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Research Article

Retention of Histidine-Rich Protein II Rapid Diagnostic Tests in Pregnant Women after Antimalarial Treatment

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ABSTRACT
Malaria is a preventable and curable disease and malaria diagnosis is critical to reduce malaria burden. This study is aimed at investigating the persistent positivity of Histidine-Rich Protein II (HRP2) rapid diagnostic test in pregnant women after antimalarial treatment. The study was carried out in Turai Yar'adua Maternity and Children's Hospital Katsina and Federal Medical Centre Katsina. Blood samples were collected with the help of trained physicians. The result from this study shows that HRP2 persist more in the blood of pregnant women after treatment with fensider. It was observed that in all the age groups, day 3, day 7 and day 14 post drug treatment, were still positive for malaria using HRP2 malaria rapid diagnostic tool. The HRP2 was positive for all the different age group after 21 and 28 days post treatment except the age group 30-34 (97.5%). The percentage in the positivity result of pregnant women testing positive after treatment with malaria drug changed at 35 and 42 days post treatment in almost all the age groups. There was no statistical significant difference between the different age groups ($p<0.05$) in HRP2. The persistent positivity decreases in all the trimester at 35 and 42 days post treatment in HRP2. There was no statistical significant difference between the age of pregnancy $p>0.05$. The highest negative result was observed in the second trimester in 42 days post treatment. This therefore provides a useful data for improved malaria diagnosis, treatment and guide for pregnant women who are susceptible to malaria parasite.

Keywords: Malaria; Histidine-Rich Protein II; Pregnant Women; Blood; Positive

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NTRODUCTION

Malaria is an acute febrile illness caused by *Plasmodium* parasites. There are five parasite species that cause malaria in humans, and two of these species are *P. falciparum* and *P. vivax* which pose the greatest threat. The parasites are spread to people through the bites of infected female *Anopheles* mosquitoes. (WHO, 2017). Malaria is a life-threatening disease caused by parasites that are transmitted to people through the bites of infected female *Anopheles* mosquitoes. It is preventable and curable. In 2019, there were an estimated 229 million cases of malaria worldwide and 409 000 estimated deaths (WHO, 2020). Children under 5 years and pregnant women are the most vulnerable group affected by malaria; in 2019, they accounted for 67% (274 000) of all malaria deaths worldwide (WHO, 2020). The African Region carries a disproportionately high share of the global malaria burden. In 2019, the region was home to 94% of malaria cases and deaths (WHO, 2020). Total funding for malaria control and elimination reached an estimated US$ 3 billion in 2019. Contributions from

governments of endemic countries amounted to US$ 900 million, representing 31% of total funding (WHO, 2020).

Accurate diagnosis is essential for effective malaria treatment. Rapid diagnostic tests (RDTs) such as the histidine-rich protein II (HRP-II) lactate and dehydrogenase (LDH) tests are commonly used methods to identify malaria infections. However, the reliability of these tests may be affected by factors such as the persistence of parasite antigens in the body after treatment (Falade, 2016).

Accurate diagnosis plays a crucial role in the effective treatment of malaria, especially given the increasing prevalence of drug-resistant strains (WHO, 2020). Early and precise identification of the parasite is vital for appropriate treatment selection and patient management. Historically, the gold standard for malaria diagnosis has been microscopy, but this method has limitations in terms of sensitivity, particularly in cases of low parasitemia (Falade, 2016). Rapid diagnostic tests (RDTs) have emerged as a valuable alternative, offering quick and reliable results, however, the accuracy of RDTs in detecting malaria antigens can be compromised after antimalarial treatment, leading to false-negative results and delayed appropriate therapy. This problem is particularly relevant in pregnant women due to physiological changes that occur during pregnancy (Amoah *et al.,* 2016). The retention of histidine-rich protein II (HRP-II) rapid diagnostic tests (RDTs) in pregnant women after antimalarial treatment is a significant problem in the diagnosis of malaria. Previous studies have shown that pregnant women are at an increased risk of malaria infection due to their immunocompromised state (WHO, 2011). This study on the retention of histidine-rich protein II in pregnant women after antimalaria treatment therefore aim at determining the extent and duration of RDTs' non-retention of antigens in this population and understanding the variations in RDT results in pregnant women thereby contributing to improved malaria diagnosis and treatment guidelines in vulnerable population.

