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Research Article

Comparative study of three diagnostic techniques for diagnosis of malaria at a Tertiary care hospital in Kanpur

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Abstract
Malaria continues to be a global public health problem. Microscopic examination of peripheral blood smear is the standard method for malaria diagnosis, which is available easily and has low cost but its reliability is questionable at low level of parasitaemia. The present study was undertaken to compare three different methods for diagnosis of malaria, i.e. peripheral blood smear and Antigen card test. In this study Peripheral blood smear (PBS), centrifuged buffy coat smear (CBS) and Antigen card test (RDT) were compared with each other taking peripheral blood smear as gold standard.
Materials and Methods: *The study was carried out over a 12 month period from January 2016 to December 2016. Blood samples (3-4 ml per patients) collected in EDTA vials from patients with clinical presentation of malaria were subjected to perform peripheral blood smear, buffy coat smear and rapid card test for the diagnosis of malaria then results were compared taking PBS as the gold standard.*
Result: *Out of 144 samples malaria was diagnosed in 53(36.80%), 69(47.91%) and 70(48.61%) by PBS, CBS and RDT respectively. The sensitivity of CBS and RDT was 76.81% and 75.71% respectively and specificity was 100%.*
Conclusion: *The development of easy, rapid and accurate test for the reliable detection of malaria infection is necessary. The centrifuged buffy coat smear technique fulfills most of these criteria and may be used at limited diagnostic source setup for low cost and reliable diagnosis of malaria.*
Keywords: *Malaria, Peripheral blood smear, Rapid card test.*

Introduction

Even in this era of newly emerging deadly diseases malaria remains the most serious parasitic disease worldwide especially in the tropical and sub-tropical countries. It is a serious, sometime fatal, parasitic disease posing a major public health problem in India[1]. There are about 380 species of *Anopheles* mosquito, but only 60 or more are able to transmit the parasite. Malaria is a life-threatening blood disease caused by parasites transmitted to humans through the bite of the *Anopheles* mosquito. Once an infected mosquito bites a human and transmits the parasites, those parasites multiply in the host's liver before infecting and destroying red blood cells[2]. According to the latest estimates from world health organization, there were 214 million new cases of malaria worldwide in 2015 (range 149–303 million). The African Region accounted for most global cases of malaria (88%), followed by the South-East Asia Region (10%) and the Eastern Mediterranean Region (2%). Among South East Asia region, India shares two-thirds of the burden (70%) followed by Myanmar (16%) and Indonesia (10%)[3]. Eighty percent of deaths only due to malaria were reported among 15 countries including India. In India Meghalaya Maharashtra, Gujarat, Karnataka, Chhattisgarh, Goa, Southern Madhya Pradesh, Jharkhand, Andra Pradesh, Assam, Odissa and Uttar Pradesh are most prevalent regions[3-4]. Malaria transmission mainly depends on two primary factors: location of mosquito breeding site and clustering of humans habitations where people serve as reservoirs of parasites for mosquito infection. A typical attack of malaria comprises three distinct stages: cold stage, hot stage and sweating stage. The clinical features of malaria vary from mild to severe, and complicated, according to the species of parasite present, the patient's state of immunity, the intensity of infection and also the presence of concomitant conditions such as malnutrition and other disease[5]. Severity of the disease depends on the interaction of a number of factors. These include the size of the infective dose of sporozoites, nutritional status of the host, level of acquired immunity, host genetic factors, parasite growth rate, drug resistance status, socio-economic condition, availability of health care and education[6].

