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ORIGINAL ARTICLE

Detection of Plasmodium DNA in Saliva of ICTPositive Malaria Patients

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Corresponding author: Asma Farid, Wali Gul, Email:drasmahameedpmc@gmail.com, waligul.kth@gmail.comAbstract

INTRODUCTION

Malaria is one of the most common and dangerous tropical infections. WHO estimates 3 million people in 24 countries are at risk for malaria. Malaria eradication is a top global health priority. Mosquitos spread Malaria, WNV, and Dengue Fever. A female Anophelesmosquito bite transmits malaria. Night and dawn are peak times. Plasmodium falciparum, malaria,vivax, ovale, and knowlesi infect humans. The deadliest parasite is Plasmodium falciparum.

Objective: To compare the sensitivity and specificity of blood and salivary PCR among malaria patients and assess saliva as a Malaria diagnostic medium.

Methods: A cross-sectional survey was conducted from March 2020 to May 2020 at Rafah Hospital Islamabad. After approval from the ethical review board, 100 subjects were approached from patients admitted to the medical /pediatrics ward, diagnosed with malaria by ICT or Microscopy. After Informed consent, ICT malaria was again performed on all of these subjects; each subject had taken paired blood and saliva samples. Data were analyzed by using SPSS version23

Results: The present study analyzed the prevalence of malarial DNA in the saliva of malaria- diagnosed patients and evaluated the efficiency of the saliva of malarial patients as an alternate medium for its diagnosis. Among 100 study participants, Blood PCR showed 100% Sensitivity and 51.9% specificity compared to salivary PCR among 100 study Participants. Moreover,Salivary PCR showed a Sensitivity of 65.8% and a Specificity of 27% compared to Blood PCR.

Practical implication: This study will provide data about the comparison of sensitivity and specificity of blood and salivary PCR among malaria patients and assess saliva as a Malaria diagnostic medium.

Conclusion: Our study concluded that Blood PCR has good sensitivity and specificity compared to saliva PCRkeeping ICT as the Gold standard. Moreover, saliva can not be used as an effective medium for the Diagnosis of Malaria.

Keywords: Malarial DNA, Saliva, Blood PCR, salivary PCR.

INTRODUCTION

Malaria is a tropical and subtropical disease. WHO says 24 malaria-affected nations are home to 3 million people. Malaria elimination is a global priority[1]. Mosquitoes transmit Malaria, WNV, and dengue. Anopheles mosquitoes transmit malaria. Bite during night and dawn[2]. Plasmodium falciparum, malaria, vivax, ovale, and knowlesi infect humans. 2017 had 219 million malaria cases and 435,000 deaths. KPK, Balochistan, Sindh, and AFTA have malaria P. falciparum has risen sixfold due to plasmodium resistance to anti-malarial and anti-insecticide drugs[3]. Malaria elimination is hard. Rapid, economical, responsive, precise, non-invasive malaria diagnostic methods are needed. Untreated P. falciparum causes brain malaria (CM) [4]. Microscopy, blood parasite antigen, and PCR may diagnose malaria. Blood film microscopy is the gold standard for malaria diagnosis, although it misses low-grade, asymptomatic infections, as proven by molecular assays[5]. Molecular diagnostics can detect malaria at low parasitemia levels. PCR amplifies malaria parasite DNA. PCR involves expensive equipment, a lab setup, and repeated tests[6].

Molecular diagnostics can detect malaria at low parasitemia levels. PCR detects plasmodium DNA best. PCR needs a thermal cycler and a clean lab. PCR requires blood and DNA[7].

Saliva is a critical, readily available medicine. Human saliva includes electrolytes, proteins, and DNA used to diagnose and monitor numerous diseases[8]. Saliva concentrations of numerous cardiovascular disease markers are linked with serum concentrations[9]. Human HIV and HPV kitsare saliva-based. HRP-2, LDH, and P. falciparum DNA have been found in malaria-infected saliva. Malaria DNA has been discovered in urine, but saliva is more sensitive[10].

Saliva beats non-blood malaria urine. Different laboratories utilize saliva versus blood for Plasmodium DNA detection using PCR [11]. Saliva influences DNA and PCR stability. Most saliva studies chill or freeze samples until DNA extraction. Valid but costly to maintain and not practical in resource-limited settings, DNA stability, and PCR sensitivity will be affected[12]. This cost- effective strategy is unfeasible in many rural and resource-limited areas. Pakistan has little P. falciparum genetic data. Malaria diagnostics involve painful blood collection, which might transmit the disease. Multiple samples reduce compliance. Minimally invasive saliva. Malaria testing seldom involves saliva. More research is required to enhance malaria saliva testing[13].