MATERIALS AND METHODS

The Study Area

The study was carried out in Turai Yar'adua Maternity and Children's Hospital Katsina and Federal Medical Centre Katsina, Katsina State. Katsina State is located on the coordinates $12^0$15`N and $7^0$30`E. It has a population of 10,368,500 and covers an area of 24,192 Km^2. It has an elevation of 519mts above sea level, with an international boundary in the north to Niger Republic. It also shares border in the East with Kano and Jigawa States, in the West with Zamfara State and in the South with Kaduna State.

Sample Collection

Blood samples were collected from pregnant women attending antenatal care at Turai Yar'Adua Maternity and Children's Hospital, Katsina and Federal Medical Centre Katsina.Samples were collected with the help of trained physicians.

Ethical Clearance and Informed Consent

Ethical clearance was obtained from the Ethical and Human Research Committee of Katsina State Ministry of Health. Consent of all participating individual was obtained in accordance with the standards of human experimentation and with the Helsinki Declaration of 1975, as revised in 2008 (Helsinki Declaration, 2008).

Study Design

A sample of 400 pregnant women was collected and diagnosed using Histidine-Rich Protein 2 Rapid Diagnostic Test kits, 250 were found to be positive with malaria and 150 were negative. Pregnant women that were positive were included in the study. Pregnant women that tested positive for malaria parasite were given a single dose treatment with fansidar (Sulfadoxine/pyrimethamine) and supervised by qualified medical personnel. The procedures were repeated at day 3, 7, 14, 21, 28, 35 and 42 days after treatment until some of the RDTs become negative. Follow-up lasted for 42 days.

Malaria Diagnosis Using HRP2 Rapid Diagnostic Test

The sample were collected by pricking the finger. A gentle prick was made on the tip of the finger with a sterile lancet at the disinfected site. Using the blood collection device provided in the RDTs kit, a required volume of blood was collected. The collected blood was transferred to the sample well on the RDT cassette, 2-3 drops of buffer was added into the buffer well and wait for 15minutes before the result was taken. The samples collection and processing were conducted concurrently. Patient's information and RDT result were recorded in the appropriate register. Cotton wool, RDT cassette and gloves were

discarded into the box for infectious waste. Empty bottles/ampulla of buffer, instructions and RDT packing were discarded into box for non-infectious waste. The sample collection has a span of 3 months, started from September, 2021 and ended in November, 2021.

Treatment

Pregnant women that tested positive for malaria parasite were given a single dose treatment with fansidar and supervised by qualified medical personnel. Fansidar tablet is an antiparasitic drug used for the treatment of malaria especially in pregnant women, each tablet containing 500mg1 – (5,6-dimethoxy-4-pyrimidiny) sulfanilamide (sulfadoxine) and 25 mg 2,4-diamino-5-(p-chlorophenyl)-6-ethylpyrimidine (pyrimethamine). Each tablet also contains cornstarch, gelatin, lactose, magnesium stearate and talc.

Data Analysis

Data obtained were entered using Microsoft Excel version 2013. Descriptive statistics was performed for different demographic characteristics. Chi square test was used to test the level of significance among categories such as age group, gender, occupation etc. Bivariate and multivariate logistic regressions were performed to measure the likelihood of infection in the different categories. All analysis were performed using the statistical package for social sciences (SPSS) version 21. All analysis were performed at $p<0.05$.

RESULTS

Demographic Characteristics of the Pregnant Women that Involved in the Study

The demographic characteristics of the pregnant women that participated in the study is showed in table 1. It was observed that the age group 15-19 has the highest number of participants (17.5%) and the age group >50 has the lowest number of participants (1.3 %.). According to the age of pregnancy the highest number of participants examined were in third trimester with 45%, it was observed that the tribe with highest participants is Hausa (71%) whereas Kanuri has the lowest participants (0.8%). House wife has the highest participants (40.8%).

