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COMPARATIVE STUDY OF INTRADERMAL SMEAR MICROSCOPY IN THE DIAGNOSIS OF MALARIA IN SYMPTOMATIC PREGNANT WOMEN

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ABSTRACT

Objectives: A comparative study of intradermal smear in the diagnosis of malaria in symptomatic pregnant women. Venous blood served as the control.

Patients and methods : Fifty consecutive symptomatic pregnant women were recruited. Thick films of both venous and intradermal blood were examined. Questionnaires were used to determine patients' preference for the two techniques. Tests of statistical significance were done with Fisher exact and Yates correlation coefficient at 95% confidence interval. Sensitivity, specificity and accuracy rates were used to assess the validity of intradermal smear.

Results: Intradermal smear more frequently diagnosed malaria parasitaemia than peripheral venous blood (66% vs 56%). This was statistically significant (P value: 0.0065). The sensitivity of intradermal smear was 85.7% while the positive predictive value was 77.4%. The accuracy rate was 76.7%. The technique of intradermal blood collection was preferred by 28% of women.

Conclusion: Intradermal smear is useful in malaria diagnosis in pregnancy and may be an additional evaluation tool for persistent fever in pregnancy.

Key Words: Intradermal smear, malaria in pregnancy, malaria diagnosis. *(Accepted 5 January 2010)*

INTRODUCTION

Malaria is a common medical complication of pregnancy in malaria endemic zones of the world where about 40% of the world's population lives[1]. Pregnant women are generally known to have an increased susceptibility as well as a more severe form of the infection[2,3]. A relative decline in immunity has been adduced for this susceptibility.

Malaria causes abortion, anaemia, preterm labour, intrauterine growth restriction and intrauterine fetal death [2,4-7]. Congenital malaria has also been reported in neonates[8].

The relative decline in immunity to malaria in pregnant women causes an increased parasitaemia as well as clinical disease. Also, Human immunodeficiency virus (HIV) infection diminishes a pregnant woman's ability to control P. falciparum infection[9]. The prevalence and intensity of malaria infection during pregnancy is higher in women who are HIV infected [9].

Peripheral blood film (PBF) microscopy is the gold standard in the diagnosis of malaria. Although rapid diagnostic tests have been used, the variable sensitivity and specificity is a limiting factor in their use[10,11].

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There are also instances when peripheral blood is negative for malaria parasites in women who present with clinical symptoms of malaria. This is particularly common in areas where there is unsupervised use of antimalaria drugs thereby posing management dilemma[12].

In these situations intradermal smear (IDS) may be useful. Intradermal blood smear microscopy for the detection of malaria pigments has been used albeit in studies involving non-pregnant patients. In malaria, parasitized red blood cells become more irregular in shape, more antigenic and less deformable causing cytoadherence to vascular endothelium. This leads to sequestration of parasitized red cells, blockage of deep vascular beds and mechanical blockage of vessels[13].

Intradermal blood smear has demonstrated a high sensitivity and specificity[13]. This is due to the sequestration of malaria parasites in the subcutaneous tissue. Dermal smear or biopsy showed a higher concentration of malaria parasites compared to venous and capillary blood film due to sequestration and also, parasitaemia persisted in subcutaneous tissue in women with a negative venous blood suggesting that intradermal smear test may be useful[13]. Macpherson et al[13] also suggested that skin smears or biopsy would more accurately represent the

total parasite load than a conventional blood smear as intradermal smear remained positive for a significantly longer period[12].

Intradermal blood smear microscopy is simple, easy to perform[13] and requires no special infrastructure for its performance and interpretation. Unlike peripheral blood film, it requires no training for venepuncture, so it would be useful in a primary health care setting[13].

All previous studies on intradermal smear were in non-pregnant subjects.

Therefore we evaluated the usefulness of intradermal smear in symptomatic antenatal women. We determined the diagnostic accuracy of intradermal smear as well as the degree of correlation with peripheral blood film microscopy in pregnancy. The patients' preference for the technique of intradermal smear was also determined.