METHODOLOGY

2020 Rifah Hospital Islamabad cross-sectional study Following ethics board approval, 100 volunteers were randomly chosen. Informed consent was obtained. Patients with more than 37.5% axillary temperature at presentation OR fever within 24 hours of enrolment and malaria confirmed by ICT or Microscopy were included. Saliva contamination is prevented by gum bleeding or pain. For ICT malaria, blood and saliva were provided. EDTA was applied to two milliliters of venous blood. Frozen blood samples were used to extract DNA. Participants in the OMNI gene®•ORAL (OM-501) kit poured 1 mL saliva (DNA Genotek, Ottawa, Ontario, Canada). DNA was extractedfrom saliva at 80°C. Malaria DNA was found in saliva. Nucleo Spin columns were used to extract blood and saliva (Macherey–Nagel, Duren, Germany). Amplification of multicopy 18s rRNA plasmodial genes using PCR. Each 20-liter PCR reaction mixture included 10 liters of 2 GoTaq®Green Master Mix (Promega, Madison, USA), 6 liters of nuclease-free water, 2 liters of template DNA, and 1 liter of 5M forward and reverse primer. Eluted DNA and genus-specific primers were employed in nested PCR. Blood and saliva DNA were examined using PCR. Confirmation with 2% agarose. 6/10 gels passed. We RT-PCR'd PCR-verified samples. In RT- PCR, the fluorescence is monitored using a heat cycler. Mic-PCR (BioMolecular System). Data were analyzed using SPSS23. P0.05 Demographics were matched to blood and salivary PCR. PCRsensitivity and specificity in blood and saliva.

Statically Calculation: Using the Open epi calculator and keeping a confidence interval of 95% and margin of error of 5%, the prevalence of malaria detected by PCR was 29%. The calculated sample size was 98 participants.

RESULTS

This study analyzed the proportion of malarial DNA in malaria-diagnosed patients'saliva and evaluated its efficacy as a diagnostic medium. In this research, there were 100

participants, the mean age was 26.0614.547, and 55% were aged 7-25. 68 (68%) live in ruralareas, whereas 32% live in urban areas we discuss in table 01 to 04

Table 1: Frequency Distribution of Demographic Variables

Variables (n=100)		Frequency(n)	Percentage(%)
Age	7-25Years	55	55%
	26-40Years	26	26%
	41-55Years	14	14%
	55 and above	5	5%
Gender	Male	81	81%
	Female	19	19%
Geographic Area	Rural	68	68%
	urban	32	32%
	Total	100	100%

Table 2 shows the frequency distribution of MP ICT malarial species, and it was reported that out of a total of 100 study participants, 89 (8 %) were P. Vivax positive, 10 (10%) were P. Falciparum Positive, and 1(1%) were both P. Vivax and P. Falciparum positive.

Table 2: Frequency Distribution of MP ICT Malarial Species

Variables (n=100)		Frequency(n)	Percentage(%)
MP ICT	P. Vivax positive	87	87%
	P. Falciparum Positive	10	10%
	P. Vivax + P. Falciparum Positive	1	1%
	Negative	2	2%
	Total	100	100%

Table 3: Frequency Distribution of Blood PCR and saliva PCR

Variables (n=100)		Frequency(n)	Percentage(%)
Blood PCR	Detected	73	73%
	Not Detected	27	27%
Salivary PCR	Detected	48	48%
	Not Detected	52	52%
	Total	100	100%

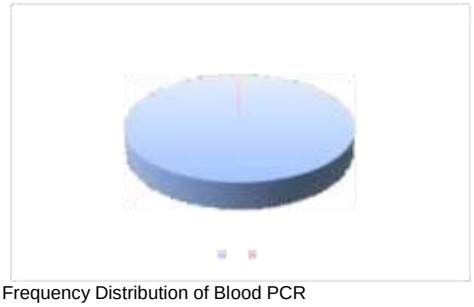

Figure 1: Frequency Distribution of Blood PCR

Figure 2: Frequency Distribution of Salivary PCR

Table 3 shows Blood PCR and salivary PCR distribution, and it was revealed that Out of a total of 100 participants, Blood PCR was detected in 73(73%) and not in 27(27%). In contrast, salivary PCR was detected in 48(48%) and not detected in 52(52%) of the study participants.

