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Original Article

Seasonal Trends with the Comparison of Venous and Capillary Blood for Diagnosis of Malaria among Children in Karachi, Pakistan

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Abstract

Background: More than 250 million people are infected by malaria parasites annually while around one million children less than 5 years of age die every year due to malaria. We aimed to assess the seasonal trends and usefulness of capillary and venous blood for rapid diagnosis of malaria.

Methods: This cross-sectional study of 18 months duration was conducted at the National Institute of Child Health (NICH), Karachi. All patients reporting fever as chief complaint were recruited as study subjects. A semi-structured questionnaire was used to collect demographic information, presenting complaints, awareness of caregivers regarding malaria, preventive measures and history of malaria fever. Three ml Venous (2-3ml) as well as peripheral blood (3-4 drops) samples of all patients were collected for microscopy and rapid diagnostic tests (RDTs).

Results: Out of total 477 patients with fever Venous and Capillary Blood RDTs methods detected 33(6.9%) and 30(6.3%) as the malaria positive while Venous and Capillary Blood Microscopy detected 30(6.1%) and 32(6.7%) cases respectively. *Plasmodium Vivax* infection was the most prevalent (87.9%) and majority (39.39%) of the cases occurred in the quarter, July to September.

Conclusion: July to September is the peak season for malaria and *P. Vivax* (87.9%) is the predominant strain in Karachi. Venous and capillary blood are equally useful for malaria diagnosis however, convenience and less invasiveness may justify the preference of capillary blood over venous blood for early diagnosis of malaria.

Introduction

Malaria is the major cause of morbidity and mortality in tropical and subtropical countries; accounts for more than 3/4th of the global infectious burden (1). Malaria remains a life-threatening issue and creates devastating effects on the health of children (2), especially, on those who are malnourished and have low immunity status (3). It is transmitted to people through bites of infected mosquitoes. Four different species are responsible *for* the spread of malaria, which includes *Plasmodium falciparum*, *P. Vivax*, *P. ovale*, and *P. malariae* (4). Malaria kills one child under the age of five every 75 seconds. Many of these deaths are avoidable and treatable. In 2019, there were 229 million cases of malaria worldwide, with 558,000 deaths. Seventy four percent (416,000) of these deaths occurred in children under the age of five. This equates to nearly 750 children under the age of five per day. According to the WHO report on malaria in 2022, more than 60% of Pakistan's population resides in malaria-endemic regions (5). It is reported that 500,000 malaria infections and 50,000 malaria deaths occur each year in Pakistan (5, 6) at a hospital in southeastern Pakistan very ill children with a microscopic diagnosis of *P. Vivax* have long been noted (7). Of all the species, *P. falciparum* reported higher mortality rates than other malarial parasites. According to published work, 31% of the total reported cases of malaria in Pakistan are due to *P. falciparum* (8).

Malaria usually predominates in areas that provide favorable ground for their breeding such as tropical and subtropical regions, as they have optimal temperature and rainfall (9). Transmission of malaria in Pakistan is predominantly seasonal and particularly occurs in Khyber Pakhtunkhwa (KPK), the Baluchistan province, and the Sindh province (10). It is believed that transmission of malaria occurs in two seasons mostly in Sep to Nov after the monsoon season and with a small period of transmission in Mar and Apr (10-12).

The accurate diagnosis of malaria is key to the effective management of malaria patients to reduce complications and mortality rates. Clinical diagnosis of malaria is often difficult as the signs and symptoms of the disease mimic other tropical infections, hence laboratory diagnosis is usually required (13). Several techniques including microscopy, serology, and PCR are available for the diagnosis but microscopic examination of Giemsa's stained blood films is the most widely used method and considered the gold standard for the diagnosis of malaria owing to its higher sensitivity, ability to differentiate between different species, ability to show the level of parasitemia and cost-effectiveness (14). Blood films can be prepared using venous blood as well as capillary blood but the sensitivity of both types of specimens is a subject of debate nowadays. Some recent studies suggest that capillary blood specimen is more sensitive as compared to venous blood (15).

