Sensitivity of Three Diagnostic Methods (PF Antigen Test, QBC and Blood Smear) in Determining Malaria Prevalence among Patients Referred to Goodnews Medical Laboratory, Umuahia, Nigeria

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ABSTRACT

Three diagnostic methods were used in malaria screening of patients referred to Goodnews diagnostic laboratory, Umuahia to determine their sensitivity as well as the infection prevalence. Blood samples were collected from 150 patients and subjected to three different malaria tests namely: PF antigen test (P.f), Blood Smear method (BS) and Quantitative Buffy Coat (QBC) method. Highest infection prevalence (60.69%) was recorded by the Pf antigen test followed by the BS method which recorded 58.67% infection rate. The least infection rate 55.33% was recorded by the QBC method and the differences were not statistically significant (X^2 = 0.90, $p<0.05$). Males were more infected (73.02%, 60.34% and 69.84%) than females (50.57%) in Pf antigen, QBC and BS methods respectively but differences were not statistically significant (X^2 = 2.25, $P<0.05$). Infection was highest in patients of 71 years and above (100%) followed by those in 1-10 years of age (70.59%). The blood smear method showed the highest sensitivity (100%) followed by Pf antigen test (96.70%) and QBC yielded 94.62%. the high sensitivity of the three methods recommends them for use, but for clinical diagnosis the Pf antigen test should be supplemented with either QBC or BS method.

Keyword: Malaria, blood smear, QBC and PF antigen

INTRODUCTION

Among human parasitic diseases, malaria remains the world's most important cause of morbidity and mortality (Nosten 2003) which are inimical to economic growth and development of the family and the nation at large. Its impact in Africa is enormous. It is responsible for the slow economic development and perpetuating vicious cycle of poverty (UNICEF, 2006). Economic losses due to malaria have been estimated to be about U.S$12 billion annually (Gallop and Sachs, 1998) in Africa where majority of the people are subsistence farmers. Impact of malaria on health is great and is still worsening due to the spread of insecticide resistant mosquitoes. Malaria reduces work capacities and undermines local efforts to live off the land in a sustainable manner. UNICEF (2005) explained that the disease impairs physical and mental development in children, diminishes returns achieved through education and limits their potentials to contribute to the social and economic growth of their country. Instant withdrawal of individual's contribution to productive processes is noticed when the infection results to mortality. In Africa, malaria is estimated to cause 400,000 cases of maternal anaemia and between 75,000-200,000 infant deaths annually. Maternal anaemia contributes to maternal deaths to an estimated tone of 10,000 deaths annually (CDC, 2005). Financial losses due to malaria in Nigeria is estimated to be 132billion naira annually, made up of treatment cost, prevention, loss of man hours etc (Federal Ministry of Health, 2008).

As the impact of malaria increases, the need to control the infection arose. Roll Back Malaria recommended among other control strategies proper diagnosis of infected individuals. This demanded the evolution of diagnostic technology which combines cost effectiveness, simplicity with rapidity of results. In the face of this challenge, biomedical science moved into action to develop new diagnostic technologies to compliment the traditional blood smear method. Presently, many diagnostic methods have emerged including the use of quantitative buffy coat (QBC), *Plasmodium falciparum* (Pf) antigen test etc. The QBC method involves the staining of the centrifuged red blood cell layer with acridin orange and its examination under UV light source. The method is easy, fast and claimed to be sensitive. The PF antigen test is a rapid diagnostic test specific for circulating parasite antigen and targeting either the histidine-rich protein-2 of *P. falciparum* or a parasite specific lactate dehydrogenase. It is fast, simple, less cumbersome and also claimed to be sensitive. We carried out this

study to determine the sensitivity of these two methods for comparison with that of traditional blood smear method in the determination of malaria prevalence in the area.

