Original Article

DIAGNOSTIC ACCURACY OF MICROSCOPY VERSUS PCR TECHNIQUE FOR THE DETECTION OF PLASMODIUM SPECIES IN PAKISTAN

Sehrish Ather[1], Romana Ayub[2], Zakria Tariq[3]

Abstract

Background: According to latest data available Plasmodium vivax and Plasmodium falciparum are the most common species of plasmodium present in Pakistan. This study focuses on the current status of malaria specie distribution across different provinces of Pakistan.

Material & Methods: This is a cross sectional study which is community based it was carried out in endemic areas of 04 provinces of Pakistan. The study was conducted stepwise by first microscopically confirming Plasmodium-positive blood samples and later these samples were reconfirmed by polymerase chain reaction (PCR) specie specific for detecting four species of human malaria.

Results: Total of 450 PCR-positive samples were collected amongst these 29 (6.4%) were P. falciparum, 386 (85.8%) were P. vivax, and 35 (7.8%) were mixed P. falciparum and P. vivax. Total 39 (8.7%) P. falciparum, 393 (87.3%) were P. vivax and mixed infections were (18%) positive in microscopically. There were no positive cases of Plasmodium malariae and Plasmodium ovale.

Conclusion: According to the study findings P. vivax and P. falciparum are most prevalent plasmodium species in in Pakistan, in addition mixed infections were also contributing to malaria prevalence in Pakistan. Regional variation in the prevalence and species composition was also found in the study.

Key Words: Plasmodium falciparum, Plasmodium vivax, Malaria, Pakistan

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INTRODUCTION

The bite of infected Anopheles mosquito causes Malaria in humans which is a life-threatening disease. Malaria is preventable and curable disease. According to reports of 2017 there were 219 million cases of malaria in 90 countries due to which death toll reached 435,000.[1] WHO reports label Pakistan as a country with highest load of malaria in EMRO region, the number of cases reported are one million annually. Some developing countries Sudan, Yemen, Somalia and Afghanistan show the comparable number of cases as of Pakistan.[2]

Different approaches are presently tried to improve malaria control activities which ultimately are intended towards the eradication of this disease. Total of four types of Plasmodium species cause malaria in humans which are P. falciparum, P. vivax, P. malariae and P. ovale. Another specie P. knowlesi, has been discovered in Southeast Asia which causes malaria in humans.[3] Plasmodium knowlesi is more lethal due to its rapid clinical course so early diagnosis and treatment can prevent

[1]Demonstrator UOL, Lahore
[2]Associate Prof. KMC, Peshawar.
[3]Orthopedic Specialist UOL, Lahore

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mortality.[4,5] In Malaysia microscopy is the method of choice for diagnosis but the limitation for P. knowlesi is that its ring stage resembles P.falciparum and in later stages the trophozooite resembles with P.malariae,[6,7] so the chances of wrong diagnosis increase. Early diagnosis and treatment in cases of malaria is the most effective way in to reduce malaria cases and burden of disease.[8]

Malaria is considered to be very intense public health issue which is common in subtropical areas of the world. Malaria is caused by mosquito having five different types. The first-line treatment depends on the type of parasite causing the disease, proper selection of anti-malarial drug is needed according to the specie seen on microscopy. Correct identification on microscopy is needed before starting the antimalarial. The common and less expensive method which can be easily followed in endemic areas anywhere is through Giemsa-stained thick blood smears by microscopy.[9]

Molecular diagnostic methods are more sensitive tests for the diagnosis of species as compared microscopic tests, however the technical expertise of the investigator is more important for the specificity & sensitivity of the test. On microscopy only half the number of patients is correctly diagnosed as compared to the molecular diagnostic methods such as polymerase chain reaction (PCR) in endemic areas.[10] Some species of Plasmodium like P. knowlesi and P. malariae, are difficult to differentiate from each other on the basis of morphological features. Cases of P. knowlesi in Malaysia and other East Asian countries are recently reported in spite of the fact that first confirm case of human P. knowlesi infection was reported in 1965. Retrospective studies done in Malaysia during 2004 made it clear that microscopy alone has given wrong diagnosis about P. knowlesi human infection cases as P. malariae.[11]

To detect Plasmodium species in human nested PCR or semi-nested PCR tests are done but none can correctly detect the separate the five species of plasmodium. As the simple molecular diagnostic method is more sensitive as compared to microscopy therefore it needs to be introduced immediately to differentiate between the different species of Plasmodium parasite.[12] 374,513 diagnosed & confirmed cases of malaria were reported among the public and private hospitals of country and so the total disease burden across Pakistan is calculated. Screening was done for 6.5 million malaria suspects at these health facilities. The number of diagnosed cases for (PV) 84.0% that is (314,574), while (PF) 14.9 % (55,639) and mixed cases were 1.1% (4,300). In 2018 cases of provincial report showed that Sindh had highest number of malaria cases 34.5% (129,085), after which was Khyber Pakhtunkhwa 31.0% (115,995), Tribal Districts (merged districts) 17.6% (65,853), Baluchistan 16.4% (61,510), Punjab 0.5% (1,875) and then AJK 0.1% (195).[13]

This study aims at explaining the burden of malaria cases in Pakistan. Studies & surveys were carried out in different years to find the rate and spread of Plasmodium species and its spread with regards to environmental and seasonal changes.

