Original Article

Comparison of Polymerase Chain Reaction (PCR) and Peripheral Blood smear (PBS) for Diagnosis of Asymptomatic Malaria Parasitemia

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Abstract

Objective: Microscopy of Giemsa-stained thick and thin peripheral blood smear (PBS) remains as the standard laboratory method for the diagnosis of symptomatic malaria. This study was done to compare PBS with polymerase chain reaction (PCR) method for detecting asymptomatic malaria parasitemia.

Patients and Method: Blood samples were collected from 900 asymptomatic school children between 7-11 years old in one of the provinces in the South of Iran.

Results: All 900 students were negative for plasmodium vivax and plasmodium falciparum by PBS. PCR method detected DNA of P. vivax in 10 blood samples (1.1%). Specificity and negative predictive value of PCR method was respectively 98.89% (95%CI: 98.0-99.5%), 100% (95%CI: 99.6-100%) compared to PBS as the gold standard method. As there was no positive case by PBS method, calculation of sensitivity, positive predictive value and likelihood ratios of the PCR method was not possible.

Conclusion: In comparison to PBS, expensive and prolongation PCR method is not suitable and cost effective for identification of malaria infection in asymptomatic.

Key words: malaria**,** peripheral blood smear, PCR

Introduction

Malaria is one of the leading infectious diseases in the world with 300-500 million clinical cases and 1-3 million deaths each year.(1,2) Areas endemic for malaria are categorized into the following types: hypoendemic, mesoendemic, hyperendemic and holoendemic. Malaria is one of the most important parasitic diseases in Iran. In our country, the areas endemic for malaria are located in the eastern and the southern parts of the country. Reported studies in 2008 showed out of 11,460 malaria cases of Iran, 8% was due to P. falciparum while 90% were infected by P. vivax.(3) About 38% of malaria cases reported in Kerman province were from Kahnuj district. The majority of malaria cases were reported from Ghaleganj and Roudbar counties which are neighboring with Kahnuj.(4) WHO recommends that the standard method for diagnosis of symptomatic malaria is the low cost thick and thin Peripheral Blood Smear (PBS). For asymptomatic malaria infections, patients with low parasitemia and patients with two or more types of plasmodium parasitemia, PCR method can be more sensitive than PBS.(5) Although the clinical important of asymptomatic malaria as a source of disease is not exactly determined, it is assumed that in endemic areas asymptomatic parasitemia result in the development of partial immunity. (6, 7) On the other hand, asymptomatic parasitemia may be source of parasite transmission. Therefore it is important to diagnose asymptomatic malaria cases as sources of infection.

Our aim was to compare the diagnostic precision of two methods (i.e., PBS and PCR) for diagnosis of malaria in asymptomatic students between 7-11 years old in one of the endemic areas of Iran.

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Materials and Methods

Asymptomatic school children, between the ages of 7-11years were selected for this study by cluster sampling in Ghale Ganj's schools in Kerman province, Southeastern part of Iran. Parental consents were obtained and a questionnaire regarding present and past history of malaria or FUO (fever with unknown origin) in the child or his immediate family member was completed for each student. A total of 900 whole-blood samples were obtained; one milliliter of venous blood sample was taken with 1%

EDTA coated syringes and placed on ice bags. Thick and thin blood smears were prepared; all 900 blood smears (PBS) were stained with Giemsa and examined by two independent experts in the field. A smear was considered negative when no parasite was detected in either of the two examinations.

Blood samples were stored at –80ºC until DNA extraction. DNA of plasmodium was extracted by high yield DNA purification kit (Catalog number DN 8115 C, Sinna Gen Inc., Iran). All PCR reactions were carried out by Master DNP TM Kit (catalog Number DN 8115 C, Sinna Gen Inc., Iran). In this study, primers were provided by the MWg Company from Germany. PCR products were analyzed by electrophoresis using 2% agarose gels.

Diagnostic test values, i.e. sensitivity, specificity, positive and negative predictive values (PPV and NPV), were calculated according their standard definitions. Confidence intervals for each value were obtained from STATA-9.1 by binomial exact method.