We focused on the pediatric population with high-grade fever visiting NICH; to assess the frequency, pattern, and seasonal variation and to compare the positivity rate of capillary and venous blood samples for diagnosis of malaria.

Materials & Methods

Study design, settings, and duration:

This was a cross-sectional study conducted at the National Institute of Child Health (NICH), Karachi, Pakistan for 18-month duration. A semi-structured questionnaire was used to record data.

Sample size

The sample size of 440 was calculated at a 95% confidence interval with 2% precision, using Epi software 6 based on our recently

published work that showed 10.3% of patients visiting OPD presented with a history of fever (16). As per hospital records, it is estimated that among the children who visited NICH OPD with complaints of high-grade fever 50% were under 5 years of age. In this way, the calculated Sample size was 440, we added 44 (10%) for non-compliance 440+44=488. Hence, the final sample size was 488.

Inclusion Criteria

Children irrespective of gender, less than 5 years of age, with a history of fever as primary complaint and visiting the Emergency department and OPD for treatment were included as study subjects.

Parents with written informed consent to provide information about the child, history, and mortality related to malaria in other family members, if any.

Exclusion Criteria

Children already taking anti-malarial drugs or those taken treatment for malaria in the last 24 h were excluded.

Methods

The study followed a purposive sampling technique. Parents/ caregivers of all children up to 5 years of age with the presentation of fever as a major cause to visit health facility; were contacted at emergency as well as OPD of NICH throughout the year (12 months). Total of 477 children < 5 years of age presented with fever as a major complaint at OPD or the Emergency department of NICH and were recruited for the study after taking informed consent(annexure-I).. Parents were interviewed for socioeconomic status, demographic information, recall of the history of malaria-related mortality, etc. (annexure-II). For the comparative part, the intravenous sample along with the peripheral blood sample of the study participant was subjected to the comparative diagnosis of microscopy and RDT, and information was collected on the additional sheet (annexure-III).

PI /CI rechecked all collected data via hospital records / via cell phones directly from the parents of participants. In case of any ambiguity about collected data, the cases were not included for final analysis and were considered dropouts.

Blood Sampling

Capillary blood was obtained from finger pricks and venous blood was obtained from cubital vein puncture. Capillary and venous sampling was performed in parallel within a 5-minute interval. Before sample preparation, blood from both sources was first collected in separate containers coated with ethylenediaminetetraacetic acid (EDTA) to prevent blood clotting (250 µL tubes for CAP blood and 3 mL tubes for VEN blood). After blood collection, the following steps were taken: For each patient, one thick two thick smears of both capillary and venous blood were made with standardized amounts of 10 µl, while 2 thin smears of capillary and venous blood were made with 5µl blood. One drop of venous blood was poured on one RDT kit while one drop of capillary blood was poured on another RDT kit (16).

Screening and comparing diagnostic sensitivity

Venous and peripheral blood of all clinically suspected malarial cases was tested for malaria using microscopy and rapid diagnostic kit (RDT) kits. Thick and thin smear slides of capillary blood were prepared for all study participants (12). The result of the RDT kit was immediately communicated to both the patient and the treating physician. The information was recorded on a pre-designed record sheet and semi-structured questionnaire.

Ethical Considerations

Ethical approval for the study was obtained from IERB of NICH via reference No. IERB/07/2015 (Annexure IV).

Data Analysis

Data entry and analysis were done using Statistical package for social sciences (SPSS) version 21.0 (IBM Corp., Armonk, NY, USA). Frequency and percentages have been calculated for categorical variables like gender, ethnicity, and test results while mean and ± standard deviation (S.D) has been calculated for numerical variables like age, number of mosquito nets, number of family members and number of family members etc. The chi-square test has been used to determine the association of different factors including socio-demographic features, knowledge and practices about Malaria, with malaria positivity. P-values less than 0.05 were considered statistically significant for all comparisons.

