic malaria in a P. falciparum/vivax endemic area. They concluded that PCR is a more accurate and reproducible method for identifying and detecting malarial parasite species, although its effectiveness significantly decreases at low parasite densities, specifically below 500/μl (23).

Conclusion

We found a slightly more significant proportion of males affected by malaria than females in the community, including children. PCR is a more accurate technique in malaria diagnosis.

Ethical Approval:
This study was approved by the Institutional Review Board, Department of Physiology, University of Sindh, Jamshoro, Pakistan.
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Data Availability: Data supporting the findings are available upon reasonable request.

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Authors' Contribution:
AMM, NS: Concept and study design, acquisition, analysis interpretation of data, drafting the manuscript, approval of the final version to be published
ZN, JAZ: Acquisition of data, drafting the manuscript, approval of the final version to be published
FI, RKA: Concept and study design, analysis and interpretation of data, critical review, approval of the final version to be published.
All Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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