1. Detected
2. Not Detected
3. Detected
4. Not Detected

Table 4 compares the sensitivity and specificity of Blood PCR with Salivary PCR. Blood PCR showed 100% Sensitivity and 51.9% specificity compared with salivary PCR among 100 study Participants.

Table 4: Sensitivity and Specificity of Blood PCR in comparison to Salivary PCR

Variables(n=100)			Saliva PCR		Total
			Detected	Not Detected	
Blood PCR	Detected	Count	48	25	73
		% within Saliva PCR	100.0%	48.1%	73.0%
	Not Detected	Count	0	27	27
		% within Saliva PCR	0.0%	51.9%	27.0%
	Total	Count	48	52	100
		% within Saliva PCR	100.0%	100.0%	100.0%

Table 5 compares the sensitivity and specificity of Salivary PCR with Blood PCR. It was reported that Salivary PCR showed a sensitivity of 65.8% and a Specificity of 27% compared to Blood PCRamong 100 study Participants.

DISCUSSION

Plasmodium vivax threatens 2.5 billion people. Biology and epidemiology of Plasmodiumfalciparum complicate management[14]. In Plasmodium vivax infections, low gametocyte parasitemia preceding illness causes relapses. Range and spread are affected. Despite low incidence and parasitemia, this parasite isn't innocuous, especially in calm persons[14]. Plasmodium vivax kills. Chloroquine's spreadability and primaquine's organization harm patients. Annually, tens of millions become sick. Plasmodium vivax-specific drugs prevent malaria transmission. Plasmodium vivax's biology requires elimination. 87 of 100 research participants had PlasmodiumVivax, 10 had Plasmodium Falciparum, 1 had both, and two were negative. &c. 2016 saw 33–35% and 35% plasmodium vivax infections by Suliman et al. 15. Hamid et al. Malaria patients had P. vivax, P. falciparum, or both. Malaria thrives in tropical settings. Exclusion programs require a diagnosis. Parasitemia and mixed infections diminish vulnerability[16]. Saliva assays detect malaria noninvasively. Low- and middle-income countries require sample handling solutions without quality loss or cold chains. The OMNI gene ®•ORAL (OM-501) kit was used to identify Plasmodium falciparum DNA in room-temperature saliva. Malarial DNA was found in 73 (73%) of 100 participants by Blood PCR and not detected in 27 (27%). In contrast, by Salivary PCR, it was detected in 48% and not detected in 52%, similar to a previous study in which malaria prevalence was 35% by PCR-blood and 28% by Salivary PCR[17]. Blood PCR revealed 73.9% Sensitivity and 33.3% Specificity, greater than salivary PCR, which exhibited 47.7% Sensitivity and 50% Specificity, matching with prior research findings [18]. PCR-blood and PCR-saliva showed 100% sensitivity in 2017. PCR-blood was 87% specific, and PCR-saliva was 93%. Blood PCR showed 100% sensitivity and 94.9% specificity, whereas salivary PCR had 92.2% and 97.4%. 73% salivary PCR sensitivity was reported by Nwakanma et al. 77% salivary PCR sensitivity was reported by Buppan et al.

Similar to a 2017 study by Kenji O. Mfuh et al., our salivary PCR was 65.8% sensitive compared to blood PCR. Saliva-based PCR exhibited 94.12% sensitivity and 97.3% specificity, according to Ofentse J. Pool et al. Salivary PCR is sensitive[19]. Our study demonstrates a feasible saliva-based PCR malaria diagnostic

method. This non-invasive method enhances safety, community participation, and bias in extensive population surveys like medicine or vaccination efficacy evaluations[20]. Malaria must be treated using asymptomatic parasite carriers. Saliva-based screening enhances community engagement, particularly for asymptomatic reservoir infections. Stable viral carriers and impoverished populations are more likely to participate[21].

CONCLUSION

Our study concluded that Blood PCR has good sensitivity and specificity compared to saliva PCR keeping ICT as the Gold standard. Moreover, saliva cannot be used as aneffective medium for the Diagnosis of Malaria.

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