Results

Out of 477 patients recruited for the study after taking informed consent 200 (41.9%) were male and 277 (58.1%) were female. The mean age of study participants was 33.6 ± 8.7 months including 33 (6.9%) patients had a positive test for malaria collectively for all tests. Thirty (6.3%) patients yielded a positive test result for malaria on microscopy while 33 (6.9%) patients were positive for malaria by the rapid immuno-chromatographic method.

Among the total 477 participants 237 (50%) patients were accompanied by their mother at the health facility followed by their fathers 169 (35.43%), uncle 20 (4.2%), etc.

Socioeconomic status was calculated based on monthly income only, which showed that 445 (93.3%) patients belonged to the low-income group with a monthly income of less than PKR 20,000/= (*i.e.* less than 200 USD). The majority of respondents *i.e.* 274 (57.44%) had no formal education at all while 88.8% had an education below or up to-matriculation level. No significant difference (*P*-value >0.05) in malaria-positive or negative cases was seen in terms of ethnicity, socioeconomic status, or literacy (Table 1).

Table 1: Association of gender, ethnicity, education level and monthly income on frequency of malaria

Variable	*Total (n=277)*	*Malaria Positive (n=33)*		*Malaria Negative (n=244)*		*P-value*
Gender						
Male	204	11	5.7	193	94.6	0.17
Female	273	22	8.8	251	91.9	
Ethnicity						
Sindhi	97	8	9.0	89	91.7	0.93
Urdu	127	8	6.7	119	93.7	
Punjabi	62	4	6.9	58	93.4	
Saraiki	15	1	7.1	14	92.9	
Pashto	108	9	9.1	99	91.6	
Balochi	12	0	0.0	12	100.0	
other	56	3	5.7	53	94.5	
Education level						
None	274	21	8.3	253	92.3	0.66
can read & write	13	0	0.0	13	100.0	
Primary	33	2	6.5	31	93.8	
less matric	31	3	10.7	28	90.0	
Matric	73	2	2.8	71	97.2	
Under	26	3	13.0	23	88.0	

graduate						
Graduate	22	2	10.0	20	90.5	
Post graduate	5	0	0.0	5	100.0	
MonthlyIncome						
<10000	87	6	7.4%	81	93.0%	0.53
11000-20000	356	26	7.9%	330	92.7%	
21000-30000	25	0	0.0%	25	100.0%	
>30000	9	1	12.5%	8	87.7%	

The mean duration of illness (*i.e.* fever) before visiting NICH was found to be 14.3± 15.1 with a minimum of 1 day and a maximum of 90 days. Mode is 7 *i.e.* most 52 (11%) patients reported on the 7th day of unresolved high-grade fever. Only 38 (5.7%) patients visited the hospital within three days of the onset of the fever and 24 (5%) tried self-treating the fever before visiting the hospital. There was a statistically significant difference (*P*-value <0.005) in the mean duration of illness between malaria-positive and malaria-negative cases, it was significantly higher among malaria-positive cases.

Regarding accessibility to NICH for patient treatment or care majority of 350 (73.4%), patients lived at a distance of more than 11 kilometers with 209 (43.8%) patients living more than 20 kilometers away from NICH and had to arrange conveyance for their visit. The majority 267 (56%) of them reported availing of public bus service for the purpose followed by motorcycles 118 (24.7%). The mean expenditure per visit per patient was PKR 155.9 ± 311.1 with the majority 365 (76.51%) having to pay less than 100 PKR (*i.e.*<1 USD). The majority 289 (60.6%) of attendants reported that it takes them more than an hour to be seen by the doctor. The majority 88.6% and 59.3% attendants reported that their children get free treatment and diagnostic tests from the hospital respectively.

