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Original Research Article

PREVALENCE OF MALARIA IN PATIENTS ATTENDING TERTIARY CARE CENTRE IN MUMBAI

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ABSTRACT

The study aimed to find the prevalence of malarial parasitic infection among patients referred to a tertiary care hospital in Mumbai between January 2021 and December 2023. A total of 61251 samples were tested during the study period of three years. Positivity for malarial antigen was detected in 7.31%, 4.65% and 3.47% samples in years 2021, 2022 and 2023 respectively. The proportion of malaria positive samples was higher in year 2021 as compared to 2023. Infection prevalence in male patients was 2.6-fold higher than in female patients. From year 2021 to 2023, there was reduction in malarial cases in males from 9.8% to 5.50 % and in females from 3.86% to 1.55%, but there was increase in male and female ratio of malarial cases. Highest malarial cases were found in age group 21 to 30 year followed by age group 31-40 in these three years. Plasmodium vivax species was found in highest number of samples in all the consecutive three years followed by Plasmodium falciparum and mixed species. These findings compared with other findings of malaria endemic populations in India.

Keywords: Retrospective, Malaria antigen, RDTs, prevalence.

INTRODUCTION

Malaria is a life-threatening disease caused by protozoan parasites of the genus Plasmodium which includes Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Plasmodium ovale and Plasmodium knowlesi.[1] Malaria remains a major public health problem in India, despite being both preventable and treatable.

In all settings, clinically suspected malaria should be confirmed with a parasitological test. Rapid diagnostic tests (RDTs) are immunochromatographic assays that detect parasite-specific antigens. Currently used tests commonly rely on detecting HRP2, which is specific to Plasmodium falciparum, while Plasmodium lactate dehydrogenase (pLDH) can be either pan-specific or species-specific.[2]

Among these. Plasmodium falciparum and Plasmodium vivax pose the greatest threat to human health. Malaria remains a major public health problem in India, despite being both preventable and treatable. Symptoms of malaria include fever, headache, vomiting and other non-specific flu-like symptoms. If appropriate drugs are not timely administered or there is parasite resistance to treatment, the infection can result in life-threatening anaemia, coma and death.[3]

In 2022, there were an estimated 249 million cases of malaria and 6,08,000 deaths across 85 countries. While in 2023, India accounted for half of all estimated malaria cases in South East Asia region, followed by Indonesia, which accounted for just under one third.[4]

In all settings, clinically suspected malaria should be confirmed with a parasitological test. Rapid diagnostic tests (RDTs) are immunochromatographic assays that detect parasite-specific antigens. Some tests detect genus-specific or species-specific antigens, such as pan-LDH (lactate dehydrogenase) and HRP2 (histidine-rich protein 2), respectively. Currently used tests commonly rely on detecting HRP2, which is specific to Plasmodium falciparum, while Plasmodium lactate dehydrogenase (pLDH) can be either pan-specific or species-specific.[2]

Prompt and accurate diagnosis of malaria is a crucial part of effective disease management, as it helps

avoid unnecessary treatment with antimalarial drugs. With the current drive towards elimination of malaria by 2027, WHO has largely advocated the policy of "test track and treat" to improve the quality of care and surveillance.

The magnitude of malaria cases, the factors contributing to demographics/seasonal trends of malaria varies from place to place. Although this is a single-centre study, understanding the prevalence of malaria over the past three years provides valuable insights into the burden of the disease, demographic and seasonal trends in the present settings.

India is slated for elimination of malaria by 2027, a retrospective study can provide useful details in analysing trends, changes in the burden of malaria and the control measures and challenges faced by the nation in elimination of the disease. Hence, such a study will facilitate in health-related planning and resource allocation, to improve accessibility and quality of health care services.

Aim and objectives of the study:

- To study the prevalence of malarial parasitic infection among patients referred to a tertiary care hospital in Mumbai.
- To study the demographics of the population and identify seasonal trends in malaria cases.
- To determine any trend in the frequency of association of the different species of malaria with seasonal variation.

