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ASSESSMENT OF HYPOGLYCAEMIA AND ANAEMIA ASSOCIATED WITH MALARIA PARASITAEMIA AMONG PREGNANT WOMEN ATTENDING SOME SELECTED HOSPITALS IN KANO METROPOLIS

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ABSTRACT

Malaria in pregnancy is one of the major factors leading to anaemia and hypoglycaemia. This study was conducted to investigate the effect of malaria, anaemia and hypoglycaemia on pregnant women attending antenatal care at Abdullahi Wase Specialist Hospital and Murtala Muhammad specialist Hospital. A total of 128 pregnant women, who granted informed consent, were recruited for the study. Demographic information such as their age, number of previous pregnancies and the age of present pregnancies were collected using structural questionnaire. Blood samples were collected and used to prepare Giemsa - Stained smears for determination of the presence of malaria parasites in pregnant women. Packed Cell Volume (PCV) on a Hawksley micro haematocrit reader using blood samples collected into capillary tubes and centrifuge at 300rpm, Haemoglobin concentration was read using Hemocue machine and Glucose level was taken using Redox Reagents according to the manufacturer's instructions. The collected data were analysed using open -Epi statistical software. Results showed that malaria was higher in youngest with (65.5%) occurrence rate, and oldest (34%) age groups while anaemia is higher in younger age group of (16-30) years (50.9%) by PCV and (47.3%) by Hb, by (31-45) years is (12.7%) and (21.8%) respectively. In contrast, pregnant women are susceptible to hypoglycaemia and based on our findings, higher positive cases were obtained in the second trimester (61.8%) followed by (23.6%) in the third trimester. The result also indicates that there is no record of a significant differences when comparing anaemia using hemocue and doing so using the PCV machine among pregnant women ($p < 0.05$). The study indicates that a large population of pregnant women in the study may be at risk of malaria, hypoglycaemia and anaemia related problems associated with child bearing. This underscores the need for integrated prevention and morbidity in pregnant women.

Key wards: - Malaria, Pregnancy, Hypoglycaemia, Anaemia

INTRODUCTION

Malaria in pregnancy is an obstetric, social and medical problem requiring multi-disciplinary and multi-dimensional solution. Pregnant women constitute the main adult risk group for malaria and 80% of deaths due to malaria in Africa occur in pregnant women and children below 5 years (Fatmi *et al.*, 2005). Malaria during pregnancy is a major health concern and ranks among the commonest complications of pregnancy in Nigeria (Omo-aghajai *et al.*, 2008). Complications of malaria in pregnancy include hypoglycemia, acute pulmonary edema, premature labour, spontaneous abortion, still births, low birth weights and anaemia (Saba *et al.*, 2008). In the light of the numerous attendant risks of malaria in pregnancy, WHO recommended a three pronged approach to the strategic framework for malaria prevention and control during pregnancy in areas of stable

transmission in Africa. These include the use of insecticide treated bed nets (ITN), intermittent preventive treatment (IPTP) and effective case management of malaria illness and anaemia (WHO, 2004). Reports show that compliance with this recommendation in Nigeria is poor (Wagbatsuma and Omoike, 2008). Additionally, Most pregnant women generally are not aware of the effects of hypoglycaemia and may not appreciate the importance of these recommendations (Fallen *et al.*, 2010).

Anaemia in pregnancy is an important public health problem worldwide (Idowu *et al.*, 2005) particularly in developing countries where nutritional deficiency, worm infestation and malaria are common (Jaleel R. Khan, 2007). Anaemia in pregnancy is a well-known risk factor for maternal death, still births, low birth weights, and fatal impairment. (Uneke *et al.*, 2007).

Management and control of anaemia in pregnancy is enhanced by the availability of local prevalence statistics, which is not adequately provided in Nigeria (Idowu *et al.*, 2005). Thus, accurate and early diagnosis of malaria illness is key to the effective management of the disease (Oshikoya, 2007). Studies have also shown that hypoglycaemia is largely unable to prevent, or treat most-life threatening obstetric complications (Fatmi *et al.*, 2005).