Results of awareness and practices regarding malaria among attendants accompanying patients showed that a majority 364(76%)of the subjects were not using mosquito nets , 341 (71.48%) attendants said they have not heard about malaria while 398 (83.43%) attendants said that malaria can be prevented by killing mosquitoes. Chi square test of association showed a significant association i.e. *P*-value 0.00001 is less than alpha that was set to be 0.05 as significant Table 2. The most common symptoms besides fever in all suspected cases included headache 193 (40.5%), rigors 193 (40.5%), nausea/vomiting 189 (39.6%), diarrhea 182 (38.2%) and chills 166 (34.81%) however, no significant different (*P*-value >0.05) existed in malaria positive and malaria negative cases regarding these symptoms.

Out of 33 positive cases, 29 (87.9%) cases were *P. Vivax*, 2 (6.1%) were *P. falciparum* while two (6.1%) patients had a mix infection of *P. Vivax*, and *P. falciparum*.

Table 2: Association of Malaria related knowledge and practices with Malaria positivity

Variable	*Malaria Positive*	*Malaria Negative*	*P-Value*
Knowledge About Malaria			
Have you Heard about malaria?			
Yes	5	74	0 .69
No	28	341	
How malaria is transmitted?			
Mosquito	05	67	
Flies	00	02	
Air	00	07	0.717
Don't Know	05	42	
Malaria can kill if not treated			
Yes	03	51	0.897
No	14	165	
Don't Know	04	39	
Is Malaria preventable			
Yes	04	51	0.621
No	12	167	
Don't Know	04	31	
How Malaria can be prevented?			
By killing Mosquito	31	398	0.891
By Medicine	00	18	
Cleanliness	00	15	
Proper treatment	00	03	
By sleeping in net	00	03	
Precaution	01	03	
Vaccine	01	04	
Malaria preventive practices			
Has your house sprayed?			
Yes	0	08	0.646
No	20	262	
Use of Mosquito nets			
Yes	3	15	0.001
No	24	364	
Type of net			
Insecticide treated	2	10	1.0
Untreated	2	10	
Quantity of nets in home			
01	00	04	0.700
02	01	08	
≥03	00	02	

Discussion

Malaria is endemic in Pakistan and a rise in malaria cases is seen every year during the rainy season, which is attributable to the increase in mosquito activity during that time. In the present study, we observed that 477 children (less than five years of age) presented with suspected malaria (high-grade fever as the primary complaint) reported in OPD, and the emergency department of NICH.

The frequency of malaria among all fever cases was found to be 6.1% - 6.9% which shows that around 7% of fever cases being reported at NICH were malaria positive. This frequency is lower than that reported in a study from Southern Khyber Pakhtunkhwa in which 28.8% of patients reporting at the hospital with fever were found to be malaria positive but they adopted a strict case definition to include only suspected cases of malaria and not all fever cases (17).

Most of the patients presenting at the hospital had an unresolved fever for more than seven days, which depicts the health-seeking behavior of parents for their children. Various national and international studies have reported low levels of care seeking from outside the home in low and middle-income countries including Pakistan (18-19). These low levels are attributed to different factors including lack of awareness, resources, cultural and sociodemographic features, and poor health services system itself (18, 19). Besides fever, other reported symptoms included rigors and nausea/vomiting to be more common among malaria-positive cases. However, no significant statistical difference (*P*-value >0.05) -was observed for these symptoms in our study which might be due to the low frequency of malaria in our selected population

Regarding socio-demographic factors, our study did not suggest any significant difference with respect to residence type, education level, monthly income and race/ethnicity. Globally, studies have shown that ethnicity is an important determinant of malaria transmission and severity of disease (20, 21). Similarly genetic studies suggests that individual risk for malaria infection is complex and multifactorial, with host genetic factors contributing to 25% of individual variation in susceptibility to clinical malaria (20). However, our findings are in consensus with earlier studies from Pakistan (22-24).

Seasonal variation in terms of malaria frequency showed that while malaria cases are seen throughout the year but a peak was recorded during the quarter from July to Sep. This is in concordance with another study where since July to Sep is considered the rainy season in Karachi, Pakistan with moderate temperatures that facilitates the growth of mosquitoes (25).

As previous studies reported from different regions of Pakistan, our study also showed a predominance of *P. Vivax* infections in our population followed by *P. falciparum* and mixed infection of *P. Vivax* and *P. falciparum* (26-32).