MATERIALS AND METHODS

Settings: This study was carried out at Department of Microbiology, a tertiary care center at Mumbai over a period of three years from January 2021 December 2023.

Study design: Retrospective observational study.

Statistical test: Descriptive statistics was applied to determine the prevalence of malaria patients tested.

Inclusion criteria

All patients whose blood specimens had been received for the investigation of acute febrile illness during the study period. All blood specimens were tested using a rapid malaria antigen test (RMAT) according to standard protocol.

Ethical clearance

Ethical clearance was obtained from the Institutional Ethical Committee. (IEC(III)/OUT/795/2024)

Sampling strategy: The patients name, age, gender was recorded in the requisition form.2-3 ml blood specimen of OPD patients was collected taking aseptic precautions as per the standard protocol. For all IPD patient's blood was collected in the ward in an EDTA container and sent to the department of Microbiology for Rapid Malarial antigen testing.

Methodology: All blood specimens were tested using a rapid malaria antigen test (RDTs) according to standard protocol. A commercially available assay Malarigen Malaria Pf/Pv Rapid Card (Aspen Diagnostics) was used to detect malarial parasite antigens from P. falciparum and P. vivax. The test was performed as per manufacturer's instructions (kit instructions). 2-3 ml of blood was collected in EDTA vacutainer as per the standard protocol.5 µL sample of whole blood was dispensed into the sample cuvette, followed by the addition of the buffer. This card detects malarial infection in human blood by using HRP2 (Histidine-Rich Protein II) for P. falciparum and pLDH (Plasmodium Lactate Dehydrogenase) for P. vivax. As the test sample flows through the membrane of the test device, the anti-pLDH and anti-HRP2 colloidal gold monoclonal antibody conjugates bind with the respective antigens, if present in the lysed sample. This complex moves along the membrane to the test region, where it is immobilized by the anti-pLDH and anti-HRP2 monoclonal antibodies coated on the membrane, resulting in the formation of pink-coloured lines, confirming a positive test result.

RESULTS

A total of 61,251 suspected cases were included over three consecutive years, out of which 3075 cases (95.02%) were positive for malaria.

Table 1: Year-wise percentage positivity of malaria cases

Year	Total tested samples	Number of Positives	Percentage positivity (%)
2021	18,690	1367	7.31
2022	19,674	914	4.65
2023	22,887	794	3.47

The proportion of malaria positive samples was higher in 2021 as compared to 2023.This difference was statically significant ($p < 0.001$)

Table 2: Male and female ratio of malarial cases in year 2021 to 2023

Sr. No.	Year	Total Samples Tested	Male : Female Ratio
1	2021	18,690	2.5 : 1
2	2022	19,674	2.3 : 1
3	2023	22,887	3.2 : 1

There is increase in male and female ratio of malarial cases from year 2021 to 2023.Malaria was found more in male population than females every year. While in year 2023 it was the highest.

Figure 1: Gender wise distribution of malarial cases (%) from year 2021 to 2023

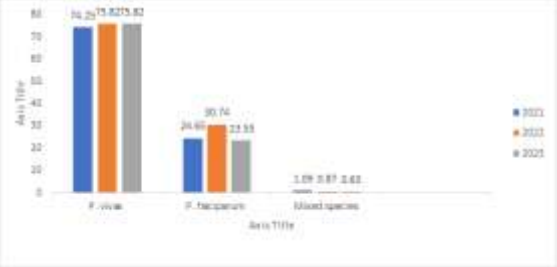

Figure 2: Various species of Plasmodium (%) detected in 2021-2023

Malarial cases were more predominant in males than females. From year 2021 to 2023, there is reduction in malaria cases in males from 9.8% to 5.50 % and in females from 3.86% to 1.55%. The proportion of malaria positive samples was higher in males as compared to females in three consecutive years. This difference was statically significant (p <0 .001). The proportion of malaria positive samples was higher in year 2021 than 2022 and 2023 in males. This difference was statically significant (p <0 .001). The proportion of malaria positive samples was higher in year 2021 than 2022 and 2023 in females. This difference was statically significant (p <0 .001).