Results

Of 900 students included, 469 students (52.1 %) were female. All 900 children were negative for P. vivax and P. falciparum by PBS. PCR method detected DNA of P. vivax in 10 blood samples (1.1%) of 871 students (96.8%) with a negative history of malaria in the last year; however in 29 (3.2%) subjects who gave a positive history of the disease, P. vivax DNA was not detected in their blood by PCR. In this study specificity and NPV of PCR method was respectively 98.89% (95%CI: 98.0-99.5%), 100% (95%CI: 99.6-100%) compared to PBS. It was impossible to determine sensitivity and PPV of the PCR method, because all 900 peripheral blood smears were negative for malarial parasites on microscopy. If the PCR method was considered as standard method, sensitivity, specificity and NPV of PBS method would be respectively 0% (95%CI: 0-30.1%), 100% (95%CI: 99.6-100%) and 98.89% (95%CI: 98.0-99.5%).

Discussion

Microscopic detection of parasites on Gimsa-Stained blood smears has been the reference standard method for the diagnosis of malaria in laboratories for more than a century; it is highly dependent on technical expertise. This method can sometimes be misleading in identifying plasmodium species especially in cases with low level of parasitemia, a mixed parasite infection or when the disease has been partially treated by anti-malarial drugs. (8-11)

As molecular methods developed and became more applicable in routine diagnosis, in addition of including the advantages of providing information about drug-resistance and genetic diversity of malaria parasites, physicians prefer to use these methods more commonly. (12)

Siddig et al. performed a study in Sudan to compare the standard microscopic examination (PBS), PCR and Immunochromatography test (ICT) to determine the best method for screening blood donors for malaria parasites. 100 blood donors were screened for malaria parasite, 21 blood samples were positive with PCR, 14 with ICT, and 13with microscopic examination. The sensitivity and specificity of PBS was 61.9% and 100%, respectively. False negativity of both the PBS and ICT was significant. (13) Roshanrovan et al. have reported the prevalence of P. vivax with PBS and PCR methods to be 2.9% and 14.2%, respectively and that of P. falciparum as 1.3% and 2.6% respectively in a region endemic for malaria. In addition, PCR method identified seven cases with mixed infections while PBS method missed them. In this study sensitivity of PBS method was 24.3%. 4 Stauffer et al. tested blood samples from newly arrived refugees to the United States by microscopy and rapid antigen capture enzyme assay (RAC), and PCR for malaria; they detected P. falciparum DNA by PCR in 9 of the 103 tested specimens, only 2 of these were positive on PBS, (sensitivity of 22%, specificity of 100%). The RAC method identified 5 samples as positive for P. falciparum, two were strongly positive and three were weakly positive. (14) Harris et al. performed a study in Temotu province of Solomon Islands that prepared for malaria elimination. During the survey 9,491 blood samples were collected and examined by microscopy for plasmodium species and density, with a subset also examined by PCR and RDT. For 1,784 samples PCR assays were done; these included samples that were positive on microscopy, samples from all febrile patients and 10% of the microscopy negative samples from each village. A total of 256 samples were positive by microscopy, giving a point prevalence of 2.7%. The species distribution was 17.5% P. falciparum and 82.4% P. vivax. A significant proportion of infections detected by microscopy, 40% and 65.6% for P. falciparum and P. vivax respectively, had parasite density below 100/microliter. PCR detected substantially more infections than microscopy (point prevalence of 8.7%), indicating a large number of subjects had sub-microscopic parasitemia. The concordance between PCR and microscopy in detecting single species was greater for P. vivax (135/162) compared to P. falciparum (39/118). (15)

All these studies compared PBS and PCR methods for detection of malaria parasite in asymptomatic subjects, and found a few of them to be positive by PBS while in our study all samples from asymptomatic students were negative by PBS. Technical errors in preparation of thick and thin blood smears and low numbers of parasite in these blood smears can explain negative results in all 900 cases. PCR method is a long procedure that requires specialized and costly equipment.

Conclusion

Our findings reveal that PCR detected the presence of P. vivax in only 1.1% asymptomatic students in whom the PBS was negative and did not require medication. In view of these results and keeping in mind the fact that PCR is

expensive requiring special equipment, we believe that PCR is not a suitable alternative to PBS for diagnosis of asymptomatic P. vivax malaria. Also, it can be recommended that blood samples of students which had positive results for P. vivax by PCR should be tested by real time PCR to be validated. (16)

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