Comparison of positivity rates of ICT, thin & thick smears, and capillary & venous blood showed a better performance of ICT in terms of positivity rate (6.9%) followed by capillary blood thick smear (6.7%) while the least positivity was observed in case of capillary blood thin smears (6.1%). However, there was no statistically significant difference between capillary and venous blood. Capillary blood microscopy is more sensitive as compared to venous blood for malaria (12) diagnosis but we though observed a comparatively high positivity rate in capillary blood thick smear but that was not significant. This may be due to a low overall positivity rate in our study since we included all fever cases not only suspected cases of malaria.

Conclusion

Quarter starting from July and ending at September is the peak season for malaria and *P. Vivax* (87.9%) is the predominant strain in Karachi. Use of either venous or capillary blood does not affect the sensitivity and specificity of the ICT or Microscopy tests, however, convenience and less invasiveness may justify the preference of capillary blood over venous blood for early diagnosis of malaria.

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Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Kifle ZD, Adinew GM, Mengistie MG, et al. Evaluation of antimalarial activity of methanolic root extract of *Myrica salicifolia* A rich (myricaceae) against *Plasmodium berghei*–infected mice. J Evid Based Integr Med. 2020; 25: 2515690X20920539.
2. Siddiqy MR. Urban environment and major challenges in sustainable development: experience from Dhaka City in Bangladesh. SE Asia J Public Health. 2017; 7(1):12-6.
3. Dapi Nzefa L, Monebenimp F, Äng C. Undernutrition among children under five in the Bandja village of Cameroon, Africa. South Afr J Clin Nutr. 2019; 32(2):46-50.
4. Khan K, Wali MN, Ayub M, ur Rehman F. 47. Malaria burden in human population of Khanozai, Pakistan. Pure and Applied Biology. 2018; 7(1):407-12.
5. World Health Organization. World malaria report 2022. World Health Organization; 2022. Available from: https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2022
6. Qureshi NA, Fatima H, Afzal M, Khattak AA, Nawaz MA. Occurrence and seasonal variation of human Plasmodium infection in Punjab Province, Pakistan. BMC Infect Dis. 2019; 19(1):935.
7. Khan W, Ur RA, Shafiq S, Khan K, et al. Malaria prevalence in Malakand district, the north western region of Pakistan. J Pak Med Assoc. 2019 ; 69(7):946-49.
8. Dayanand KK, Kishore P, Chandrashekar V, et al. Malaria severity in Mangaluru City in the southwestern coastal region of India. Am J Trop Med Hyg. 2019; 100(2):275-79.
9. Al-Awadhi M, Ahmad S, Iqbal J. Current status and the epidemiology of malaria in the Middle East Region and beyond. Microorganisms. 2021 9; 9(2):338.
10. Mironova VA, Shartova NV, Beljaev AE, Varentsov MI, Korennoy FI, Grishchenko MY. Re-introduction of *vivax* malaria in a temperate area (Moscow region, Russia): A geographic investigation. Malar J. 2020 ;19(1):116.
11. Umer MF, Zofeen S, Majeed A, Hu W, Qi X, Zhuang G. Spatiotemporal clustering analysis of malaria infection in Pakistan. Int J Environ Res Public Health. 2018;15(6):1202.
12. Woday A, Mohammed A, Gebre A, Urmale K. Prevalence and associated factors of malaria among febrile children in Afar region, Ethiopia: a health facility based study. Ethiop J Health Sci.2019; 29(5):613-622.
13. Njunda AL, Assob NJC, Nsagha SD, Kamga FHL, Mokenyu MD, Kwenti TE. Comparison of capillary and venous blood using blood film microscopy in the detection of malaria parasites: A hospital based study. Sci J Microbiol. 2013; 2(5): 89-94.
14. Mbanefo A, Kumar N. Evaluation of malaria diagnostic methods as a key for successful control and elimination programs. Trop Med Infect Dis. 2020; 5(2):102.
15. Fitri LE, Widaningrum T, Endharti AT, et al. Malaria diagnostic update: From convention-