MATERIAL AND METHODS

After getting the ethical approval from University of Lahore Pakistan, this study was carried out as community-based Cross-sectional study in endemic areas of all four provinces of Pakistan. The 2019 data related to Malaria from public and private facilities was utilized, and also census of 2017 population data was taken into account. Federally administered tribal areas of Khyber Pukhtunkhwa now known as the merged districts were excluded from the study because of the restrictions due to political instability and violence. [14]

Four most endemic cities were included in

this study from every province and the samples were collected from following
District Dir (Khyber Pakhtunkhwa province), District Mirpur khas (Sindh province), District Loralai (Balochistan province), District Jhang ((Punjab province), Samples were collected from all sites during the period of April-Oct 2020 which was considered to be peak malaria season. People of all ages with positive signs of malaria were included in research.
Blood was taken from patients who gave consent. Patient age and gender were also noted and then 03 ml of blood was taken from the vein and put into EDTA tube. 10% Giemsa solution was used for thick and thin films staining and examined at 100 x under oil immersion. The smears were examined according to WHO guidelines by trained technicians. [15]

RESULTS

A total of 450 microscopy-positive samples were done by PCR along with positive and negative controls. By PCR technique, 6.4% (29) were identified as P. falciparum, 85.8% (386) were P. vivax, and 7.8% (35) were mixed P. falciparum and P. vivax. Total 39 (8.7%) P. falciparum, 393 (87.3%) were P. vivax and mixed infections were 18 % positive in microscopy (Table 2). There were inconsistencies found among the PCR and microscopy results. PCR has shown precise results while identifying with exact number of patients with a particular specie of plasmodium like there were total 29 P. falciparum identified by PCR, rather than 39 P. falciparum specie identified by microscopy means 10 more patients were diagnosed with falciparum infection in the same way, 386 P. vivax were identified by PCR rather 393 were diagnosed microscopically means seven less patients were specified with P. vivax, and so for mixed infection number 35 were identified by PCR but only 18 were diagnosed on microscopy means 12 patients were more detected on PCR. Slides were not available for re-examination in the case of discrepancies between microscopy and PCR, but PCR was carefully done with all samples.
Of the 450 microscopy-positive samples, according to the latest data available the highest number of cases were in Sindh, next in Khyber Pakhtunkhwa then in Baluchistan and finally least cases were from Punjab. These numbers of cases were selected on the basis of data available in 2019 disease burden calculation in different provinces of Pakistan. The highest to lowest number of cases were already discussed in the above part. The results of the study showed that P. vivax was the most common malaria specie in study area while Falciparum stands second in line.

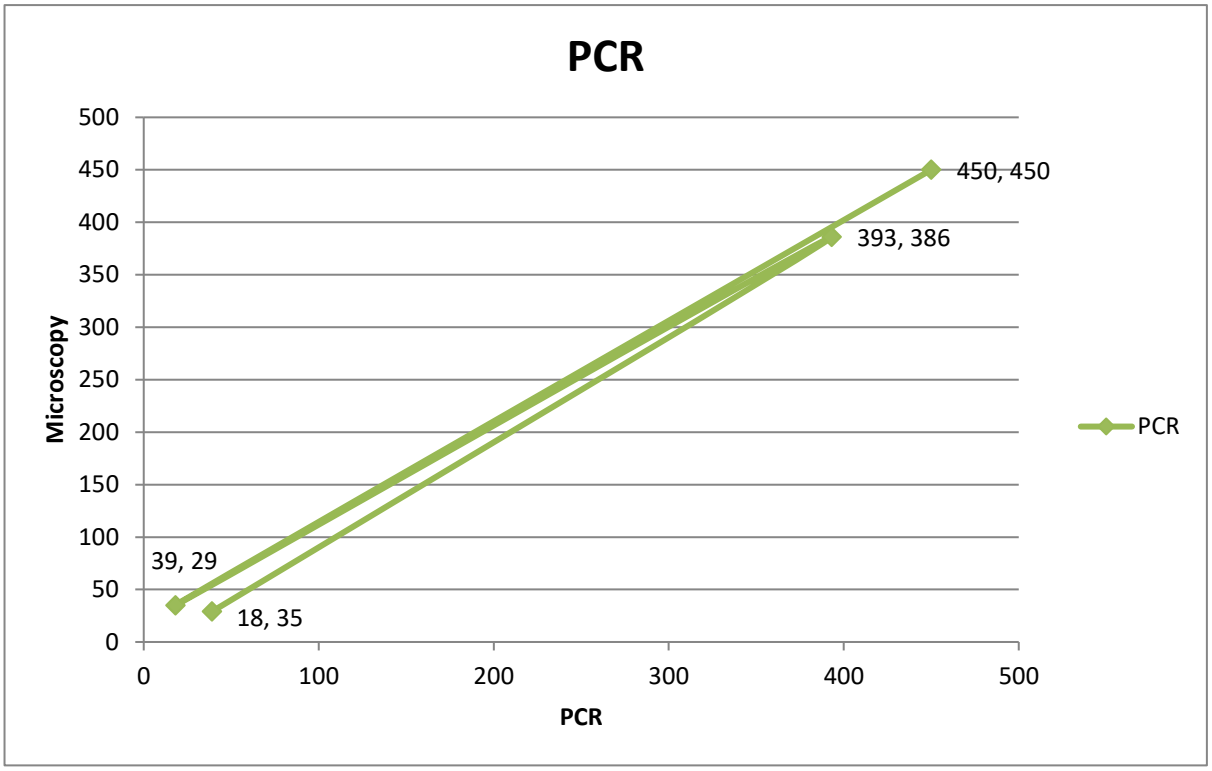
Table 1: PCR Positive tests for malaria.