All age groups may be affected. Maximum mortality occurs in young children< 5 years of age in endemic areas but in case of non-immune persons mortality occurs regardless of age. In addition to children, pregnant women, non-immune travelers, refugees, displaced persons and laborers are at highest risk of severe disease[7]. Infection with malarial parasites may result in a wide variety of symptoms, ranging from absent or very mild symptoms to severe disease and even death[8]. Common symptoms and signs are: fever, chills, sweating, headache, nausea and vomiting, body aches, malaise, weakness and splenomegaly. In falciparum malaria, additional findings are: mild jaundice, hepatomegaly and tachypnoea. If falciparum malaria is not treated properly following complications may occur: cerebral malaria, severe anemia, hemoglobinuria, pulmonary edema, thrombocytopenia, cardiovascular collapse, shock, kidney failure, hyperparasitemia, metabolic acidosis and hypoglycemia[9]. Diagnosis of malaria based on clinical grounds aloneis unreliable and should be confirmed by laboratory tests. The accurate diagnosis of malaria depends on the demonstration of parasites in stained blood smears. Microscopic examination of blood film is regarded as the gold standard Method[10]. This method is relatively simple and has low cost. Several other reliable techniques have been used in recent time to diagnosis of malaria in clinical set-up like quantitative Buffy coat assay and the rapid diagnostic tests. Rapid diagnostic tests always based on detection of parasite antigen such as the histidine-rich protein-2(HRP-2) and plasmodium lactate dehydrogenase (PLDH) and specific aldolase. Others newest advance techniques include the detection of parasite specific nucleic acid sequences in the sample by polymerase chain reaction or by using specific

complementary biotin late probes[11]. Smear microscopy remains the gold standard against which all other tests have been evaluated. All these tests vary in their sensitivity and specificity. Keeping in mind the seriousness of the condition and the current availability of diagnostic facilities across India we decided to conduct a comparative study of the peripheral blood smear, centrifuged buffy coat smear and antigen card test to find out low cost, reliable and easy to perform at source limited diagnostic setup.

Materials and Methods

Place and the period of the study: The study was carried out in the Department of Microbiology, Rama Medical College Hospital and Research Centre for a period of one year from January 2016 to December 2016.

Type of study: The study was designed as Prospective type.

Inclusion criteria for cases: Blood samples from suspected cases were selected irrespective of age and sex, on the basis of following clinical findings from both OPD and IPD patients. (1) Fever associated with chill and rigor (2) Sweating (3) Splenomegaly (4) Hepatomegaly (5) Headache (6) Fatigue and (7) Abdominal discomfort.

Exclusion criteria for cases: All patients who were diagnosed and/or treated with antimalarial drugs within last six months were excluded from the study.

Sample collection: 3-4 ml blood specimen was collected from anti-cubitalvein in EDTA vial of all patients by taking sterile precaution.

Preparation of thin film: After collection of one drop of blood on a clean grease free slide, thin film was made by spreading the blood using a smooth edged slide or spreader at an angle of 45º from the horizontal plane. A well-prepared thin blood film should have a smooth tail end and free of vertical lines and holes. The slide was then labeled properly and allowed to air-dry[12].

Preparation of centrifuged buffy coat smear: Buffy coat smear were prepared by hematocrit tube. Tube filled with blood up to mark and centrifuged it for 30 minutes at 1500-2000 rotation per minute. Once process is done there layer of blood will appers at top plasma layer at bottom red blood cells and in middle there is a another layer that is forms by deposition of white blood cells known as buffy coat layer. Discard the plasma layer by picking out by lumber puncture needle without disturb buffy coat layer. After discarding plasma layer pick buffy coat layer and prepare smear.

Staining of the film: Peripheral blood film smear and Centrifuged buffy coat smear were stained with leishman stain following kit procedure.

Microscopic examination of the stained film: The thin film and cenetrifuged coat smear were examined by the 40 X objective first. Then 100X oil immersion objective was used. After applying immersion oil the film was examined by moving along the edge of the film. Then moving the slide inward by one field, returning in a lateral movement and so on. At least 100 fields were examined before a slide was considered as negative for malarial parasite.

Detection of Antigen: Antigen was detected by Rapid card test method with Alere True line malaria antigen test kit. The kit was manufactured by Standard Diagnostics, Inc.

Interpretation of the result:

Negative: The test was considered as negative, if only the control band appeared.

Positive: The test was considered as positive for P. *falciparum*, if one or two colored line appeared in addition to the control line. Similarly the test was considered as positive for P.*vivax*, if a single line just next to the control line appeared.

Invalid: The test was considered as invalid, if no control (C) line appeared.