Age of Pregnant Women that remain Positive for Histidine Rich Protein 2 after Treatment with Antimalarial Drugs

The prevalence in positivity of different age group that participated in the study was recorded in table 2. After administering drug to the pregnant women found to be positive for malaria, it was observed that in all the age groups, day 3, day 7 and day 14 post drug treatment were all still positive for malaria using HRP2 malaria rapid diagnostic tool. It was also observed that after 21 and 28 days post treatment, all the different age groups were still positive for malaria except the age group 30-34 (97.5%). The percentage in the positivity result of pregnant women testing positive after treatment with malaria drug changed at 35 and 42 days post treatment in almost all the age groups. There was no statistical significant difference between the different age groups ($p<0.05$), however, age group >50 shows the highest negative result 66.7% in 42 days post treatment as observed in Table 2.

Prevalence of Pregnant Women Positive for Histidine Rich Protein 2 Rapid Diagnostic Test after Treatment with Antimalarial Drugs According to the three stages of Pregnancy.

Table 3 reveals that at day 3, 7 and 14 post treatment they were all positive. It was also observed that at day 21 and 28 those in first and second trimester were still positive whereas those in third trimester reduced in their positivity to 99.1%. However, the persistent positivity decreases in all the trimester at 35 and 42 days post treatment. There is no statistical significant difference between the age of pregnancy $p>0.05$. Therefore, the highest negative result was observed in second trimester in 42 days post treatment as seen in Table 3.

Percentage Rate of Antigen Detection using Histidine Rich Protein 2 Rapid Diagnostic Test in Different Tribes Sampled

The prevalence in positivity of different tribes of pregnant women that participated in the study after administering antimalarial drug to the pregnant women found to be positive for malaria is presented in Table 4. It was observed that in all the tribes' day 3, 7 and 14 post drug treatment were all positive for malaria using HRP2 malaria rapid diagnostic tool. At day 21 and day 28 post drug treatment it was observed that all the tribes were positive except in

Fulani with 97.2%. Furthermore, at day 35, the positivity decreases in Hausa, Fulani and Yoruba whereas the Igbo, Kanuri and Nupe remain positive. It was also observed that at day 42 post drug treatment, all the tribes decreased in positivity except in Igbo. There is no statistical significance difference between the tribes except in day 42 which is 0.045. However, Nupe shows the highest negativity result in day 42 which is 75.0% (Table 4).

Prevalence of Pregnant Women that remain Positive for Histidine Rich Protein 2 antigen after Treatment with Antimalarial in Different Occupation

The Percentage Prevalence in positivity of pregnant women in various occupation that were diagnose for malaria using HRP2 RDT after drug treatment is presented in table 5. It was observed that in all the various occupations, day 3, 7 and 14 post drug treatment, they were all still positive for malaria using HRP2 malaria rapid diagnostic tool. More so, day 21and 28, the positivity change only in pregnant women that were civil servant with 98.1% both. It was also observed that at day 35, the percentage in the positivity change in almost all the occupation except in pregnant women that were teachers and students which were still 100% positive. The percentage in the positivity result change completely after 42 days post treatment in all the occupation. There is no statistical significant difference between the occupation $p>0.05$. Hence, the occupation teacher shows the highest negative result 75.0% in day 42 post treatment as observed in Table 5.

Table 1. Demographic Presentation of the Pregnant Women that Participated in the Study

Demographic Characteristics	Number Examined (%)
Age Groups (Years)	
15 – 19	42 (17.5)
20 – 24	35 (14.6)
25 – 29	34 (14.2)
30 – 34	40 (16.7)
35 – 39	32 (13.3)
40 – 44	34 (14.2)
45 – 49	20 (8.3)
> 50	3 (1.3)
Total	**240 (100%)**
Age of Pregnancy	
1st Trimester	48 (20.0)
2nd Trimester	84 (35.0)
3rd Trimester	108 (45.0)
Total	**240 (100%)**
Tribe	
Fulani	36 (15.0)
Hausa	171 (71.3)
Igbo	8 (3.3)
Kanuri	2 (0.8)
Nupe	4 (1.7)
Yoruba	19 (7.9)
Total	**240 (100%)**
Occupation	
Business	48 (20.0)
Teaching	4 (1.7)