al to advanced method. J Clin Lab Anal. 2022; 36(4):e24314.
16. Chagas CR, Binkienė R, Ilgūnas M, Iezhova T, Valkiūnas G. The buffy coat method: a tool for detection of blood parasites without staining procedures. Parasit Vectors. 2020; 13(1):104.
17. Kaboré JM, Siribié M, Hien D, et al. Attitudes, practices, and determinants of community care-seeking behaviors for fever/malaria episodes in the context of the implementation of multiple first-line therapies for uncomplicated malaria in the health district of Kaya, Burkina Faso. Malar J. 2022; 21(1):155.
18. Mischlinger J, Pitzinger P, Veletzky L, et al. Use of capillary blood samples leads to higher parasitemia estimates and higher diagnostic sensitivity of microscopic and molecular diagnostics of malaria than venous blood samples. J Infect Dis. 2018; 218(8):1296-1305.
19. Memon AA, Shahid A, Mustufa MA, et al. Pattern of disease, reason of visiting and waiting time at various stages of tertiary care hospital. Isra Medical Journal; 2013;5(3): 200-203.
20. Cassy A, Chicumbe S, Saifodine A, Zulliger R. Factors associated with malaria care seeking among children under 5 years of age in Mozambique: a secondary analysis of the 2018 Malaria Indicator Survey. Malar J. 2022; 21(1):1-8.
21. Arama C, Maiga B, Dolo A, et al. Ethnic differences in susceptibility to malaria: what have we learned from immuno-epidemiological studies in West Africa? Acta Trop. 2015; 146:152-6.
22. Ramdzan AR, Ismail A, Zanib ZM. Prevalence of malaria and its risk factors in Sabah, Malaysia. Int J Infect Dis. 2020; 91:68-72.
23. Haque U, Soares Magalhães RJ, et al. The role of age, ethnicity and environmental factors in modulating malaria risk in Rajasthali, Bangladesh. Malar J. 2011;10(1):367.
24. Khattak AA, Venkatesan M, Nadeem MF, et al. Prevalence and distribution of human Plasmodium infection in Pakistan. Malar J. 2013;12:297
25. Karim AM, Yasir M, Ali T, et al. Prevalence of clinical malaria and household characteristics of patients in tribal districts of Pakistan. PLoS Negl Trop Dis. 2021; 15(5): e0009371.
26. Hussain I, Qureshi N, Afzal M, Shaheen N, Ali A, Ashraf A. Prevalence and distribution of human *Plasmodium* infection in Federally Administrative Tribal Areas of Pakistan. Acta Parasitol. 2016; 61(3): 537-543.
27. Cassy A, Saifodine A, Candrinho B, et al. Care-seeking behavior and treatment practices for malaria in children under 5 years in Mozambique: a secondary analysis of 2011 DHS and 2015 IMASIDA datasets. Malar J. 2019; 18(1):1-0.
28. Khan NU. Frequency and seasonal variation of *Plasmodium* species in southern districts of Khyber Pakhtunkhwa. Pakistan Armed Forces Medical Journal. 2014; 64(4):518-23.
29. Regmi K, Kunwar A, Ortega L. A systematic review of knowledge, attitudes and beliefs about malaria among the South Asian population. Infect Ecol Epidemiol. 2016;6(1):10.
30. Ullah Z, Khattak AA, Aziz N. Haematological outcomes in progression of malaria: A cohort study from district Dera Ismail Khan, Pakistan. JPMA. 2020; 70(10):1830-3.
31. Khan MW, Khan MN, Khan RA. Epidemiology and parasitological survey of malarial parasites in Khyber Pakhtunkhwa, Pakistan. J Pak Med Assoc. 2018; 68(1):145-6.
32. Kalra S, Khandelwal D, Singla R, Aggarwal S, Dutta D. Malaria and diabetes. J Pak Med Assoc. 2017; 67(5):810-3.