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Full Length Research Paper

Effects of malaria on the indices of anaemia of Plasmodium parasitized pregnant subjects

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Malaria infection during pregnancy is a major public health problem in tropical and subtropical regions of the world. This study evaluated the effect of malaria on the indices of anaemia of 50 *Plasmodium* parasitized pregnant subjects. Fifty non- malaria parasitized pregnant and fifty non- pregnant and non-parasitized subjects served as control. The mean haematological values was significantly lower among parasitized pregnant women compared to non–parasitized pregnant and non pregnant subjects (p = 0.001) . The incidence of anaemia among parasitized and non parasitized pregnant subjects was (66 and 48%). A positive correlation was observed between the level of parasitaemia and anaemia (r = 0.67, p = 0.04). Microcytic and hypochromic anaemia was significantly higher in pregnant and parasitized subjects (p = 0.001). Burden of malaria infection and anaemia was higher in primgravidae compared with multigravidae subjects. Preventative strategies including regular chemoprophylaxis, intermittent preventative treatment with antimalarials, provision of iron supplementation and insecticide-treated bed nets should be implemented.

Key words: *Plasmodium* parasitaemia, anaemia, pregnancy, Niger Delta, Nigeria.

INTRODUCTION

Malaria in pregnancy is a major public health problem in endemic tropical and subtropical countries and a major cause of fetal and maternal morbidity and mortality (Bergstrom et al., 2004). Malaria and pregnancy are mutually aggravating conditions, the physiological and pathological changes in pregnancy due to malaria have a synergistic effect on the course of each other. In pregnant women the morbidity due to malaria are complications arising from febrile illnesses, hypoglyceamia, cerebral involvement, pulmonary oedema and puerperal sepsis (Face and Jenkins, 2002). Anaemia is the most prominent haematological manifestation of malaria infection. It is most marked with *Plasmodium falclparum,* which invades erythrocytes of all ages (Snow et al., 2004). Anaemia (defined by the World Health Organization as haemoglobin levels of < 12 g/dl in adult non-pregnant females and haemoglobin <11 g/dl in pregnant females) is one of the world's leading causes of disability (UNICEF, 2001), and thus one of the most serious global public health problems. The prevalence of anaemia in pregnancy varies considerably because of differences in socioeconomic conditions, lifestyles and health- seeking behaviours across different cultures. Anaemia affects 42% of all women in the world and 52% of pregnant women in developing countries compared with 23% in the developed world (UNICEF, 2001). The most common causes of anaemia are poor nutrition, deficiencies of iron and other micronutrients, malaria, hookworm disease, and schistosomiasis. HIV infection and haemoglobinopathies are additional factors (van den Broek et al., 1998). Anaemia is one of the most prevalent nutritional deficiency problems affecting pregnant women (Thangaleela and Vijayalakshmi, 1994). The high prevalence of iron and other micronutrient deficiencies among women during pregnancy in developing countries is of concern and maternal anaemia is still a cause of considerable perinatal morbidity and mortality (Cutner et al., 1999).The high frequency of iron deficiency anaemia

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in the developing world has a substantial health and economic cost. In an analysis of 10 developing countries, the median value of physical productivity loses per year resulting from iron deficiency anaemia was about US$0.32 per head or 0.57% of the gross domestic product (Horton and Ross, 2003). Women in most developing countries are in a state of precarious iron balance presenting with iron and folate deficiency during their reproductive years (Mukherji, 2002). This is usually due to poor nutritional intake, menstrual blood loss, recurrent parasitic infection (malaria, hookworm) and repeated pregnancies. It is estimated that anaemia accounts for 3.7 and 12.8% of maternal death during pregnancy and child birth in Africa and Asia respectively (Khan et al., 2006) .Women were classified as iron deficient when ferritin level was less than 30µg/L (William, 2000). In Africa alone, an estimated 275 million people are infected with malaria (Najera et al., 1993).

Malaria infection has a complex effect on iron metabolism that may affect the interpretation of haemoglobin and serum ferritin (Hastka et al, 1993). The spectrum of iron status covers several well-defined stages, ranging from adequate iron storage to depleted iron stores, iron-deficient erythropoiesis, and iron deficiency anaemia. To characterize the iron status of a population or to monitor changes in status, it is desirable to use a combination of indicators that provides information about this entire spectrum. Serum ferritin is a particularly useful indicator of iron status, because it is linearly related to iron stores when stores are present (Jeremiah et al., 2007).