House wives	98 (40.8)
Students	37 (15.4)
Civil Service	53 (22.1)
Total	**240 (100.0)**

Table 2. Age of Pregnant Women That Remain Positive Using Histidine Rich Protein II Rapid Diagnostic Test after Treatment with Antimalarial Drugs (Fansidar)

Age Groups (Years)	Number Examined	Number Tested Positive (%) for HRP2						
		Day 3	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
15 – 19	42	42 (100.0)	42 (100.0)	42 (100.0)	42 (100.0)	42 (100.0)	41 (97.6)	37 (88.1)
20 – 24	35	35 (100.0)	35 (100.0)	35 (100.0)	35 (100.0)	35 (100.0)	33 (94.3)	28 (80.0)
25 – 29	34	34 (100.0)	34 (100.0)	34 (100.0)	34 (100.0)	34 (100.0)	33 (97.1)	27 (79.4)
30 – 34	40	40 (100.0)	40 (100.0)	40 (100.0)	39 (97.5)	39 (97.5)	38 (95.0)	32 (80.0)
35 – 39	32	32 (100.0)	32 (100.0)	32 (100.0)	32 (100.0)	32 (100.0)	32 (100.0)	29 (90.6)
40 – 44	34	34 (100.0)	34 (100.0)	34 (100.0)	34 (100.0)	34 (100.0)	32 (94.1)	25 (73.5)
45 – 49	20	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	18 (90.0)	17 (85.0)
> 50	3	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	2 (66.7)
Total	**240**	**240 (100.0)**	**240 (100.0)**	**240 (100.0)**	**239 (99.6)**	**239 (99.6)**	**230 (95.8)**	**197 (82.1)**
χ^2					5.021	5.021	4.220	5.298
Df					7	7	7	7
p Value					0.657ns	0.657ns	0.754ns	0.624ns

NA – Not applicable, df – Degree of freedom, ns – Not significant at p>0.05, HPR2 – Histidine-Rich Protein II, χ^2 – Chi-square

Table 3. Prevalence of Pregnant Women Positive for Histidine Rich Protein 2 Rapid Diagnostic Test after Treatment with Antimalarial Drugs According to the three stages of Pregnancy

Age of Pregnancy	Number Examined	Number Tested Positive (%) for HRP2						
		Day 3	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
1st Trimester	48	48 (100.0)	48 (100.0)	48 (100.0)	48 (100.0)	48 (100.0)	47 (97.9)	42 (87.5)
2nd Trimester	84	84 (100.0)	84 (100.0)	84 (100.0)	84 (100.0)	84 (100.0)	83 (98.8)	65 (77.4)
3rd Trimester	108	108 (100.0)	108 (100.0)	108 (100.0)	107 (99.1)	107 (99.1)	100 (92.6)	90 (83.3)
Total	**240**	**240 (100.0)**	**240 (100.0)**	**240 (100.0)**	**239 (99.6)**	**239 (99.6)**	**230 (95.8)**	**197 (82.1)**
χ^2		NA	NA	NA	1.227	1.227	5.226	2.335
Df		NA	NA	NA	2	2	2	2
p Value		NA	NA	NA	0.541ns	0.541ns	0.073ns	0.311ns

NA – Not applicable, df – Degree of freedom, ns – Not significant at p>0.05, HPR2 – Histidine-Rich Protein II, χ^2 – Chi-square

Table 4. Percentage Rate of Antigen Detection using Histidine Rich Protein II Rapid Diagnostic Test in Different Tribes Sampled

Tribe	Number Examined	Number Tested Positive (%) for HRP2						
		Day 3	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Hausa	171	171 (100.0)	171 (100.0)	171 (100.0)	171 (100.0)	171 (100.0)	167 (97.7)	142 (83.0)
Fulani	36	36 (100.0)	36 (100.0)	36 (100.0)	35 (97.2)	35 (97.2)	32 (88.9)	29 (80.6)
Yoruba	19	19 (100.0)	19 (100.0)	19 (100.0)	19 (100.0)	19 (100.0)	17 (89.5)	15 (78.9)
Igbo	8	8 (100.0)	8 (100.0)	8 (100.0)	8 (100.0)	8 (100.0)	8 (100.0)	8 (100.0)
Kanuri	2	2 (100.0)	2 (100.0)	2 (100.0)	2 (100.0)	2 (100.0)	2 (100.0)	0 (0.0)
Nupe	4	4 (100.0)	4 (100.0)	4 (100.0)	4 (100.0)	4 (100.0)	4 (100.0)	3 (75.0)
Total	**240**	**240 (100.0)**	**240 (100.0)**	**240 (100.0)**	**239 (99.6)**	**239 (99.6)**	**230 995.8)**	**197 (82.1)**
χ^2					5.690	5.690	8.311	11.336
Df					5	5	5	5
p Value					0.338ns	0.338ns	0.140ns	0.045*