MATERIALS AND METHODS

This study was done at the antenatal clinic of Irrua Specialist Teaching Hospital, Edo state, Nigeria. It was a comparative study and peripheral venous blood served as control. The study was approved by the Medical Ethics Committee of the hospital and informed consent was obtained from all women.

Fifty consecutive symptomatic pregnant women were included in the study. All women had both peripheral venous blood and intradermal blood collected and made into thick films which made up a total of 100 slides. The sample size was calculated by the statistical formula[14] for comparative study based on 3% prevalence of acute malaria in pregnancy. The confidence level was set at 95%.

In order to determine the women's preference for the technique of intradermal blood collection, a questionnaire was administered.

Indices of test validity used were sensitivity, specificity, positive predictive value and accuracy rate.Tests of statistical significance were done with Fisher exact and Yates correlation coefficient at 95% confidence interval.

Exclusion criteria

Urine microscopy and culture was done to exclude urinary tract infection. Respiratory examination was also done to exclude infection of the respiratory system. The other exclusion criterion was prolonged prelabour rupture of membranes >24hours (PPROM).

Procedure for intradermal smear

The intradermal blood smear was made by making several small intradermal pricks with a No 23 gauge needle within a small area (1× 1 cm) on the outer aspect of the upper arm. The area was gently squeezed and the blood made into a thick film on the slide. The slides were allocated numbers and thereafter stained with Giemsa stain.

Slide reporting:

Thick films of both the venous and intradermal blood were examined under the light microscope for evidence of malaria parasite or pigment with oil immersion technique at 10 × 100 magnification. Quantitative reporting of the slides was done per 100 high power fields (HPF). The slides were reported negative, if after viewing 100 fields, no parasites were seen. However, for the positive slides, the number of parasites/100 leukocytes was counted.

Two laboratory scientists viewed the slides and these were classified as positive or negative. By the allocated study numbers on the slides, they were blinded such that they did not know which was intradermal or peripheral smear. Neither did they know which smear was from which patient. The average of the two independent reports was used for the study.

RESULTS

Intradermal smear more frequently diagnosed malaria parasitaemia than peripheral blood (66% vs 56%). The ability of intradermal smear to diagnose malaria in pregnancy was statistically significant (P value: 0.0065). Thirty nine (78%) symptomatic women presented with complaints of fever although only 46% of them had a temperature of 37.2^{0}C and above at presentation. Other presenting symptoms included headaches, chills and body aches. The age distribution of study population is shown in table 1. As in table 2, nulliparous women constituted the majority (32%) of the women. Most of the women (54%) were in the third trimester of pregnancy.

Intradermal blood smear had a sensitivity of 85.7%. The specificity, however, was 56.3%. Table 3 shows the comparison of IDS to BPF microscopy. The positive predictive value was 77.4% while the negative predictive value was 69.2%. Intradermal smear had an accuracy rate of 76.7%.

The false positive and false negative rate of intradermal blood smears in these women was 22.6% and 25% respectively. Twenty eight per cent of the women preferred the technique of collecting intradermal blood. 48% did not prefer the technique while 14% were indifferent.

The ability of intradermal smear to diagnose malaria was statistically significant (P value 0.0384; CI 95%) compared to peripheral venous blood film only in nulliparous women.

Table 1: **This Table shows the age Distribution of Participating Women.**

Age	Number	Percentage (%)
<20	1	2
20-24	11	22
25-29	15	30
30-34	9	18
35-39	14	28
>40	0	0
Total	**50**	**100**

Table 2: **Parity of Women in the Study.**

Parity	Number	Percentage (%)
0	16	32
1	11	22
2	10	20
3	6	12
4	4	8
>5	3	6
Total	**50**	**100**

Table 3: **Comparison of Intradermal Smear with Peripheral Blood Film.**

		Intradermal smear	
		Positive	Negative
Peripheral venous blood	Positive	24	4
	Negative	10	12

P- value 0.0065; CI 95%

DISCUSSION

The desire for a total eradication of malaria parasitaemia in pregnancy[9] in order to avoid attendant consequences on the mother and her fetus has led to the evaluation of different diagnostic techniques for malaria detection in pregnancy. Peripheral blood film microscopy is the gold standard for malaria parasite detection because of the various limitations of other forms of diagnosis, hence, there arises a need to optimize microscopy for the diagnosis of malaria parasitaemia in pregnancy.