There appear to be a dearth of information on the effect of malaria parasitaemia on the haematological parameters of infected pregnant women in the Niger Delta of Nigeria. The Niger Delta of Nigeria is said to be the world's largest wetland. These 36,000 sqkm (14,000 square miles) of marshland, creeks and tributaries and lagoons drain the Niger River into the Atlantic at the Bight of Biafra. Transportation through this ecosystem is usually via rivers and creeks that snake through dense mosquito- infested swamps. There is high incidence of malaria in the area. Indeed early European visitors to the area described it as the 'white man's grave-yard' because of the high malaria-related mortality rate they experienced. The aim of this study was to investigate the effect of malaria on the indices of anaemia of pregnant women in the Niger Delta of Nigeria.

MATERIALS AND METHODS

Subjects/settings/design

Between June - September, 2006, complete blood count (CBC) measurements results were evaluated for 50 malaria parasitized, 50 non- parasitized pregnant and 50 non-pregnant and non-parasitized subjects who were enrolled into this multi centre observational study. Five study centers were used for the collection of samples namely; Nigerian National Petroleum Company (NNPC) Clinic, University of Port Harcourt Teaching Hospital (UPTH), Braithwaite Memorial Hospital (BMH), Ogbunabali health centre and development of medical laboratory science, Rivers State University of Science and Technology, all in Port Harcourt the heart of the Niger Delta of Nigeria. Subjects were women of child bearing age. The median age and range was 25 years and [18 - 45] years respectively. Subjects who were 18 years and who showed clinical and laboratory evidence of malaria were enlisted into the study as test group while aged - matched non - parasitized pregnant and non-pregnant subjects who showed no clinical or laboratory evidence of malaria were included as controls. The institution ethical committee granted ethical approval before the commencement of the study and written informed consent was received from the study participants. Non-parasitized women were defined as pregnant/non-pregnant women without malaria or other febrile illness at booking consultation. Malaria parasitaemia was defined by the presence of asexual forms of *P. falciparum* on QBC malaria test and confirmed by microscopic examination of the peripheral blood in pregnant women with elevation of temperature (aural < 37.5°C), history of fever or any of the following symptoms: headache, dizziness, joint pain, anorexia, nausea, spontaneous bleeding.

Laboratory analysis

Blood collection

Blood samples were collected by venipuncture into Ethylene Diamine Tetracetic Acid (EDTA) anticoagulated tubes and used for the determination of complete blood count, and malaria testing using the QBC ^{TM} Autoread ^{TM} plus centrifugal haematology and malaria analyzer.

Haematological analysis and malaria parasite detection

Indices of anaemia of red cell count, hematocrit, haemoglobin, mean cell volume, mean cell haemoglobin, mean corpuscular haemoglobin concentration was examined. Complete Blood Count (CBC) were measured by the QBC Autoread plus haematology analyzer (model 428576, 2006) (QBC Diagnostics Inc, USA) using venous blood. The QBC Autoread plus centrifugal Haematology system yields quantitative values for haematological indices from venous or capillary blood. Red cell count (RBC) was counted manually according to method described by Dacie and Lewis (2001). Red cell indices; mean cell haemoglobin (MCH) and mean cell volume (MCV) were calculated. Malaria diagnosis was made using the QBC malaria test. Fifty five micro litre of blood is taken in a QBC™ tube coated with Acridine Orange (AO- fluorescent dye) and centrifuged for 5 min. The parasites in a QBC tube, when viewed with the Paralens microscope adaptor, are seen concentrated in the area below the Buffy Coat, fluorescing in the background of Dark Red Blood Cells. Blood smears (thin and thick films) were prepared for all QBC malaria positive samples and stained using Giemsa stain (for confirmation, speciation, study of red cell morphology and parasite load determination). The thin film was made by the push wedge technique. Parasite counts were reported per 500 white blood cells (WBC) and for counts above 1,000 parasites per 500 WBC by the percentage of infected red cells (RBC). All stages of the parasites were recorded (asexual and gametocytes). Previous study (Achidi et al., 2005) found diagnosis by direct centrifugation using QBC malaria to be at least 8 times as sensitive as conventional microscopy when applied to serially diluted samples of malaria-infected blood. About 10% of infections diagnosed by direct centrifugal microscopy in a clinical setting were not detected by conventional examination of stained thick films. Superior sensitivity, together with the one step, solid state nature of the direct centrifugal procedure, provides important advantages for malaria diagnosis. Testing was carried out at the research laboratory in the Depart-ment of Medical Laboratory Science in the Rivers State University of Science and Technology, Port Harcourt.