P. vivax species was found in highest number of samples in all the three years. This difference was statically significant (p <0 .001). There was an increase in P. falciparum in 2022 as compared to its previous and subsequent years.

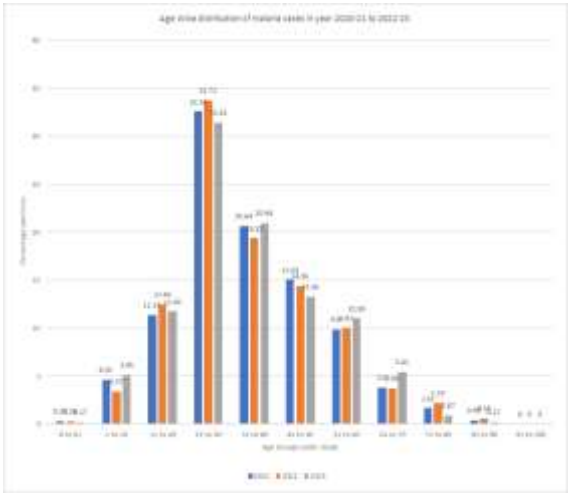

Figure 3: Age wise distribution of malarial cases from 2021 to 2023

Highest malarial cases were found in age group 21 to 30 year followed by age group 31-40 in these three years.

Table 3. Seasonal trend of Malaria from 2021 to 2023

Month	2021 (% Positivity)	2022 (% Positivity)	2023 (% Positivity)
January	5.20%	3.56	1.33
February	4.70%	2.60	0.92
March	7.28%	4.99	1.17
April	6.40	6.63	3.85
May	5.18	5.98	3.28
June	5.49	3.90	3.11
July	9.70	6.56	1.97
August	8.00	8.29	4.72
September	7.27	4.06	4.74
October	9.56	2.86	4.58
November	7.06	1.83	4.48
December	5.45	2.09	3.84

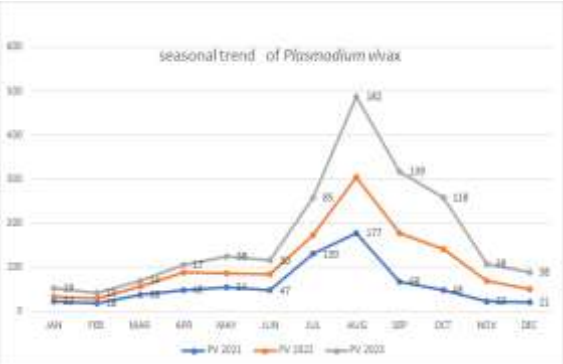

Figure 4: Seasonal trend of Plasmodium vivax from 2021 to 2023

Figure 5: Seasonal trend of Plasmodium falciparum from 2021 to 2023

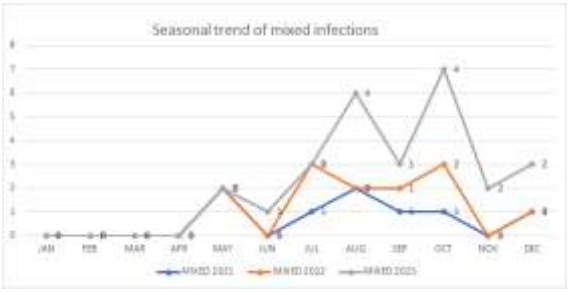

Figure 6: Seasonal trend of mixed infections (Plasmodium falciparum & Plasmodium vivax) from 2021 to 2023

DISCUSSION

The present retrospective study was carried out to determine the prevalence of malarial parasitic infection among patients attending a tertiary care hospital in Mumbai, who were referred for malaria antigen test. Further, to study the demographics of the population by correlating positive malarial parasitic infection findings with age and gender.