Malaria and pregnancy are mutually aggravating conditions. The physiological changes of pregnancy and the pathological changes due to malaria have a synergistic effect on the course of each other, thus making the life difficult for the mother, the child and the treating physician. *P. falciparum* malaria can run a turbulent and dramatic course in pregnant women. The non-immune, primi gravidae are usually the most affected (Adam *et al.*, 2008). In pregnant women, the morbidity due to malaria includes anemia, fever illness, hypoglycemia, cerebral malaria, pulmonary edema, puerperal sepsis and mortality can occur from severe malaria and hemorrhage. The problems in the new born include low birth weight, prematurity, IUGR, malaria illness and mortality (Greenwood, 1997).

Hypoglycaemia is the clinical syndrome that results from low blood sugar. The symptoms of hypoglycaemia can vary from person to person, as can the severity. Classically, hypoglycaemia is diagnosed by a low blood sugar with symptoms that resolve when the sugar level returns to the normal range. The medical term for blood sugar is blood glucose (Kochar *et al.*, 1998).

This is another complication of malaria that is peculiarly more common in pregnancy. The following factors contribute to hypoglycemia:

1. Increased demands of hypercatabolic state and infecting parasites.
2. Hypoglycemic response to starvation.
3. Increased response of pancreatic islets to secretory stimuli (like quinine) leads to hyper insulinemia and hypoglycemia.

In these cases, patients can remain asymptomatic and may not be detected. This is because all the symptoms of hypoglycemia are also caused by malaria such as tachycardia, sweating, giddiness etc. Some patients may have abnormal behavior, convulsions, altered sensorium, sudden loss of consciousness etc. These symptoms of hypoglycemia may be easily confused with cerebral malaria. Therefore, in all pregnant women with falciparum malaria, particularly those receiving quinine, blood sugar should be monitored every 4-6 hours.

Hypoglycemia can be recurrent, and therefore, constant monitoring is needed.

In some, it can be associated with lactic acidosis and in such cases mortality is very high. Maternal hypoglycemia can cause fetal distress without any signs.

Pregnant women are more prone to anaemia. Anaemia refers to a range of problems in red blood cells and the major symptoms of anaemia are fatigue. Glucose or blood sugar can indirectly contribute to anaemia in few ways; the most common pathway is through kidney. Excessive glucose can clamp down on the production of kidney hormone that triggers the production of red blood cells. (Uzma and Robert, 2009).

Anaemia Due to Malaria

There are about 400 different types of anaemia. The condition generally means a person has a lower than normal number of healthy red blood cells. That is lower than normal amounts of haemoglobin inside red blood cells. Haemoglobin is a protein that helps deliver oxygen to the tissues within body and 2/3 of iron body is found in haemoglobin. Iron deficiency is one of the major causes of anaemia especially in pregnant women. About 1/3 of the global population suffers from iron deficiency anaemia. (Cleveland Clinic, 2009).

When the infected female Anopheles mosquito bites a human being, sporozoites are introduced with the saliva that the mosquito uses as an anticoagulant. This anticoagulant prevents the blood from clotting in the mosquito's very small, tube-like proboscis or mouth parts. Once inside the human being, the sporozoites move quickly to the liver, where they try to invade liver cells (John Storey, 1991).

Hypoglycaemia Due to Malaria

Hypoglycaemia can contribute to anaemia through reducing the absorption of iron, gastrointestinal bleeding and through other complications that cause anaemia especially in women during pregnancy period. For instance, low glucose can lead to kidney nerve damage both of which contribute to anaemia in pregnant women; in hypoglycaemia kidney disease, the filtration mechanisms become disordered (Robert and Toto, 2009).