Table 1. Some haematological parameters in malaria parasitized and non-parasitized subjects.

Parameter	*Plasmodium* parasitized pregnant women (Mean ± SD)	Non –parasitized pregnant women (Mean ± SD)	Non-parasitized and non-pregnant women (Mean ± SD)	P-value
Haemoglobin (g/dl)	8.8 ± 0.86 a, b	11.6± 0.69	12.7 ± 0.9	0.001
Packed cell volume (l/l)	0.25 ± 0.025 a, b	0.34± 0.03	0.40 ± 0.04	0.001
Mean cell volume (fl)	76 ± 1.8a, b	80 ± 2.8	88.8 ± 5.6	0.001
Mean cell haemoglobin (pg)	26.4 ± 2.2a, b	28.6 ± 2.6	29.2 ± 1.6	0.01
Mean cell haemoglobin concentration (g/L)	312 ± 6[c, d]	326 ± 7	330 ± 6	0.05
Red cell count (×10^9/L)	3.8 ± 0.5[c, d]	4.0± 0.4	4.2 ± 0.5	0.06

[a] Significant difference observed between Malaria infected and non-infected pregnant women
[b] Significant difference observed between malaria infected pregnant women and non-pregnant control
[c] No significant difference observed between malaria infected and non-infected pregnant women
[d] No significant difference observed between malaria infected pregnant women and non-infected and non- pregnant control

Table 2. Blood picture among anaemic non-pregnant and plasmodium parasitized and non -parasitized pregnant subjects.

Blood picture of anaemic Subjects	Plasmodium parasitized pregnant women N (%)	Non –parasitized pregnant women N (%)	Non-parasitized and non-pregnant women N (%)	P-value
Microcytic and hypochromic	30 (90.9)[a, b]	17 (70.8)	7 (43.8)	0.001
Normochromic and normocytic	3 (9.1)[a, b]	7 (29.2)	9 (56.3)	0.001

[a] Significant difference observed between Malaria infected and non-infected pregnant women.
[b] Significant difference observed between malaria infected pregnant women and non-pregnant control.

Statistical analysis

Statistical Package for Social Sciences version 10 (SPSS Inc, Chicago, IL.) was used to generate frequency distribution and percentage prevalence of the various parameters. Descriptive analysis of percentages of categorical variables was reported.

Comparisons were assessed using mean and chi-square test. Correlations were compared by a model of linear regression analysis. A p-value of < 0.05 was considered statistically significant in all statistical comparison.

RESULTS

Effect of malaria on indices of anemia

Plasmodium falciparum was responsible for all the cases of parasitaemia in subjects studied. The mean values, and standard deviations of the haematological variables for all subjects are shown in Tables 1. The mean values of Haemoglobin concentration in g/dl was significantly lower among *Plasmodium falciparum* parasitized pregnant women compared to non –parasitized pregnant and non pregnant women (8.8 ± 0.86, 11.6 ± 0.69 and 12.7 ± 0.9) respectively (p = 0.001) . Similarly, there was a statis-tically significant difference in the mean values of PCV (l/l), MCV and MCH of parasitized pregnant and non parasitized pregnant and non-pregnant women (0.25 ± 0.025 l/l, 76 ± 1.8 fl and 26.4 ± 2.2 pg), (0.34 ± 0.03 l/l, 80 ± 2.8 fl and 28.6 ± 2.6 pg) and (0.40 ± 0.04 l/l, 88.8 ± 5.6 fl and 29.2 ± 1.6 pg) respectively (p = 0.001). The mean values of the MCHC (g/l) for the parasitized pregnant and non parasitized pregnant and non-pregnant women were 312 ± 6, 326 ± 7 and 330 ± 6 g/l for the (p = 0.06). The incidence of anaemia defined as haemoglobin value of < 11 g/dl among parasitized and non-parasitized pregnant subjects was 33(66%) and 24(48%) respectively. Comparatively the incidence of anaemia defined as haemoglobin < 12 g/dl among non –pregnant and non- parasitized subjects was 16(30%) as shown in Table 4. The mean cell haemoglobin concentration and red cell count were only marginally lower in parasitized pregnant women compared to non parasitized pregnant and non pregnant women (p > 0.05).