There are no previous published studies on intradermal smear involving pregnant women despite their susceptibility to malaria. Intradermal smear represents a sequestered biomass of malaria parasites[13]and demonstrates more frequent and a higher parasite load than peripheral venous blood[2]. These characteristics are similar to those of placenta parasitaemia.

In this study, intradermal smear more frequently diagnosed malaria parasitaemia compared to peripheral venous blood (66% vs 56%).This was statistically significant (P value: 0.0065).

Participants in this study were mainly nullipara (32%) and primipara (20%) with most attacks of acute malaria (54%) occurring in the third trimester of pregnancy. This conforms to an earlier report that malaria in pregnancy is more common in the last trimester of pregnancy than the first[15]. The women presented mainly with fever, headaches, chills and body aches. Although 39 women complained of fever at presentation, fever (Temp >37.2°C) could be objectively demonstrated in only 46% of them, as most immune pregnant women present with mild febrile episodes[2].

The sensitivity of intradermal smear depends on the level of parasitaemia and symptomatic women are more likely to have parasitaemia. The ability of intradermal smear to detect malaria parasitaemia when it was actually present was 85.7%. The import of this is intradermal smear for malaria parasite will test negative in 14.3% of symptomatic pregnant women with malaria when the converse was the case. The reliability of the test, the positive predictive value, was 77.4%. This is a measure of the probability of intradermal smear to detect malaria parasitaemia in the presence of malaria parasites. That is, it will correctly detect malaria parasitaemia in greater than seven (7) out of ten (10) cases. This study indicates that intradermal smear is useful in the symptomatic pregnant women.

Nigeria has a dearth of trained personnel to perform venepuncture at rural communities due to its technically demanding requirements, hence, intradermal smear microscopy could be useful in these settings. It is simple, easy to perform and requires no special infrastructure[13]. It also requires less training. Once the dermal blood is collected, however, the technique of staining is essentially the same as that of venous blood and the cost is same. This is particularly important in low resource settings as ours. Intradermal smear may also be useful in obese women in whom venepuncture could be very difficult.

Moreover, in settings with relative unrestricted access to antimalaria drugs as ours, intradermal smear may be an additional investigation for malaria workup as it demonstrated a persistence of parasitaemia when venous blood was negative[13]. This is particularly important in pregnancy, especially, as pregnant women with persistent fever may be inappropriately treated for Typhoid fever.

The preference for the collection technique of intradermal blood by 28% of the women was due to the resentment of multiple skin pricks by majority of women.

In this study we did not evaluate species identification. Previous works demonstrated more of P. falciparum in intradermal smear than other species[13]. This is not only because *P. falciparum* is the most widely distributed[12] and most common species in endemic areas of Africa[8], but also because mature forms of P. falciparum sequestrate in tissues[16].

The findings from this study suggest that intradermal smear is useful in the evaluation of women with symptoms suggestive of malaria in pregnancy.

Intradermal smear may find great usefulness in primary health care and rural settings where there are few trained personnel to perform venepuncture. Even in urban areas, it may be a good alternative to difficult venepuncture in obese women and those with persistent fever following treatment.

Intradermal smear is a useful technique for detecting malaria parasitaemia in symptomatic pregnant women. It may also be useful as an additional evaluation tool in pregnant women with persistent fever. We therefore recommend its introduction in the evaluation of pregnant women with malaria symptoms due to its simple, easy technique and high sensitivity. Since previous studies on intradermal smear were in non-pregnant women, there is a need for more research on its usefulness in pregnancy. Its role in the detection of malaria parasitaemia in asymptomatic pregnant women needs to be evaluated.

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