A total of 61,251 febrile cases were tested for three consecutive years using the Rapid Malaria Antigen Test. Prevalence of malarial infection in the present study was 7.31%, 4.65% and 3.47% in years 2021,2022 and 2023 respectively. The proportion of malaria positive samples was higher in 2021 as compared to 2023.This difference was statically significant ($p < 0.001$). Hadiya et al (2015),[5] from Gujrat and Karlekar SR et al,[6] from Gadchiroli (Maharashtra) have reported a prevalence of 2.10 % and 4.28% respectively. However, in a study by Singh G et al,[7] in New Mumbai and Pandey S et al,[8] at Bilaspur reported 16.58 % and 24.74 % respectively. This difference in prevalence could be due to the geographic differences and the seasons in which study was carried out.

The proportion of malaria positivity was higher in males as compared to females in all three consecutive years. This difference was statically significant ($p <0.001$). The ratio of male to female was found to be 2.6 :1, which is consistent with findings from studies by Mehta N et al,[9] Lavanya J et al,[10] Singh G et al,[7] Kannabath R et al,[11] Karlekar SR et al,[6] Joshi MM et al,[12] and Dayanand KK et al.[13]

Male preponderance in malaria cases is often attributed to factors such as body odour and hormonal influences, which may attract mosquitoes. Their increased exposure to mosquito bites is due to their travel habits, migration, movement in wider areas and outdoor working conditions. Also due to unknown inherent susceptibility and different healthcare facilities male preponderance may be observed.[7]

Over the past three years, (year 2021 to 2023), a gradual decline in malaria positivity has been observed. Lavanya J et al,[10] reported a decline in positivity in 2021 due to the COVID-19 pandemic, where overlapping clinical symptoms and the diversion of healthcare resources for pandemic control contributed to significant underreporting of malaria. However, no such experience was encountered at the present tertiary care institute. While there was a decrease in the number of patients seeking laboratory services, the percentage positivity remained relatively stable. Studies by Kannabath R et al,[11] and Chaya AK et al,[14] showed a gradual decline in cases too.

The present study reported, the highest number of malaria cases in the age group of 21 to 30 years, followed by the 31 to 40-year category. Joshi MM et al,[12] Singh G et al,[7] Lavanya J et al,[10] Chaya et al,[14] and Jivabhai HT et al,[15] reported maximum number of cases in age group of 21 to 30 years. Karlekar SR et al,[6] reported mean age group of 24.8 years and Saher S et al,[16] reported age group of 16 to 30 years. The reason of higher prevalence of malaria in age group of 21 to 30 year could be due to movement in wider areas, possibly endemic and constant migration in search of livelihood, which exposes individuals to more mosquito bites.

The predominant malaria parasite in the present study was P. vivax (75.12%), followed by P. falciparum (23.96%) and mixed infections (0.91%). The proportion of malaria positive samples with P. vivax was higher as compared to P. falciparum in three consecutive years. This difference was statically significant ($p <0.001$). The differences in prevalence of P. vivax and P. falciparum in different areas can be due to presence of endemicity of a particular type and high relapses in vivax type. Various other studies in India have been compared with the present study in [Table 4].

Table 4: Comparison of the present study with others.