MATERIALS AND METHOD

Study Area

This study was carried out in Goodnews Medical Diagnostic Laboratories at No.11 Kaduna Street by Okigwe Road Umuahia. Umuahia is a develop town that shares boundaries with Uzoakoli, Aba, Enugu, Imo and Bende etc. It is also the State capital of Abia State. The population is predominantly traders. Others are public servants, artisans, farmers, students and housewives. The town is endowed with abundant natural resources. Rainfall is always high during rainy season and the temperature ranges from 27^0C-32^0C. Blocked drainage systems create stagnant water for mosquito breeding. Goodnews medical laboratory was selected because it is well patronized by clinicians and other health practitioners due to their good facilities and expertise of their technical staff. The study was conducted between June and August 2011 which was the peak period of malaria transmission.

Study Population and Ethical Clearance

A total of 150 patients referred to Goodnews Medical Laboratory, Umuahia from both public and private clinics were randomly selected for the study. These were made up of males, females, adults and children. A letter seeking for approval and co-operation was written to the management of the establishment explaining the objective of the study. After series of subsequent consultations, approval was received. On each day of sample collection, the randomly selected patients were briefed on their involvement in the exercise and their consent received.

Sample Collection

Information relating to names, age and sex of each randomly selected patient was recorded. About 2mls of venous blood was collected from each patient using disposable syringes and needle. This was emptied in EDTA bottle, corked and labeled with the tag of the patient.

Blood Smear Preparation and Examination

A capillary tube/pipette was used to collect and mount a drop of blood on a clean grease-free slide to prepare a thick smear. This was allowed to dry in a room temperature and fixed for 3mins in methanol before staining. Staining was done according to Ochei and Kolhatkar (2008) using field stain. The fixed thick blood smear was dipped into field stain A for about 3mins. This was then

removed, washed in clean water and dipped into field stain B solution for 5secs. The slide was then removed, washed and kept in a slanting position to dry. Examination of slides was done using high power light microscope, viewed under emersion oil to detect positive cases.

Plasmodium Falciparum (PF) Antigen Test

The test was conducted using the SD BIOLINE Malaria PF/PV test kit manufactured by SD STANDARD DIAGNOSTICS, INC. It is a immunochromatographic test for qualitative detection of antibodies of all iotypes (IgG,IgA,IgM) specific for *P. falciparum* and *P. vivax* simultaneously in whole blood, serum or plasma. The kit contains a membrane strip pre-coated with recombinant malaria P.f capture antigen (MSP) on test band 1 region and with recombinant malaria P.v capture antigen (MSP) on test band 2 region. The recombinant malaria P.f/P.v antigen (MSV)-colloid gold conjugate and serum sample moves along the membrane chromatographically to test regions 1, 2 and forms a visible line as the antigen-antibody-antigen gold particle complex forms with high degree of sensitivity and specificity. Two drops of whole blood is placed in the sample well of the test kit marked "S" and two drops of assay diluent was dropped on it and observed for 10mins to see colour bands appearing within the result window at positions marked 1 for *P. falsiparum*, 2 for *P. vivax* and C for control. This is interpreted according to the manufacturer's instruction.

Quantitative Buffy Coat (QBC) Method

Blood from the remaining blood samples of each patient was filled in a high precision glass capillary tube pre-coated internally with acridine orange stain and potassium oxalate, sodium heparine and K2EDTA acting as anti-coagulants. The blood is filled to $^3/_4$ of the length of the capillary tube. The tube is then rolled between the fingers to mix the blood with anti-coagulants and to area coated with acridine orange stain. Then a precisely made cylindrical float designed to be suspended in the packed red blood cells is inserted. The blood filled tube was centrifuged at high speed of 12,000rpm. This separated the different blood components and the floats moved down the capillary pushing the white cells, red cells, platelets, and plasma towards the capillary surface making it easier to view under white light microscope equipped with UV adapter. Fluorescence parasites were then observed at red blood cell/white blood cell interface.

