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ORIGINAL ARTICLE

Diagnosis of Malaria Gametocytes Using MP ICT Vs PCR in Infected Patients: A Cross-Sectional Study of Asymptomatic Relatives

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ABSTRACT

In high-risk situations, asymptomatic malarial gametocyte carriers promote malaria transmission. It may spread illness if it is constantly available as a vast undetected reservoir of Malaria. Subjects with malarial gametocytes in their circulation but no symptoms may be a hidden reservoir of the illness.

Objectives: To determine the prevalence rate of subjects without symptoms who have gametocytes in their blood and live in the same housing as a malaria patient.

Methodology: This study was carried out at Mercy Teaching Hospital Peshawar and Khyber Teaching Hospital Peshawar. The study duration was 10 months from October 2 to December 28, 2020. Once malaria cases were identified, patients were asked about nearby family members who did not have symptoms or tested negative for malarial infection using ICT. All the data was collected and then analyzed by SPSS version 23.

Results: A total of 100 participants were included in the current study. Our results revealed that all participants showed a negative result for MP ICT. In contrast, Malarial gametocyte in blood PCR was detected among 18(18%) and not detected in 82(82%) of the study participants)

Conclusion: According to the study results, many asymptomatic close relatives of patients living in the same house have malarial gametocytes. The most often afflicted direct family members were the mother and father, followed by sisters. PCR is the only toolfor identifying malarial gametocytes, while ICT is ineffective at detecting carriers.

Keywords: Diagnosis, Malaria Gametocytes,Mp,Pcr,A Cross-Sectional Study

INTRODUCTION

The malaria cases in Pakistan, Afghanistan, Somalia, Sudan and Yemen account for more than 95% of the regional malaria burden. It has been reported that the incidence rate was 1.66/in 100 population, where Plasmodiumvivax accounted for more than 80% of the incidence; however, in Pakistan, P.falciparum is the most frequent species and has expanded substantially over the last several decades [1]. Tropical and subtropical nations are affected by Malaria. 90% of malaria cases occur in sub-Saharan Africa, where P. falciparum is the most frequent species [2]. P. falciparum is spread via female Anopheles mosquito bites, usually indoors [3]. It reproduces in humans after finishing its life cycle. Khyber Pakhtunkhwa (KP) has the most significant infectivity in Pakistan due to higher infectivity and negative repercussions [4]. Current district map stratification and delineation show 66 high tolerance districts and institutions. Malaria incidence in KP varies with climatic variances, increasing outbreak risk. Warm autumns (which extend transmission) and less vector control have increased malaria incidence. Geographic and temporal variations in malaria transmissionneed identifying risk variables to apply tailored control strategies[5]. Improved sanitation and pesticide sprays in regions with high malaria incidence may interrupt the disease cycle by removing mosquitoes (female anopheles vector for malaria dissemination). Malaria patients often have high-grade fever (>=101F) but sometimes have uncommon symptoms [6]. This circumstance provides a platform for cross-infection among family members living in the same residence since they have higher exposure than general individuals. Houses with malaria patients are more likely to have a cluster of infections than those without, and asymptomatic caremight represent a significant risk of disease transmission in the family and community [7]. Microscopy of peripheral blood film and the immune-chromatographic test (ICT) is used to identify gametocytes in the host's blood. Giemsa-stained microscopy is still the gold standard in malaria diagnosis since it's sensitive, quantitative, and species-specific. Low-income nations lack diagnostic competence and lab equipment. Microscopy detects low-level parasite infestation [8]. ICT techniques are more reliable than slide methods. However, they still create false negatives, resulting in parasitemia mistakes [9]. Asymptomatic malaria infection results from no fever or other acute symptoms and no current anti-malarial therapy. Some patients (careers) have enough parasites in their blood to be readily recognized ('sub-microscopic infections) PCR-based techniques may identify low parasite levels and mixed infections. PCR can identify malaria infections with as little as 0.01–0.2 helminths/L of blood. Due to the lack of information and speciation in asymptomatic individuals, PCR is more sensitive than microscopy for diagnosing P. falciparum infection [10]. Undiagnosed malaria cases may cause transmission. Malaria parasites with low prevalence must be found by screening many peopleto find a minority infected. Ultra-sensitive molecular approaches may identify lower parasite densities than regular HMIS Malaria'sasymptomatic gametocyte carriers might be a hurdle to elimination. To successfully eradicate Malaria, studies must identify asymptomatic carriers[11].

MATERIALS AND METHODS

This study was carried out at Mercy Teaching Hospital Peshawar and Khyber Teaching Hospital Peshawar. The study duration was 10 months from October 2 to December 28, 2020. The study approval was taken from the Riphah International University's Institutional Review Board (IRB). Keeping a 5% margin of error, 95% confidence level, and a 6.5% response distribution, the sample size was (n=94). 5% of the participants (symptomatic carriers) were added to overcome the dropout. Therefore the ultimate sample size was 100.