Results

The present study was conducted on a total of 144 subjects; the prevalence rate was to be found 48.61%. [Fig 1] Fig 2 shows the age and sex distribution of the study population. Among the cases, 80 were male and 64 were

female. Majority of the cases (32) were in the age group of 11-30. Minimum number of cases (14) were in the age group <10 year. Fig 3 shows the rate of detection of malaria case by peripheral blood film centrifuged buffy coat smear and rapid card Method. Out of suspected cases 53 were positive and the rest 91 were negative by microscopic Examination of peripheral blood film. The rate of positivity and negativity were 36.80% and 63.19% respectively.

In case of centrifuged buffy coat smear out of suspected cases 69 were positive and 75 were negative. The positivity and negativity of centrifuged buffy coat smear were 47.91% and 52.08% respectively. In case of rapid card out of suspected cases 70 were positive and 74 were negative. The positivity and negativity of rapid Card for malaria were 48.61% and 51.38% respectively. Fig 4 shows the comparison between Centrifuged buffy coat smear and Rapid card test when compared with cases. Centrifuged buffy coat smear shows high sensitivity for malaria while specificity is equal to rapid card test.

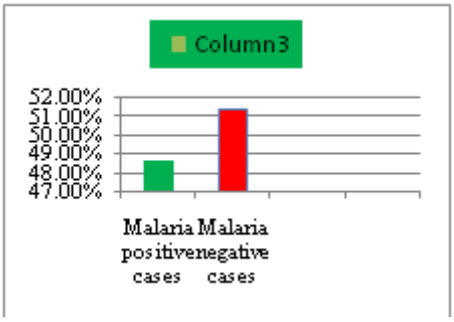

Figure 1: Prevalence of Malaria in Kanpur

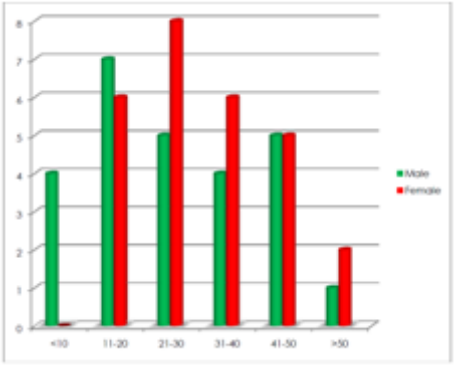

Figure 2: Age and Sex wise distribution Male and Female

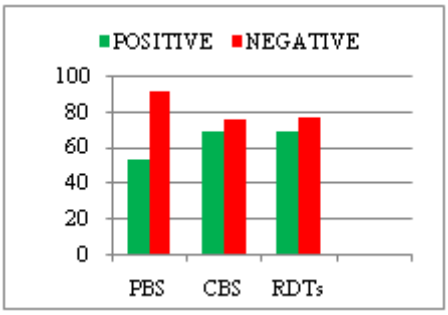

Figure 3: Detection of Malaria by PBS, CBS and RDTs methods

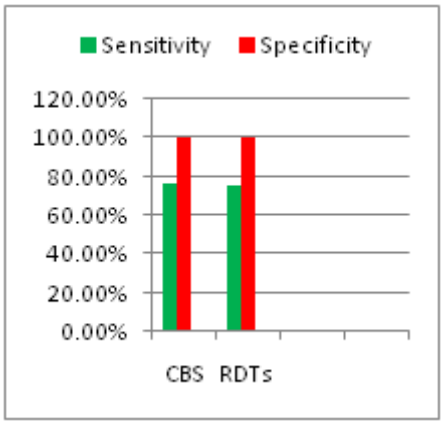

Figure 4: Comparison between Centrifuged buffy coat and Rapid card test

Discussion

In many parts of the world, physicians often go with presumptive malaria diagnosis based on clinical symptoms and signs. Various clinical algorithms have been suggested and the best of them predict only up to 50% of true malaria cases. This method as poor specificity and positive predictive value. It does not allow differentiation of different species of malaria infection[14]. In the present study, we found 80 (55.56%) males and 64 (44.45%) females in clinically suspected cases. This result is nearly similar with the study from Saudi Arabia by Malik *et al.* in 1998; they found 231 (69.17%) males and 103 (30.83%) females in the clinically suspected cases[15]. We also found 40 (67.80%) males and 19 (32.20%) females positive cases. This result is also similar with the study from Uganda by Estacio *et al.* in 1993, they found 15 (60%) males and 10 (40%) females in the microscopy positive cases[16]. In another study from Saudi Arabiain1994, reported that 132 (56.66%) males and 101 (43.34%)