Statistical Analysis

Data generated from the results were transformed into simple percentages and analyzed using chi-square statistics. Diagnostic performance involving the calculation of sensitivity was done according to Ochei and Kolhatkar (2008). True positive (TP), true negatives (TN), false positives (FP) and false negatives (FN) were calculated and used to determine sensitivity. Using the formula:

$$Sensitivity = \frac{TP}{(TP + FN)} \times \frac{100}{1}$$

RESULTS

Results of the three diagnostic methods showed that of the 150 patients subjected to the three test methods 91 (60.67%) were positive by Pf antigen test, 83 (55.33%) were positive by QBC and 88 (58.67%) were positive by blood smear method (table 1). Differences were not statistically significant $(x^2 = 0.90, P < 0.05)$. Taking the result of the blood smear method as an index of active infection, the sensitivity of the three diagnostic methods were calculated. The sensitivity of the blood smear method was highest (100%) followed by Pf antigen test (96.70%) while QBC recorded the least sensitivity (94.62%).

Table 1.1: Malaria Prevalence Using the (3) Three Different Diagnostic Methods

Methods	No. Examined (%)	No. Infected (%)	No. Uninfected (%)
PF antigen	150	91(60.67%)	59 (39.33%)
QBC	150	83(55.33%)	67 (44.67%)
BS	150	88(58.67%)	62 (41.33%)

Gender related issues raised in this study indicated that out of the 63 males involved in the study, 46 (73.02%) were positive by Pf antigen tests, 38 (60.32%) were positive by QBC while 44 (69.84%) were positive by BS method. Among the females, there were no differences in the diagnostic performance of the three methods as each detected 50.57% infection among them. Differences in gender related prevalence were not statistically significant $(x^2 = 2.25,\ P < 0.05)$.

TABLE 2: Sex-Related Prevalence of Malaria Infection Using the (3) Three Diagnostic Methods

Sex	No. sampled	No. infected (%)		
		PF Antigen	QBC	PS
M	63	46 (73.02%)	38 (60.34%)	44 (69.84%)
F	87	44 (50.57%)	44 (50.57%)	44 (50.57%)
Total	**150**	**90 (60.00%)**	**82 (54.67%)**	**88 (58.67%)**

Infection rate by age-group revealed that patients in the 71-80years age-group were the most infected with malaria as all 4 (100%) patients were positive were positive by both Pf antigen test and BS method. Pf antigen test showed second highest infection rate (73.33%) among 21-30 years age-group followed by those in 1-10 years age-group who recorded 70.59% infection. The least infection was recorded in the 61-70 years age-group with 44.44% prevalence. QBC recorded highest infection rate (73.0%) in the 71-80 years followed by those in 1-10 years (64.71%). Next was those in 41-50 years age group which recorded 57.14%. In these infection rate by QBC method was in the 31-40 years (44.44%)

For the blood smear method, highest prevalence was found in the 71-80 years age-group (100%) followed by 1-10years (67.65%) and 41-50 years (64.29%). The least infection was found in the 11-20 years age-group (26.32%). Differences in infection

rates in the various age-groups were not statistically significant (P<0.05).

TABLE 3: Age-Related Prevalence of Malaria Infection Using the (3) Three Diagnostic Methods

Age (years)	No. Examined	No. infected (%)			No. uninfected (%)		
		PF Antigen	QBC	PS	PF Antigen	QBC	PS
1-10	34	24(70.59)	22(64.71)	23(67.65)	10(41.67)	12(35.29)	11(32.35)
11-20	19	10(52.63)	9(47.37)	5(26.32)	9(47.37)	10(52.63)	14(73.68)
21-30	30	22(73.33)	17(56.67)	19(63.33)	8(26.67)	13(43.33)	11(36.67)
31-40	27	14(51.85)	12(44.44)	13(48.15)	13(48.15)	15(55.56)	14(51.85)
41-50	14	8(57.14)	8(57.14)	9(64.29)	6(42.86)	6(42.86)	5(35.71)
51-60	13	6(46.15)	7(53.84)	5(38.46)	7(53.85)	6(46.15)	8(61.54)
61-70	9	4(44.44)	5(55.56)	5(55.56)	5(55.56)	4(44.44)	4(44.44)
71-80	4	4(100)	3(73.00)	4(100)	-	1(25.00)	-
Total	**150**	**92(61.33)**	**83(55.33)**	**83(55.33)**	**58(38.67)**	**67(44.67)**	**67()44.67**