After DNA extraction using QIAGEN MINI BLOOD KIT, asymptomatic ICT-negative patients underwent RT-PCR. Aseptically collected intravenous blood was kept at four °C for DNA extraction. Alpha Genomic Laboratory Rawalpindi did PCR and DNA extraction. 300l of whole blood was obtained and homogenized with RBC's lysis buffer to guarantee maximal RBC lysis. Five minutes of incubation followed by 5 minutes of 3000g centrifugation. The particle was treated while the supernatant was discarded. After incubation at 60oC, 1.5ul of RNase A (10mg/ml) was added and vortexed. The sample lysate was mixed with 100ul of PPT Buffer for 10 seconds. 3,000 x g for 5 min formed a protein pellet. The supernatant was transferred to a clean 1.5 ml tube, and 300ulof isopropanol was added. 100 l Rehydration Buffer is added,

and the DNA pellet is incubated at 60oC for 30-60 min [12].

2l of extracted DNA was combined with 2l of 6X bromophenol blue dye (loading dye) and placed into wells. UV Trans-Illuminator bio DocAnalyzer showed the gel.

Integrity and impartiality of research were assured by the Declaration of Helsinki in 1964. The study's participants remained anonymous and private, following research procedures[13].

SPSS version 23 was used to analyze the data. Age, weight, and height were presented as mean+/-sd. Variables such as gender, relation to malaria-positive patients, MP-ICT, and Blood PCR distribution were expressed as frequencies and percentages. The Chi-square test is used to compare qualitative attributes. A T-test was used to compare quantitative attributes. 0.05 was considered significant.

RESULTS

The present study analyzed the prevalence of malarial gametocytes among the relatives of 100 malaria-diagnosed patients. The mean age BMI and relation with patients of the study participants were 30.43±18.61, 22.34 ± 4.89 and 7.33±5.87, respectively (see Table 1 A).

Table 1 (A): Mean ± Std. deviation of Demographic Variables

Variable (n=100)	Mean ± Std. deviation
Age	30.43±18.61
BMI	22.34± 4.89
Relation with patient	7.33±5.87

Demographic characteristics reported that most participants (36%) were aged between 26 and 40. The study participants were (52%) females and (48%) males. Moreover, the most affected relative were sisters (22%), followed by mothers (16%), fathers (15%), andbrothers (10%), respectively (Table 1 B).

In addition, our results revealed that all participants showed a negative result for MP ICT. In contrast, Malarial gametocyte in blood PCR was detected among 18(18%) and not detected in 82(82%) of the study participants (Table 2).

Table 2: MPICT and Blood PCR distribution of the subjects studied

MP ICT	N	%
Negative	100	100%
Blood PCR	N	%

Table 1 (B): Distribution of Demographic variables

Variables			N	(%)
Age		6- 25 Years	33	33%
		26-40Years	36	36%
		41-55Years	20	20%
		56 and above	11	11%
Gender		Male	48	48%
		Female	52	52%
Relation with Malaria positive patient		Father	15	15%
		Mother	16	16%
		Husband, male cousin, and son	3(each)	3%
		Wife, father in law and Female cousin	1 (each)	1%
		Brother	10	10%
		Sister	22	22%
		Sister in law, Mother in law, Grand Mother, and Daughter	2(each)	2%
		Grandfather	4	4%
		Uncle	8	8%
		Aunt	5	5%
Total			100	100%
	Detected	18	18%	
	Not Detected	82	82%	
	Total	100	100%	

Paired statistics of MPICT and Blood PCR tests revealed that there is statistically a significant difference (p <0.001) between MPICT and Blood PCR tests (see Table 3).

Table 3: Paired Sample Statistics

Variables (n=100)	Mean ± Std. Deviation	p-value
MP ICT	2.00 ± 0.000	<0.001
Blood sample PCR	1.82 ± 0.386	

A direct comparison of the alternative diagnostic methods, ICT and PCR, was also performed. When ICT

results were compared to PCR results, ICT had a sensitivity of 19 % and a specificity of 18.2 %. In contrast, PCR had a sensitivity and specificity of 84%, indicating that ICT was as reliable as PCR and that ICT could be used as an alternative method in remote areas, as shown in Table 4.

Table 4: Specificity of PCR and MP ICT (n=100)

	PCR	MP-IT
Positive	18/100	0/100
Negative	82/100	100/100
Sensitivity %	83%	19%
Specificity %	84%	18.2%

The sensitivity and specificity of MP-ICT and PCR taking PCR is a gold standard. The formula for specificity was (Number of actual negative/number of true negatives + Number of false positives). The formula for sensitivity was (Sensitivity =no of actual positive/number of accurate positive + numbers of false negative).

Correlations of the diagnostic tests and other parameters: Pearson correlation was made between different variables such as BMI, MP-ICT, and gender. There was a strong correlation between age and BMI as correlation is near 1, there was no correlation, and linear relationship of MP-ICT with BMI and age as correlation is 0.00, correlation of gender with age and BMI is also <0.00 which means there was negative correlation while with MP-ICT it was >0.00 which means there was a weak correlation. When a correlation of PCR withother variables was made, it was found that PCR has a negative correlation with age and gender while no correlation withBMI and MP-ICT. When a correlation of relation with parents was made, it was found that there was a negative correlationwith age, MP-ICT, and gender (<0.00), while with PCR (>0.00 but less than 1), there was a positive but weak correlation (Figure 1 and Table 5).