females were in the microscopy positive cases[17]. From the present and other studies, it is suggested that males are more frequently infected by malaria than females. This may be due to males are more frequently exposed to the risk of acquiring malaria than 82 females because of their outdoor life they lead. Moreover, females are usually better clothed than males, particularly in our country, which provides them some sort of protection from mosquito bites[18]. In present study, we found 36.80% was positive for malaria by microscopic examination of peripheral blood film, 47.91% was positive by centrifuged buffy coat smear and 48.61% was positive by rapid card test in clinically suspected cases. In a study by Shambhavi singh et al in 2016 from India found among 1982 clinically suspected cases 2.92% was positive by microscopic examination of peripheral blood film, 3.33% was positive by centrifuged buffy coat smear and 4.09% was positive by rapid card test[19]. Another study by J. jofar Ebrahim et al in 2013 from Saudi Arabia found 19.9% was positive by microscopic examination of peripheral blood film, 24.13% was positive by centrifuged buffy coat smear and 27.09% was positive by rapid card test[20]. Another study done by S Mohanty et al in 2015 from India found17.1% was positive by microscopic examination of peripheral blood film, 21.9% was positive by centrifuged buffy coat smear and 23.2% was positive by rapid card test[11].

Another study done by Akhtar et al in 2010 from India found 82.8% was positive by microscopic examination of peripheral blood film, 92.18% was positive by centrifuged buffy coat smear and 93.7% was positive by rapid card test[13]. When we compared sensitivity and specificity of centrifuged buffy coat smear we found following results. [Table 1] When we compared sensitivity and specificity of Rapid card test which is mentioned in table 2.

Table 1: compared sensitivity and specificity of Centrifuged buffy coat smear to different studies

Study	Findings
Present Study in 2016	Sensitivity of centrifuged buffy coatwas 76.81% And specificity was 100%
Study by S Mohanty et al 2015	Sensitivity of centrifuged buffy coat was 91.9%and specificity was 99.2%
Study by Akhtaret al in 2010	Sensitivity of centrifuged buffy coat was 93.3%and specificity was 95%
Study by Adeoveet al in 2007	Sensitivity of centrifuged buffy coat was 55.91%and specificity was 88.8%
Study by BhandariPL in 2008	Sensitivity of centrifuged buffy coat was 96.22% and specificity was 93.6%

Table 2: compared sensitivity and specificity of Rapid card test to different studies

Study	Findings
Present study in 2016	Sensitivity of rapid card was 75.71% and specificity was 100%
Study by Akhtar et al in 2010	Sensitivity of rapid card was 98.24% and specificity was 93.65%
Study byJ. Jafar Ebrahimet alin 2013	Sensitivity of rapid card was 98.2% and specificity was 100 %
Study by Panigrahi K in 2013	Sensitivity of rapid card was 93.0% and specificity was 94.67%
Study by Praveen K Bharti et al in 2010	Sensitivity of rapid card was 93.0% and specificity was 85.0%

Conclusion

From the present study it may be concluded that, Rapid card for antigen detection can diagnose malaria reliably. In case of Rapid card for antigen sensitivity is low in comparison to Centrifuged buffy coat smear and specificity is equal to Centrifuged buffy coat smear (100%). It can be performed in peripheral laboratories in rural areas where people cannot afford for Rapid card due to its high cost. Centrifuged buffy coat smear examination is more reliable and cost effective method than Rapid card test and it is substitute as alternate to rapid card test in detection of malaria due to its high sensitivity in resource limited settings.

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