Malaria parasitaemia is implicated for most of the applications recorded among pregnant women, especially due to lowered immunity. These complications are fetal and are rare investigated during antenatal care. It is important to monitor the two (2) fundamental complications; anaemia and hypoglycaemia.

The aim of this study is to assess hypoglycaemia and anaemia associated with malarial parasitaemia among pregnant mothers.

MATERIALS AND METHODS

Study Population

All subjects included in the study were informed about the procedure and the possible outcomes. Also, their informed consent (verbal) was obtained prior to sample collection. The subjects were then divided into two groups based on their parity (Group-1: Primigravidae; Group-2: Multi gravidae). A questionnaire was administered to the participants at enrolment to capture demographic characteristics, socio-economic factors, malaria prevention behaviours and clinical history. All subjects were selected based on a systematic random sampling method to avoid bias.

Ethical Consideration

Ethical approval was obtained from Hospitals Management Board, Kano State, Patient's consent was sought for during the study.

Sample Size

The minimum sample size was calculated to be 128 using Open Epi Version 2.3 statistical software.

Laboratory Analysis

Malaria Detection and Density of parasitaemia Specimen Collection

About five millilitre (ml) of whole blood was collected by vein puncture from each patient for malaria parasite screening, blood glucose level (hypoglycaemia), packed cell volume (PCV) and haemoglobin estimation. The blood samples were then collected in an EDTA (Ethyl diamine tetracetic acid) container. The sample was transported to the laboratory and processed immediately (Lewis *et al.*, 2008).

Microscopy and Parasite Identification

Blood films (thick and thin) were made with the collected blood using the standard method and stained was collected using Giemsa staining technique. The presence of malaria parasites was identified and the speciation of the parasites to the specie level was done using

their morphological characteristics (WHO, 2000; NMCP, 2005; IMMC, 2011).

Thin Film: Thin film was made by putting a drop of the blood on a glass slide, and using another clean slide as the spreader. The blood was allowed to run along clean slide and the spreader was firmly pushed along the slide keeping it at an angle of 45^oC (WHO, 2013).

Thick Film: A drop of blood was placed on a slide and spread to make an even thick film. Circular thick film was about 1cm in diameter. The film was then stained with Giemsa stain.

Giemsa Staining Techniques

A standard rapid (10%) method of staining techniques was adopted as recommended by (WHO, 2013).

- i. The thin film was fixed by dabbing it with a pad of cotton wool dampened with methanol or by briefly dipping the film into methanol.
- ii. The Giemsa solution of about 10% in the buffered water was made by mixing three drops of Giemsa from the stock solution, using the Pasteur pipette, with 1 ml of buffered water.
- iii. The slide was stained face upwards on the rack. The stain was poured gently until each slide was covered with the stain and was allowed to stay for 10 minutes.
- iv. The slide was washed gently with buffered distilled water pH 6.7.
- v. The slide was placed upright in a draining rack to air dry. (WHO, 2014)

Examining Blood Film

The slide was examined microscopically under X100 oil immersion objective. A drop of immersion oil was added. (WHO, 2014)

Thick Film: The density of the Malaria parasite was read against the leucocytes and an approximate parasite count was calculated.

The asexual form of the parasite (rings, trophozoites, and schizonts) was count against 200 White Blood Cell. Malaria density calculation was based on the assumption of 8000 WBC per microliter of blood. (WHO, 2014)

$$\frac{\text{Number of parasites counted} \times 8000}{\text{Number of leukocytes}} = \text{parasite per m}$$

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Determination of Blood Glucose (Random Blood Glucose)

Collection of Blood sample:

About 2ml each of patient's blood was collected by venipuncture into a tube containing a mixture of ethylene diamine tetra acetic acid and sodium fluoride in the ratio of 1:2 (W/W). Five mg of the mixture is adequate for 2ml of blood. The tube was thoroughly shaken for complete mixing.