NA – Not applicable, df – Degree of freedom, ns – Not significant at p>0.05, * - Significant at p<0.05, HPR2 – Histidine-Rich Protein II, χ^2 – Chi-square

Table 5. Percentage Positivity of Antigen of Histidine Rich Protein II Detected after Treatment in Pregnant Women in Different Occupation

Occupation	Number Examined	Number Tested Positive (%) for HRP2						
		Day 3	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Business	48	48 (100.0)	48 (100.0)	48 (100.0)	48 (100.0)	48 (100.0)	45 (93.8)	38 (79.2)
Teacher	4	4 (100.0)	4 (100.0)	4 (100.0)	4 (100.0)	4 (100.0)	4 (100.0)	3 (75.0)
House wife	98	98 (100.0)	98 (100.0)	98 (100.0)	98 (100.0)	98 (100.0)	95 (96.9)	82 (83.7)
Student	37	37 (100.0)	37 (100.0)	37 (100.0)	37 (100.0)	37 (100.0)	37 (100.0)	33 (89.2)
Civil Servant	53	53 (100.0)	53 (100.0)	53 (100.0)	52 (98.1)	52 (98.1)	49 (92.5)	41 (77.4)
Total	**240**	**240 (100.0)**	**240 (100.0)**	**240 (100.0)**	**239 (99.6)**	**239 (99.6)**	**230 (95.8)**	**197 (82.1)**
χ^2					3.543	3.543	4.121	2.657
Df					4	4	4	4
p Value					0.471ns	0.471ns	0.390ns	0.617ns

NA – Not applicable, df – Degree of freedom, ns – Not significant at p>0.05, HPR2 – Histidine-Rich Protein II, χ^2 – Chi-square

Determination of the Duration of Persistence of Histidine Rich Protein II in the Blood of Pregnant Women after Treatment with Antimalarial Drug

Table 6 shows the Persistence of HRP2 in the Blood of Pregnant Women after Treatment with Antimalarial drug. It was observed that, using HRP2 rapid diagnostic tool, for 0.01% of malaria to clear in the blood it takes 21days.

Bivariate and Multivariate Analysis of Risk Factors Associated with Malaria Detection Using Histidine Rich Protein 2 Rapid Diagnostic Test in Pregnant Women after Treatment with Antimalarial.

Table 7 shows the Bivariate and Multivariate Analysis of Risk Factors Associated with Malaria in Pregnant Women after Treatment with Antimalarial. The age group of 35-39 years had highest likelihood of detecting malaria parasites using HRP2 RDT with odds ratio of 4.833, followed by the age group of 15-19 years with odds ratio of 3.700. The likelihood of infection with malaria was least in the age group of 40-44 years with odds ratio 1.389. During the trimesters of the pregnancy, women in the first trimester had highest likelihood of detecting malaria parasites using HRP2 than the other trimester with the odds ratio of 1.533., Hausa tribe has the highest likelihood detecting malaria infection with odds ratio of 1.632 while the Yoruba had the least. , Students are at highest risk of detecting infection with malaria with odds ratio of 2.415, followed by housewives with odds ratio of 1.500. The likelihood of infection among teachers was the lowest with odd ratio of 0.878. Combined comparison of the age groups revealed higher likelihood of detection of infection of malaria in 35-39, 15-19 and 45-49 with adjusted odds ratio of 2.302, 1.758 and 1.259 respectively. Also, the highest adjusted odds ratio was observed from women in the first and third trimesters with adjusted odd ratio of 1.671 and 1.168 respectively.

