Utility of Intradermal Blood Smear in the detection of Asymptomatic Malaria Parasitaemia in Pregnancy

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Summary

Objectives: *We evaluated the usefulness of intradermal smear microscopy (IDS) in the detection of asymptomatic malaria parasitaemia in pregnancy. Peripheral venous blood (PVB) served as control. The preference for the collection technique of dermal blood was also assessed.*
Patients and methods: *One hundred and fifty (150) asymptomatic women were recruited. They had both intradermal smear and peripheral venous blood smear were made for all patients. Measures of test validity included sensitivity, specificity and accuracy rate. Test of statistical significance was with Yates correlation at 95% confidence limit.*
Results: *The prevalence of asymptomatic malaria parasitaemia was higher using intradermal smear (35.3% vs 33.3%) though this was not statistically significant. Intradermal smear had a sensitivity of 40% and specificity of 67%. The positive predictive value was 37.8% with accuracy rate of 58%. 41% of participants preferred the technique of collection of intradermal blood.*
Conclusion: *Intradermal smear appears to have no usefulness in the detection of asymptomatic malaria parasitaemia in pregnancy. However, we recommend more studies on its value in pregnancy, especially amongst symptomatic pregnant women.*

Key words: *Intradermal smear, malaria in pregnancy, malaria diagnosis*

Introduction

Pregnant women are known to demonstrate an increased susceptibility to malaria[1]. The relative decline in immunity to malaria in pregnant women causes an increased parasitaemia as well as clinical disease[1].

Malaria parasitaemia in pregnancy could be asymptomatic or symptomatic. Pregnant women living in holo-endemic or hyperendemic areas develop asymptomatic parasitaemia. This elicits both the humoral and cellular immunity which takes 5 to 10 years to develop[2]. Unfortunately, the Human Immunodeficiency Virus (HIV) infection erodes this immunity and diminishes a pregnant woman's ability to control *Palsmodium falciparum* infection[1].

Also the consequences of malaria parasitaemia on the foetus are same in both symptomatic as well as in asymptomatic pregnant patients. Where there is a high level of maternal immunity, malaria parasitaemia is associated with an increased risk of maternal anaemia, abortion and preterm labour. The tendency to intra-uterine growth restriction and low birth weight infants is also higher[3-7].

Treatment of malaria during pregnancy aims at a complete elimination of the infection because any level of parasitaemia is of consequence to the mother and her foetus[1], thereby making accurate diagnosis of malaria essential.

The diagnosis of malaria is traditionally by peripheral blood smear examination by microscopy (thick & thin) after it has been stained with Giemsa. This is the gold standard[3,8]. At microscopy, parasite quantification is done while serial evaluation may be used to monitor response to treatment[8].

Rapid diagnostic tests, RTDs, have been reported to find usefulness when discrepancy is observed at microscopy[9].

However, their use is limited by their varying sensitivity and specificity[10] and they may not also detect parasitaemia in the blood of pregnant women with low levels of malaria parasites[11], hence there is a need to increase the options available for blood film microscopy for the diagnosis of malaria parasitaemia in pregnancy.

Intradermal blood smear microscopy for the detection of malaria pigments has been used albeit in non-pregnant patient studies. In malaria, parasitised red blood cells become more irregular in shape, more antigenic and less deformable causing cytoadherence to vascular endothelium. This leads to sequestration of parasitised red cells, blockage of deep vascular beds and mechanical blockage of vessels[17–20].

Intradermal blood smear has demonstrated a high sensitivity and specificity[12] because of 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 and also parasitaemia has been reported to persist in subcutaneous tissue in patients with a negative venous blood suggesting that intradermal smear test may be useful[12]. Macpherson *et al* [12] 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[13].

Intradermal blood smear microscopy is simple, easy to perform[12] 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[12]. It is also an important diagnostic tool when malaria is suspected but repeated peripheral blood film shows negative results[12,14].

Moreover, the cost implication of treatment, side effects of medication and unnecessary treatment for suspected malaria may be avoided if accurate detection of malaria parasites can be achieved in pregnancy.

We therefore evaluated the usefulness of intradermal smear microscopy in asymptomatic pregnant women and also determined the preference for the technique of collection of intradermal blood.

Materials and Methods

This was a comparative study done at the antenatal clinic of Irrua Specialist Teaching Hospital, Edo State, Nigeria. Peripheral venous blood served as the control. One hundred and fifty consecutive asytomatic antenatal patients were recruited into the study. All patients had both peripheral venous blood and intradermal blood collected and made into thick film which made up 300 slides. A questionnaire was also filled to have the patient's biodata and record patient's preference between technique of collection of intradermal blood and peripheral venous blood.

Test of statistical significance was done with Chi square with Yates correction at 95% confidence limit. Validity of Intradermal smear was done with sensitivity, specificity, positive predictive value and accuracy rate.

Inclusion criteria: Women were regarded as asymptomatic if they had no symptoms of malaria and were also afebrile. They were also included if they had not used antimalaria drugs, for treatment or prophylaxis, in the preceding four (4) weeks.

Procedure for Intradermal Blood Smear: The intradermal blood smear was made by making several very small intradermal pricks with a No 23 gauge needle within a small area of (1× 1 cm) on the outer aspect of the 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.