A positive and significant correlation was observed between the level of parasitaemia and anaemia among *Plasmodium falciparum* parasitized pregnant subjects (r = 0.67, p = 0.04). Red cell morphology among the anaemic subjects showed that 30(90.9%) of pregnant and parasitized, 17 (70.8%) pregnant non-parasitized and 7(43.8%) non-pregnant and non- parasitized subjects had a microcytic and hypochromic red cell picture while 3 (9.1%), 7 (29.2%) and 9 (56.3%) had a normocytic and normochromic picture respectively as shown in Table 2. The incidence of severe anaemia (HB 8.0 g/dl) was significantly higher in *Plasmodium falciparum* parasitized pregnant subjects compared to non-parasitized pregnant

Table 3. Degree of anaemia in Plasmodium parasitized and non-parasitized pregnant women

Degree of anaemia (Hb g/dl)	Plasmodium parasitized pregnant women N (%)	Non –parasitized pregnant women N (%)	P-value
Mild (10.1 - 10.9 g/dl)	8 (24.2)	14 (58.3)	0.001
Moderate (8.0 - 10.0)	10 (30.3)	10 (41.6)	0.002
Severe (< 8.0 g/dl)	15 (45.5)	0 (0)	0.001

[a] Significant difference observed between malaria infected and non-infected pregnant women.

Table 4. Degree of anaemia in non-parasitized and non-pregnant women.

Degree of anaemia (Hb g/dl)	Non –parasitized and non- pregnant women N (%)
Mild (10.1- 11.9 g/dl)	14 (85.7)
Moderate (8.0-10.0)	2 (12.5)
Severe (< 8.0 g/dl)	0 (0)

and non-pregnant subjects as shown in Tables 3 and 4.

Effect of malaria and gravidae status on indices of anaemia

Table 5 shows the mean values and standard deviations (SD) of the haematological values based on gravidae status. The mean haemoglobin, packed cell volume, mean cell volume and mean cell haemoglobin concentration was significantly higher among multigravidae subjects compared to primigravidae subjects (10 ± 0.71 g/dl, 0.027 ± 0.03 l/l, 76 ± 2.8 fl and 28.5 ± 2.2 pg) compared to (8.35 ± 0.68 g/dl, *0.025 ± 0*.03, 74 ± 1.8 fl and 26.8 ± 2.5 pg) respectively p = 0.001. The mean cell haemoglobin concentration (MCHC), red cell count of multigravidae although marginally higher but not signi-ficantly different from those in primigravidae women (325 ± 5 g/l, 4.0 ± 0.4 x 10^{12}/L) compared to (316 ± 7 g/l and 3.8 ± 0.5 x 10^{12}/L) (p > 0.05). The mean (SD) parasite load was significantly higher in plasmodium parasitized primigravidae compared to multigravidae 2150 (256) parasites/µl compared to 1826(198) parasites/µl as shown in Table 5.

The mean parasite count in *Plasmodium falciparum* parasitized subjects was 2650 ± 234 parasites/µl, 95% confidence interval (2092 - 3118).

DISCUSSION

Our study shows that anaemia is a common haematological condition in developing countries and that it is more pronounced in pregnant subjects particularly those infected with *Plasmodium falciparum* malaria. The prevalence of anaemia observed among plasmodium parasitized pregnant, non- parasitized pregnant and non-parasitized and non-pregnant subjects was (66%), (48%) and (30%) respectively. This observation is in agreement with previous report which indicated that 42% of all women in the world and 52% of pregnant women in developing countries are anaemic. It is however significantly lower than prevalence observed in the developed world among pregnant women (23%) (UNICEF, 2001). Our observed prevalence is consistent with prevalence reported in a group of pregnant women in Malawi (57%) (Van Den Booek et al., 2000), Tanzania (69.7%) (Mattelli et al., 1994) and Burkina Faso (66%) (Meda et al., 1999).