Table 6: Duration of Persistence of Histidine Rich Protein II

% Probability of clearance	Number of Days of Persistence in the Blood
	HRP2
0.01	21.19
0.02	25.18
0.03	28.10
0.04	30.51
0.05	32.63
0.06	34.54
0.07	36.31
0.08	37.98
0.09	39.56
0.10	41.07
0.15	47.97

Table 7. Bivariate and Multivariate Analysis of Risk Factors Associated with Malaria Detection Using Histidine Rich Protein 2 Rapid Diagnostic Test in Pregnant Women after Treatment with Antimalarial

Demographic Characteristics	Number Examined	HRP2 (%)	Odds Ratio (95% C.I.)	Adjusted Odds Ratio (95% C.I.)
Age Groups (Years)				
15 – 19	42	37 (88.1)	3.700 (0.282 - 48.623)	1.758 (0.647 - 4.771)
20 – 24	35	28 (80.0)	2.000 (0.158 - 25.343)	0.852 (0.345 - 2.102)
25 – 29	34	27 (79.4)	1.929 (0.152 - 24.463)	0.817 (0.330 - 2.021)
30 – 34	40	32 (80.0)	2.000 (0.161 - 24.912)	0.849 (0.360 - 1.998)
35 – 39	32	29 (90.6)	4.833 (0.332 - 70.403)	2.302 (0.668 - 7.924)
40 – 44	34	25 (73.5)	1.389 (0.112 - 17.236)	0.549 (0.236 - 1.280)
45 – 49	20	17 (85.0)	2.833 (0.191 - 41.995)	1.259 (0.352 - 4.504)
> 50	3	2 (66.7)	1	0.431 (0.038 - 4.862)
Total	**240**			
Age of Pregnancy				
1st Trimester	48	42 (87.5)	1.533 (0.570 - 4.126)	1.671 (0.661 - 4.225)
2nd Trimester	84	65 (77.4)	0.684 (0.333 - 1.405)	0.622 (0.318 - 1.217)
3rd Trimester	108	90 (83.3)	1	1.168 (0.599 - 2.278)
Total	**240**			
Tribe				
Hausa	171	142 (83.0)	1.632 (0.164 - 16.250)	1.246 (0.613 - 2.535)
Fulani	36	29 (80.6)	1.381 (0.124 - 15.361)	0.888 (0.361 - 2.185)
Yoruba	19	15 (78.9)	1.250 (0.101 - 15.500)	0.804 (0.253 - 2.553)
Igbo	8	8 (100.0)	NA	NA
Kanuri	2	0 (0.0)	NA	NA
Nupe	4	3 (75.0)	1	0.650 (0.066 - 6.399)
Total	**240**			
Occupation				
Business	48	38 (79.2)	1.112 (0.431 - 2.871)	0.789 (0.358 - 1.740)
Teacher	4	3 (75.0)	0.878 (0.082 - 9.233)	0.650 (0.066 - 6.399)
House wife	98	82 (83.7)	1.500 (0.649 - 3.465)	1.203 (0.610 - 2.376)
Student	37	33 (89.2)	2.415 (0.712 - 8.186)	1.962 (0.656 - 5.864)
Civil Servant	53	41 (77.4)	1	0.679 (0.321 - 1.437)
Total	**240**	**197 (82.1)**		

C.I. – Confidence Interval

DISCUSSION

Pregnant women were examined for the persistence of this protein using HRP2 RDTs. It was observed that HRP2 were detected in the blood for up to 42 days in over 80% and 60% respectively. This observation differs from the report of Poti *et al.* (2019) where they reported that HRP2 antigens were positive from 1 to 12 days post-treatment in individuals treated with artemether lumefantrine and ativequone-proquanil. Antigen of HRP2 were identify in infected Red Blood Cells (RBC) gotten from infected with *Plasmodium falciparum* three days after treatment with artesunate (Ndour *et al.* (2017). The continues positivity of the antigen HRP2 might probable as a result of the parasites that are non-viable by the development of erythrocyte which is opposing in the spleen, where the malaria parasite is detached by splenic macrophages from an intact red blood cell that reoccurred into circulation as infected Red Blood Cell (Ndour *et al.* 2017 and Ayona *et al.,* 2006). Poti *et al.,* (2019) also suggested that the reduced