Reporting of the slides: Thick films of both the venous and intradermal blood were then 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.

Ethical Consideration: In line with ethical principles in research works involving human participants, patients were informed about the study, highlighting its essence. Permission was obtained from the Medical Ethics Committee of Irrua Specialist Teaching Hospital and informed consent was obtained from all the participants. In this study results were made available to the patients and treatment prescribed to all with positive results. They were prescribed Sulfadoxine, Pyrimethamine combination, in this case Fansidar® after excluding allergy to sulfonamides.

Results

The prevalence of asymptomatic malaria parasitaemia using intradermal smear was 35.3% while that for peripheral venous blood was 33.3%. However, the higher prevalence detected by intradermal smear was not statistically significant. Tables i and ii show the age distribution and parity of the participants respectively. Most were in the age range 25-29 years (44%) and many (35%) were nulliparous patients.

The sensitivity and specificity of intradermal blood smear was 40% and 67% respectively. It had a positive predictive value of 37.8% while its negative predictive value was 69%.

The accuracy rate was 58% while the false positive rate and false negative rate of intradermal blood smear was 62% and 31% respectively. The technique of collecting intradermal blood was preferred by 41% of the women.

The relationship between parity and the results of microscopy for both source materials is depicted in table iii. Both intradermal smear and venous blood were positive mainly in nulliparous patients. This was not further subjected to test of statistical significance because of the low sensitivity of 40%.

Table i:
Age distribution of the sample population.

Age (years)	Number	%
<20	2	1
20-24	24	16
25-29	65	44
30-34	42	28
35-39	15	10
≥40	2	1
Total	**150**	**100**

Table ii:
Parity of participants

Parity	Number	%
0	52	35
1	37	25
2	25	17
3	11	7
4	14	9
≥5	11	7
Total	**150**	**100**

Table iii:
Microscopy result in relation to patient's parity.

	Peripheral venous Blood		Intradermal blood smear	
Parity	Positive	Negative	Positive	Negative
0	19	33	19	33
1	8	29	13	24
2	11	13	7	17
3	5	6	6	5
4	4	8	3	9
≥5	2	9	4	7

Discussion

The susceptibility of pregnant women to malaria parasitaemia has made its diagnosis important as this will reduce the attendant consequences on the mother and her fetus. The prevalence of asymptomatic malaria parasitaemia using intradermal blood smear of 35.5% was slightly higher than 33.3% diagnosed with peripheral venous blood. The statistically insignificant difference of the ability of intradermal smear to detect asymptomatic parasitaemia confers no advantage over the gold standard.

The prevalence of asymptomatic parasitemia of 33.3% by venous blood and 35.5% by intradermal smear were both higher than earlier reports using peripheral venous blood[4,5]. This may reflect seasonal variation as this study was done during the rainy season when malaria parasitaemia is highest. This conforms to seasonal variation which correlates with both venous blood parasitaemia and sequestered malaria parasites in tissues as earlier reported [4].

Placenta malaria parasitaemia has been reported to be more frequent and heavier than maternal peripheral parasitaemia and up to 50% of women with placenta parasitaemia do not show peripheral malaria parasitaemia[3]. Since the dermis also sequesters malaria parasites, its no surprise that intradermal smear more frequently diagnosed malaria parasitaemia than peripheral venous blood in this study. Moreover, 22% of participants who had positive intradermal smear had negative peripheral venous blood.

The sensitivity of intradermal smear in asymptomatic patients depends on the level of parasitaemia and asymptomatic patients are less likely to have parasitaemia anyway. Hence the low sensitivity of 40%. This implies that intradermal smear will test negative in 60% of asymptomatic pregnant women when the converse was the case.

The reliability of the test, the positive predictive value, was 37.8%. This measures the probability of intradermal smear to detect malaria parasitaemia in the presence of malaria parasites. From this study, intradermal smear will correctly detect malaria parasitaemia in less than four (4) out of ten (10) cases. This work indicates that intradermal smear has no usefulness in the detection of asymptomatic parasitaemia.

Intradermal smear is simple, easy to perform and requires no special infrastructure [12]. It also requires less training unlike venepuncture which is more technically demanding, requiring intense training of medical personnel. Unfortunately, despite its ease of performance, the results of this study do not justify its use in asymptomatic pregnant women.

This study has, however, posed a question. Should intradermal smear positive patients be treated with antimalaria drugs? The peculiarity of pregnancy has made this question relevant especially in asymptomatic patients. The importance of this cannot be overemphasised as the new WHO approach to the management of malaria in pregnancy recommends intermittent preventive treatment (IPT) with Sulfadoxine Pyrimethamine combination from second trimester of pregnancy in asymptomatic patients[3,8].

In this study, results were made available to the patients and treatment prescribed for all positive patients. They had Sulfadoxine Pyrimethamine combination, in this instance, Fansidar®, if they were in the second trimester after excluding allergy to Sulfadoxine.

This study suggests that intradermal smear may have no usefulness in the detection of asymptomatic malaria parasitaemia in pregnancy. More research needs to be done to evaluate the usefulness of intradermal smear in pregnancy, especially, in symptomatic pregnant women.

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