Similarly of the 1118 women whose haemoglobin levels were analyzed at first antenatal enrolment in Cameroon, 68.9% were anaemic and the mean haemoglobin level of malaria parasite positive pregnant women and parturient women were significantly lower than those who were malaria parasite free (Achidi et al., 2005). The high incidence of anaemia observed among *Plasmodium falciparum* parasitized pregnant women in this study underscores the need for regular malaria chemoprophylaxis for all pregnant women particularly in malaria endemic countries.

The World Health Organization has recommended a package of interventions to prevent malaria during pregnancy.

This includes; the promotion of insecticide-treated bed nets (ITNs), intermittent preventive treatment in pregnancy (IPTp), and effective case management of malarial illness. It is recommended that pregnant women malaria-endemic areas receive at least two doses of sulphadoxine- pyrimethamine in the second and third trimesters of pregnancy. Severely anaemic pregnant women may require blood transfusion, which is not always feasible in under- resourced settings, and it may even carry some risks of transfusion-transmissible infections for the woman.

Table 5. Effect of gravidae status on some haematological parameters of malaria parasitized subjects.

Parameter	Primgravidae (Mean ± SD)	Multigravidae (Mean ± SD)	P-value
Haemoglobin (g/dl)	8.35±0.68[a]	10.0 ± 0.71	0.001
Packed cell volume (l/l)	0.025 ± 0.03[a]	0.027± 0.03	0.04
Mean cell volume (fl)	74 ± 1.8 a,	76 ± 2.8	0.04
Mean cell haemoglobin (pg)	26.8 ± 2.5 [a]	28.5± 2.2	0.04
Mean cell haemoglobin concentration (g/l)	316 ± 0.7 [b]	325 ± 0.5	0.06
Red cell count (x 10^9/L)	3.8 ± 0.5 [b]	4.0 ± 0.4	0.05
Parasite count (parasites/µl)	2150 ± 256 [a]	1826± 198	0.001

[a] Significant difference observed between Malaria infected primgravidae and multigravidae women
[b] No significant difference observed between malaria infected primgravidae and multigravidae women.

Red cell morphology among the anaemic subjects showed that the incidence of iron deficiency anaemia defined as a microcytic and hypochromic red cell picture in pregnant (parasitized and non-parasitized) with haemoglobin level < 11 g/dl and haemoglobin < 12 g/dl for non-pregnant subjects was significantly higher among parasitized pregnant women compared to non parasitized (pregnant and non-pregnant) subjects (90.9%) compared to (70.8) and (62.5%) respectively. We observed a positive and significant correlation between the level of parasitaemia and anaemia among *Plasmodium falciparum* parasitized pregnant subjects.

This finding is consistent with previous studies which observed that anemia is one of the most frequent complications related to pregnancy (Mendez et al., 2009), (Sifakis, 2000), (Baig-Ansari et al., 2008) and that it is often complicated by *Plasmodium parasitaemia* (Tarimo, 2007), (Awah et al., 2009), (Philips and Pasvol, 1992) particularly in developing malaria endemic countries. Iron deficiency anemia (IDA) remains a world public health problem, particularly in child-bearing-age and pregnant women and is a major risk factor for unfavorable pregnancy outcomes.

The pathophysiology of the anaemia of falciparum malaria is both complex and multifactorial. Among several factors, the major mechanisms are those of red cell destruction and decreased red cell production. Poten-tial causes of haemolysis include loss of infected cells by rupture or phagocytosis, removal of uninfected cells due to antibody sensitization or other physicochemical membrane changes, and increased reticuloendothelial activity, particularly in organs such as the spleen.

Decreased production results from marrow hypoplasia seen in acute infections, and dyserythropoiesis, a morphological appearance, which in functional terms results in ineffective erythropoiesis, specific/nonspecific immune responses whereby red cell survival is shortened and the potential role of parvovirus B19 as a possible cause of bone marrow aplasia has been postulated (Menendez et al., 2000).

The destruction of erythrocytes (RBCs) is the most frequently observed cause of severe malarial anaemia and the removal of non-parasitized RBCs is thought to be the most important, accounting for approximately 90% of the reduction in haematocrit in acute malaria. Iron demands need to be covered in adolescent women due to the increased physical growing, menstruation, pregnancy and fetal growing tissues.