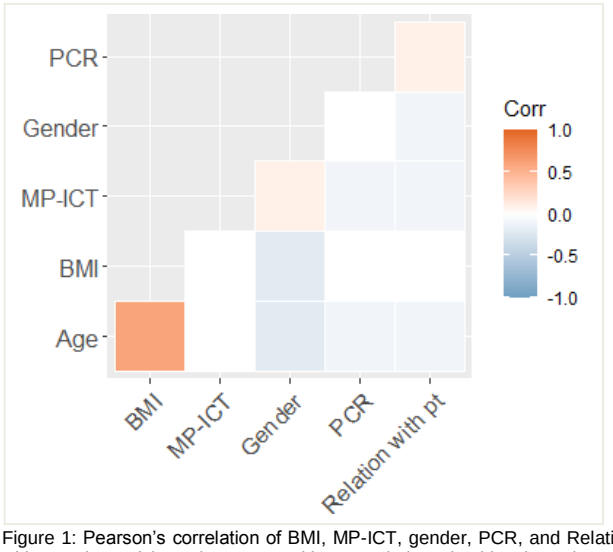

Figure 1: Pearson's correlation of BMI, MP-ICT, gender, PCR, and Relation with pt. The red box shows a positive correlation, the blue box shows a negative correlation, and the color's intensity indicates the correlation's strength.

DISCUSSION

Because trained microscopists are hard to come by in malaria-free areas, nonmicroscopic techniques were devised. This study looked at the number of asymptomatic people who had gametocytes in

their blood and lived with a malaria patient[14]. Our demographics revealed that women outnumbered males. The average BMI of the individuals was Sisters, mothers, fathers, and brothers were the most affected. Surprisingly, demographic factors did not affect the number of gametocytes in the bloodstreams of study participants[15]. Malaria is a worldwide and Pakistani issue. Ghanchi et al. (2019) revealed that gametocytes are required for parasite transmission. In mosquitos, they produce sporozoites. Male and female gametocytes are found in infectious Plasmodium parasites. Malaria gametocytes are crucial in disease transmission from humans to Anopheles mosquitos. Malaria is a seasonal disease, and climate determines the Anopheles mosquito's life span, population, and survival rate. Pakistan is mesoendemic, meaning seasonal transmission occurs under normal rainfall conditions[16]. From December through February, gametocyte carriage facilitates malaria transmission. Gametocytemia season lasts from March through May. Although all participants tested negative for MP-ICT, 18% had Malarial gametocytes in their blood PCR. According to Ishengomaetal (2018) and Zhongetal. (2018), asymptomatic Plasmodium spp. Infections vary by endemicity. The frequency is 73.7 percent and 82 percent at high transmission conditions [17]. According to PCR, almost 60% of Plasmodium carriers in low transmission areas are asymptomatic [16]. In Choco,Colombia, a thick blood smear detected 1% of asymptomatic infections while PCR found nine percent[18]. Malaria has varying effects on hematological indicators. The most common Malaria hematological abnormalities are anemia and thrombocytopenia. According to blood PCR, 18% of relatives of malaria patients had asymptomatic Malarialgametocytes. In contrast, all study participants had adverse MPICT outcomes. The Paired sample T-test revealed a significant difference between MP ICT and Blood PCR among research participants. Based on field research, the current findings show that asymptomatic patients have sub- microscopic Plasmodium. According to this study, MPICT understates the prevalence of malaria parasites[19]. Consequently, blood PCR must be employed for parasite field detection in malaria eradication. Pearson correlation was also performed between the different variables. It was discovered that BMI with age was a close correlation, as BMI increases with age. Various demographic and socioeconomic factors are responsible for increased BMI with age. At the same time, there was weak or no correlation between the other variables, indicating that these variables have no correlation and no effect on each other. These findings show the frequency of asymptomatic sub-microscopic Plasmodium infection and essential demographic elements of Plasmodium-infected individuals, such as age groups, gender, weight and height, and relationship with malaria patients [20]. Understanding possible infection origins may lower the risk of sickness in certain hamlets. This has significant implications for malaria-eradication strategies such as mass screening and medication administration. The study only covered the relatives of hospitalized patients and had a limited sample size [21].

CONCLUSION

Based on the study results, it is concluded that the prevalence of malarial gametocytes among asymptomatic close relatives of patients living in the same house is high. The sisters were most commonly affected among the close relatives, followed by the mother and father. PCR is the only procedure to be done to detect malarial gametocytes, while ICT has zero sensitivity in detectingthe carriers. Because of the low level of parasitemia, RT-PCR may correctly detect as a molecular technique capable of detecting the low level of parasitemia.

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