Author	Year	Place	Observation
Mehta N et al.[9]	2011-12	Mumbai	P. vivax 64.13%. P.falciparum 32.6% Mixed 3.25%
Singh G et al.[7]	2013	Navi Mumbai	P. vivax 54.76% P. falciparum 17.8% Mixed 27.44%
Chaya AK et al.[14]	2014	Mumbai	*P.* vivax 71%, P. falciparum 25% Mixed 3.8%
Lavanya J et al.[10]	2015-2021	Chikkamangalaru	P. vivax 70% , P. falciparum 25% Mixed 5%
Hadiya et al.[5]	2015	Gujrat	P. vivax 61.41%, P. falciparum 38.56%
Naz R et al.[17]	2016	Haryana	P. vivax 73.9% , P. falciparum 24.6% Mixed 1.9%
Thavare s et al.[18]	2016-2017	Mumbai	P. vivax 78.7%,P. falciparum 13.8% Mixed 7.5%
Kk Dayanand et al,[13]	2019	Mangalore	P. vivax 64.13%, P. falciparum 32.6% Mixed 3.25%
Kannabath et al.[11]	2021	Pondicherry	P.vivax 64.13%. P. falciparum 32.6% Mixed 3.25%
Present study	2021-2023	Mumbai	P. vivax 75.12%, P. falciparum 23.96% Mixed 0.91%

Peripheral blood smear examination has always been considered the gold standard for the detection of malaria. The limit of detection of the thick smear is approximately 5–10 parasites/µL, while that of the

thin smear is around 50–100 parasites/µL. Microscopy remains the most affordable, widely available, and cost-effective diagnostic tool, enabling quantification of parasitemia and monitoring of parasite clearance. It also facilitates differentiation between asexual and sexual stages of the parasite. However, distinguishing morphological stages of Plasmodium knowlesii and Plasmodium falciparum can be challenging, and accurate diagnosis requires a well-trained and experienced microscopist.

Rapid diagnostic kits are easy to perform, require minimum training and expertise and can be used in settings where microscopy is unavailable and where diagnosis is required by clinicians without delaying the treatment of patients. Antigens commonly available used are

1. P. falciparum specific antigen HRP2.
2. A pan P. falciparum specific lactic acid dehydrogenase.
3. A pan specific P. vivax specific lactic acid dehydrogenase.
4. A pan plasmodium lactic dehydrogenase.
5. Aldolase which is a pan plasmodium antigen.

In the present study, we used Rapid Malaria Antigen Tests (RDTs) based on HRP2 & pan LDH was used. This test took 15-20 minutes and easy-to-use. It allowed rapid diagnosis. Rapid tests can be used for diagnosis, outbreak investigation, and surveys. However, RDTs may have limitations in sensitivity and do not provide parasite density data, so they are often used in conjunction with other diagnostic methods, when appropriate.[7]

Limitations: Malaria prevalence is influenced by local factors such as climate, vector density, altitude, and water bodies. A single-centre study may not capture these environmental variations that exist across different regions. Being a single centric study, it is difficult to ascertain the actual prevalence/burden of the disease. However, in the present study patients with acute febrile illness were included,70% of whom were from Mumbai and other 30% were from the other parts of the state with the data available as per records. Therefore, this could represent the regional prevalence. Histidine-rich protein 2 (HRP2), a commonly targeted antigen in Plasmodium falciparum rapid diagnostic tests (RDTs), is known to persist in the bloodstream for up to 3 weeks or more following effective treatment. This extended circulation can lead to false-positive RDT results, making it challenging to differentiate between active infection and recently treated cases. Therefore, persistent positivity couldn't be ruled out in HRP2 positive cases. Microscopy was not carried out to confirm the positive results.

CONCLUSION

The present study reveals a high rate of malarial infection in all three consecutive years and also demonstrate a downwards trend in positivity over 3 years. This decline may reflect improvements in vector control, public health interventions, or changes in health-seeking behaviour.

Together these findings indicate that the routine "Test track and treat" policy and vector control in Mumbai are yielding gains. Transmission remains amplified in the monsoons. Plasmodium vivax remains the most prevalent species), consistent with increased vector breeding and transmission during this period. Though there was decline in the total number of cases, the proportion of P.falciparum malaria remains almost the same.

The study also emphasizes the importance of species identification for appropriate clinical management and prognosis, especially in cases of severe malaria. This study reinforces the need for robust surveillance, rational antimalarial use, and sustained investment in diagnostic infrastructure to support national malaria control and elimination goals.

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