This finding underscores the need for iron supplementation in pregnancy. During pregnancy the total maternal need for extra iron averages to 800mg of which about 300mg is for the fetus and the placenta and the rest is for the maternal haemoglobin mass expansion. Maternal haemoglobin is higher and haemoglobin level > 11g/dl at birth was more frequent in pregnant women treated with intravenous iron, as compared with oral iron (Bayoumeu et al., 2002). Compared with oral iron, haematological parameters were better with intramuscular iron and compared with intravascular iron, the parameters were better with intravenous iron (Al et al., 2005).

Our study shows that *Plasmodium falciparum* – parasitized primigravidae seemed to be at a significantly greater risk of anaemia and malaria compared to multigravidae women. The mean haemoglobin, packed cell volume, mean haemoglobin concentration and mean cell volume was significantly lower in primgravidae compared to multigravidae. The mean parasite count in *Plasmodium falciparum* parasitized subjects was significantly higher in primgravidae compared to multigravidae. This finding is consistent with previous reports which found *Plasmodium falciparum* - parasitized primigravidae at a significantly greater risk of anaemia compared to multigravidae women (Desai et al., 2007), (Kabanywanyi et al., 2008), (Shulman and Dorman, 2003). A recent study of 300 women delivering in rural Ghana showed higher rates of anemia, clinical malaria, and placental burden of infection among primigravidas compared with multigravidas (Ofori et al., 2009). The majority of the pregnant women, mainly primigravidae, were anaemic. Similarly a previous report by Enato and co-workers evaluated the prevalence of malaria parasitaemia and anaemia among pregnant women attending antenatal clinics in two tertiary health-care

facilities in Edo State, Nigeria and observed that primigravidae were more predisposed to anaemia and malaria (Enato et al., 2009).

Nnaji and co-workers (2006) observed a statistically significant difference between the prevalence rate of malaria parasitaemia in the primigravidae (87.9%) and grand multigravidae (63.6%) among their cohort of malaria infected pregnant subjects. The reason for this gravidae associated predisposition to malaria and anaemia may be due to the fact that adults who live in malaria-endemic regions generally have some acquired immunity to malaria infection as a result of immunoglobulin production during prior infections. This immunity diminishes significantly in pregnancy, particularly in primigravidas.

The mean (SD) parasite load was significantly higher in *Plasmodium* parasitized primigravidae compared to multigravidae 2150 (256) parasites/µl compared to 1826 (198) parasites/µl. The mean parasite count in *P. falciparum* parasitized subjects was 2650 ± 234 parasites/µl, 95% confidence interval (2092 – 3118). The level of parasitaemia observed among the subjects studied may be due to the fact that subjects received adequate care taken by early detection and prompt treatment.

This study had demonstrated the effect of malaria on haematological parameters in pregnancy. It has once again demonstrated the need for the inclusion of malaria parasite screening among the panel of routine investigation for antenatal clinics, especially in our malaria endemic setting. Current prevention of malarial disease in pregnancy relies on 2 main strategies: providing pregnant women with insecticide-treated bed nets (ITN) and intermittent presumptive treatment (IPT) with antimalarial medications. IPT refers to the administration of 2 or more doses of chemoprophylaxis after 20 weeks of gestation in an attempt to reduce subclinical malarial load.

There is the urgent need to avoid the effect of anaemia in pregnancy on health services and expenditure particularly in developing countries by implementing a strategy for the control anaemia among pregnant women, including education combined with dietary modification to improve iron intake and bioavailability, Iron and folate supplementation, iron fortification of foods and early detection and appropriate management of anaemia. Although the result of massive efforts in highly endemic tropical areas with regards to costly eradication projects undertaken in the past has proved unsuccessful, the major World Health Organization programme to eradicate malaria tagged "roll back malaria" that is presently ongoing in Nigeria requires serious collective effort from relevant scientific researchers to allow for locally generated public health data which is likely to be of tremendous benefit. There is the need for increased awareness of the problem of malaria and for the control of anaemia among communities in the Niger Delta of Nigeria, integration of malaria control tools with other health programmes targeted to pregnant women and newborns, strengthening of the antenatal care system and involvement of traditional birth attendants as part of health service delivery.

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