DISCUSSION

The result of this work showed that malaria is still of public health importance in the study area. Prevalence rate of 58% average of the three diagnostic methods used in this study was recorded. This is higher than the results obtained by some scientists in their work elsewhere. For instance Ibeziako *et al.,* (1980) in their work in Port Harcourt observed 7.3% among pregnant women. Other scientists obtained results that are lower than that obtained in this study. For instance, Mockenhaupt *et al.,* (2002) in their study of pregnant women in Ghana recorded 90% prevalence. The high infection rates recorded in the three diagnostic methods used in this study is indeed a proof of the endemicity of the disease in the area which may not be unconnected with the result of interaction between the climatic elements and the behavioural attitude of the people living in different communities of Abia State as well as their degree of responsiveness to ITNs use.

Diagnosis also revealed that more males were infected than females which agreed with the work of Ukpai and Njoku (2000) who found out also that more males were infected than females in their work and attributed it to more exposure of males to mosquito bite than females. They believed that males expose their body more frequently than females especially during hot weather. Another reason could be because men engage in tedious jobs and return late in the evening tired. The result is that they sleep off without taking any precaution against mosquito vectors of malaria that

bite later in the night. However, the result of this work contrasts with those of Oparaocha (2003) who reported that more females were infected in his study than males and attributed it to lower red blood count associated with females.

Females according to him, is associated with lower RBC count due to excessive loss of blood during their menstrual cycles which predispose them to severe malaria symptomatolgy. Prevalence by age-group revealed that patients of 70 years and above had the highest infection rates (100%,73% and 100%) by Pf antigen test, QBC and BS methods respectively. The reason for higher prevalence among the very aged group is not properly understood. However one may say that people of that age-group are losing their consciousness for protection from mosquito attack, experience immunodepression and produce certain body odour that may attract mosquito vectors to them. This work also recorded low prevalence to malaria (53%, 47% and 26%) using Pf antigen, QBC and BS method respectively among 11-20 years age-group. This may have to do with the higher immune response associated with people in this age-group.

The development of rapid diagnostic tests (RTDs) to identify individuals infected with malaria has been of paramount importance in the effort to control malaria. This is because the blood smear method though remains; the gold standard is cumbersome and time wasting. The use of QBC and PF antigen test is a new development in diagnostic technology with obvious advantages over the BS method. In this study, highest infection rate (60.67%) was recorded by PF antigen test followed by blood smear (58.67%) and the least rate (55.33%) was recorded by the QBC method. Since the PF antigen test identifies antibodies only and not the parasite we decided to use the gold standard BS method as an index of active infection for the determination of the diagnostic performance. True positives and negatives, false positives and negatives of the three diagnostic methods were determined and used in calculating the sensitivity of these methods. Blood smear method yielded the highest sensitivity of 100% followed by PF antigen method with 96.70%. QBC method yielded 94.62% sensitivity. Difference in the sensitivity of the three diagnostic methods were not statistically significant ($P<0.05$) indicating that the three methods are efficient diagnostic tools in malaria detection. The PF antigen test though unable to identify malaria parasites are however useful in community-wide epidemiological investigation requiring quick intervention. Its use is therefore

recommended with caution as it could identify patients with cleared parasitemia as positive on the basis of circulating antibody of the previous infection. For clinical use, the PF antigen tests should be supplemented with either the blood smear technology or the QBC methods for parasite identification.

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