	Frequency	Percent
Plasmodium Falciparum	29	6.4
Plasmodium Vivax	386	85.8
Mixed Infections	35	7.8
Total	450	100.0

Table 2: Microscopy Positive Lab test for malaria

	Frequency	Percent
Plasmodium Falciparum	39	8.7
Plasmodium Vivax	393	87.3
Mixed Infections	18	4.0
Total	450	100.0

Figure 3: Diagnostic accuracy of Microscopy verses PCR Technique



This above figure shows that among total 450 respondents microscopy results are very close to PCR, that is 386 vivax are identified by PCR and microscopy detected 393 vivax species positive in the same way 29 verse 39 falciparum specie and 35 verses 18 mixed infection were detected by PCR and microscopy respectively.

DISCUSSION

Among other serious public health problems malaria is still a major health hazard in Pakistani community. According to study reports of 2008, mortality from malaria was almost 50,000/year from a total 2.6 million reported malaria cases.[16] In 2010 EMRO reported over one million confirmed malaria cases out of which 22% were from Pakistan. [17] Exact estimation of prevalence of specie was difficult due to lack complete coverage and resources. The focus of this study was to get up-to-date information of malaria cases in highly endemic areas of Pakistan. Samples were collected from patients coming with symptoms, health seeking attitude and regional variation was a strong limitation. During the transmission season all patients were sampled coming to health facility but particular species are on peak during different months of the year. The month of April till September is peak season for P. vivax, whereas P. falciparum peak season is August till December.[18] These points were taken into account while assessing small reporting differences in the prevalence of malaria and in the proportions of both species among regions. In spite of limitations of the study this data contributed a lot in current condition of malaria in Pakistan.

According to results of previous studies the cases of malaria in males in their twenties were found to be higher as compared to females.[19] Some hypotheses for the unequal distribution of malaria disease among the males as compared to females is that the males are more exposed to Anopheles and they were more prone to be infected by the bite of mosquitoes. The study main finding depicts that malaria is highly prevalent in Sindh then Khyber Pakhtunkhwa followed by Baluchistan, with lowest prevalence in

Punjab. Few years back Punjab had highest number of malaria cases but with good case management techniques the number declined.

This study findings are consistent with the studies done before which claimed that the cases of P.vivax and Falciparum are the two major species prevalent in Pakistan, whereas P.vivax is more common as compared to falciparum.[20] According to studies done in past show the nearly quarter to one half of malaria cases were attributed to P. falciparum in these cities.[21] The discrepancies were found while detecting samples for microscopy-positive and PCR negative for Plasmodium, for samples that were P. falciparum or P. vivax mono infections by microscopy and mixed species infections by PCR. Repeated PCR test result showed that few error lies in diagnosis by microscopy. The major factors found to influence results accuracy of microscopy are use of polluted slides, faulty stains, artifacts, lack of experts in dealing manually and also the services provided. It has always been misdiagnosed that where P. vivax is high, P. falciparum must also be present in this patient, which will result in misinformation and false treatment.

CONCLUSION

Management and control of malaria is always a challenge in Pakistan as different tropical regions have different distribution of species, so lack of information poor skills for diagnosis lead to wrong diagnosis in Pakistan. This study results indicate that Sindh, Khyber Pakhtunkhwa and Baluchistan provinces should be the focus areas because prevalence of malaria is highest and in these endemic areas of Pakistan, proper laboratory facilities should be provided to improve species diagnosis while using microscopy technique in the areas where PCR facility is not available right now.

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AUTHOR'S CONTRIBUTION

SA: Research proposal development, Data collection, Analysis, Article writing and Reviewing
RA: Research proposal development, Analysis, Article writing and Reviewing
ZT: Research proposal development and Data collection

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