protein permission is from the red blood cell part of the blood when compare to the plasma part of the blood. They also suggested that parasite DNA may persist in the blood even after parasites are killed. They also added that HRP2 might be binding RBCs that are no infected in circulation as it is released from diseased RBCs, therefore causing persistence of positivity (Poti *et al.* (2019). HRP2 stages in individuals RBCs seems to be 20 to 40 times more than in the plasma. Post parasite clearance and persistence of HRP2 is slower in RBCs fraction than in plasma fraction (Poti *et al.,* 2019). Swarthout *et al.* (2007) reported a similar result, where 73% of cases with RDT test were still positive for malaria parasite 35 days after treatment and opined that the positivity might be due to parasite density in the blood. In another study Iqbal *et al.* (2004), found that 35% of patients still had HRP2 antigenaemia 14 days after treatment. Their findings suggested that continues positivity of HRP2 which was 90.4%, three days after decrease slowly to 34.9% after day 14. They likewise reported that HRP2-based RDTs presents more false positive results than pLDH-based RDT. A study by Hopkins *et al.* (2007) in Kampala, Uganda reported that HRP2 assay showed superior sensitivity but inferior specificity compared with the pLDH assay. This observation was in conformity with the current study as to why more false positive was reported using HRP2. Abeku *et al.* (2008) further stated that the HRP2 antigens can stay in blood for more than thirty days when the parasites has cleared, and persistence of HRP2 has been reported to rely on the existence of the antigen in and parasite and erythrocytic density at the onset of treatment. Kozycki *et al.* (2017) in their study in Rwanda also reported that HRP2-based RDTs were more sensitive than pLDH-based RDTs. This is consistent with the findings where extended positivity of HRP2 was observed in all the age groups and trimesters. Abeku *et al.* (2008) reported that sensitivity of RDTs was not affected by age of patients or fluctuation in parasite during different months but by parasite density. The age of the individuals is not significantly associated with HRP2 protein prevalence from this study and is corroborated by Alemayelu *et al.* (2021) who reported non-significant difference in the prevalence of HRP2 among age groups of study population in Assosa zone, Ethiopia. Similarly Oladosu *et al.* (2021) reported that the prevalence of malaria parasites in Osun state Nigeria using HRP2-based RDTs showed no significant difference among the age groups of the participants whose age group range from 15 to 50 years. The trimester, tribe and occupation of pregnant women were significantly not associated with the persistence of either HRP2. Although, a study by Kattenburg *et al.* (2012) in Nanoro, Burkina Faso reported statistically significant difference in the persistence of HRP2 persistence between primigravidae and multigravidae, and also between pregnant women below the age of 25 years and those greater than or equal to 25 years. In their report, primigravidae women had high parasite density than multigravidae, while pregnant women less than the age of 25 years had higher parasite density than those above the age of 25 years. They attributed the differences to parasite density.

HRP2 RDTs as observed from this study were found to be a beneficial diagnose of peripheral in fection of *P. falciparum* in pregnant women with symptom of malaria. Nevertheless, they are not adequate enough as a diagnostic tool in screening amongst women that don't have symptoms of malaria. These findings have implications for the management of malaria in pregnancy in order to curb the adverse effect of multiple treatments.

CONCLUSIONS

The result from this study indicated that histidine rich protein II persist in the blood of pregnant women after treatment with fansider (antimalaria drug). The age groups had no changes in the positivity after day 3, 7 and 14 post drug treatment for all the RDTs. The positivity start changing after 21days post drug treatment in most cases. The results of the research therefore indicate that the performance of the RDTs was high in HRP2 based RDTs (80%). HRP2 proteins persisted up to 42 days' post antimalarial treatment. Age, tribe, trimester of pregnancy and occupation were statistically not associated with the persistence of HRP2. The parasite density might be responsible for the persistence of the protein.

RECOMMENDATION

It is suggested also in addition that microscopy should be used as a diagnoses for post-treatment diagnosis of malaria in pregnancy especially during the peak periods of malaria transmission in malaria prevalent communities.

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