Procedure:

The preparation of test was obtained by pipetting 0.1ml of blood into 1.8ml of sodium sulphate-zinc sulphate reagent in a centrifuge tube. This was followed by adding 0.1ml of 2N Sodium hydroxide and the solution were centrifuged at 3000rpm for 5 minutes and then 0.5ml was taken for supernatant in duplicate.

The blank preparation and the standard preparation were then taken as follows: (0.5ml of distilled water). This was followed by the preparation of standard concentration of glucose (200mg/dl), 0.5ml of a range of glucose solutions (50mg/dl, 100mg/dl, 150mg/dl and 200mg/dl) is used suitably diluted from standard. This was done in the following ways:

I. The first preparation involves 50mg/dl - 125µl glucose standard + 375µl distilled water

II. The second one was of the following kind; 100mg/dl - 250µl glucose standard + 250µl distilled water

III. The third one is characterised by the preparation of 150mg/dl - 375µl glucose standard + 125µl distilled water

IV. While the fourth one brings together 200mg/dl - 500µl glucose standard

- the glucose oxidase reagent of 5ml was added and incubated for 1h at 37°C, the reading of extinction at 540nm against the reagent blank was then taken.
- In a situation whereby absorbance reading of the sample was too high, the supernatant was diluted with distilled water and the subsequent steps were also repeated.

Determination of Packed Cell Volume (PCV).

The hemotocrit can be calculated by an automated analyser and PCV can be determined by centrifuging heparinized blood in the capillary tube at 10,000 revolution per

minute (RPM) for five (5) minutes (Herbert *et al.*,1982).

The Determination of Haemoglobin (hg) Level

The determination of haemoglobin level was done by spectrophotometry using cyanireth method. (Liposky *et a.*,1982).

RESULTS

From the results obtained in this study, fifty five (55) pregnant women fulfilled one or more of the WHO criteria for scanty, mild and severe malaria parasites. On the occurrence, based on the trimester 40 (72.8%) were positives in the second trimester and 15 (27.3%) in the third trimester. Table 2 shows the density of parasitemia based on the trimester, (WHO, 1991). It shows that 3(5.5%) and 1(1.8%) had scanty parasitaemia in the second and third trimester, 26 (47.3%) and 10 (18.2%) had mild parasitaemia and 8 (14.5%) and 3 (5.5%) had severe anemia. Table 3 shows anemia due to malaria based on the trimester using PCV >35% and Hb of <11.5g/dl as the standard value (Jaleel, 2008). In this study, 30 (54.5%) pregnant women in the second trimester and 28(50.9%) shows anemia. While 1(1.82%) and 8(14.5%) in third trimester. Anemia associated with parasitaemia based on age group was presented in Table 4. The prevalence of malaria parasite and anaemia in pregnant women in relation to age shows that malaria was higher in younger (65%) than older age groups (34.5%) while anaemia was higher in younger age group of 16-30 years, (50.9%) using PCV and (47.3%) using Hb and 31-45 years (12.7%) PCV, and (21.8%) for Hb respectively. Based on gravidity, 30 (54.5%) primagravidae pregnant mothers were observed in the second trimester and 11 (20%) multigravidae, 13(23.6%) primagravidae were observed in the third trimester, and only 2 (3%) multigravidae. Table 6 shows Hypoglycemia associated with malaria parasite based age group where 31(56.4%) pregnant women were observed with hypoglycaemia between the age of 16 - 30 years, and 15 (27.3%) were between the age of 31-45years. Hypoglycaemia associated with malaria based on the trimester was discussed as shown in Table 7 which indicates a higher percentage of positive cases in the second trimester 34 (61.8%) followed by the third trimester 15 (23.6%).

Table 1:- Occurrence of Malaria Based on Trimester

S/N	Trimester	No. Positive
1	First	0
2	Second	40 (72.8%)
3	Third	15 (27.3%)

Table 2:- Density of Parasitaemia Based on Trimester

S/N	Density of Parasitaemia / μ l	Trimester		
		1	2	3
1	1-4999	0	3	1
2	5000-9999	0	26	10
3	> 10,000	0	8	3

Table 3:-Aneamia Due to Malaria Based on Trimester

S/N	Trimester	Anaemia	
		PCV (<35%)	Hb (11.5g/dl)
1	1	0 (0%)	0 (0%)
2	2	30 (54.5%)	28 (50.9%)
3	3	1 (1.8%)	8 (14.5%)

Table 4: Anaemia and Malaria Parastaemia Based on Age Group

S/N	Age Group (Years)	No. of Malaria Cases (%)	Anaemia	
			PCV (<35%)	Hb (<11.5g/dl)
1	0-15	0 (0%)	0 (0%)	0(0%)
2	16-30	36 (65.5%)	28 (50.9%)	26(47.3%)
3	31-45	19 (34.5%)	7 (12.7%)	12(21.8%)

Table 5: Anaemia AssociatedwithMalaria Parasitaemia Based On Gravidity

Trimester	Primagravida	Multigravida
1	0 (0%)	0 (0%)
2	30 (54.5%)	11(20%)
3	13 (23.6%)	2 (3.6%)

Table 6: Hypoglycaemia Associated with Malaria Parasitaemia Based on Age Group

S/N	Age Group (Years)	No. of Malaria Cases (%)	Hypoglycaemia (< 75mg/dl)
1	0-15	0 (0%)	0 (0%)
2	16-30	36 (65.5%)	31(56.3%)
3	31-45	19 (34.5%)	15 (27.3%)

Table 7:- Hypoglycaemia Associated with Malaria Based on Trimester

S/N	Trimester	No. of Positive cases (%)
1	First	0 (0%)
2	Second	34 (61.8%)
3	Third	13 (23.6%)

DISCUSSION

This study has revealed a 55 (42.9%) prevalence of malaria among 128 randomly selected pregnant women attending Abdullahi Wase Specialist Hospital and Murtala Muhammad Specialist Hospital.

On the occurrence of the infection based on trimester, 40 (72.8%) were positive in the second trimester and 15 (23.3%) were so in the third trimester. This indicates that malaria is

regularly found among a particular people in the study area. This is particularly among the pregnant women and it may be due to lack of antimalarial drugs, increase in unsanitary conditions that may favour the breeding of mosquitoes, the vector of malaria parasite, non-adherence to intermittent preventive treatment during pregnancy and lack of other malaria preventive measures for pregnant women.

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Plasmodium falciparum was identified as the major cause of malaria within the study population. Jumbo *et al.*, (2011) also reported in an earlier study that 98% of malaria parasitaemia in pregnant women in Otukpo was caused by *P. falciparum*. The prevalence of this study (42.9%) is similar to what was reported by Gajida *et al.*, (2010) who estimated the rate of (39.5%) in the study he carried out at some primary health care centers in Kano State.

This study involved pregnant women in Kano metropolis from October 2016 - July 2017 and the density was low. This may be due to the timing of the study which contributed to the great variance between the prevalent rates. Higher percentage was obtained among those with moderate parasitaemia (47.3% and 18.2%) followed by severe (14.5% and 5.5%) then mild (5.5% and 1.8%) respectively. Additionally the prevalence of the study is lower than what was established by Dantata, Oyeyi, and Galadanci.,(2017) who reported the prevalence of (59.7%).

Anaemia due to malaria based on trimester using PCV > 35% and Hb of <11.5 g/dl as the standard value according to the (Jaleel R. Khan 2008), pregnant women in second trimester shows the highest percentage of 54.5% using PCV and 50.9% using Hb. This clearly indicates that there is no significant difference when comparing the level of blood using Hemocue or PCV machine at P value > 0.05. However, the prevalence of anaemia in pregnant women is reiterated with malaria infection with regard to trimester. Higher percentage of parasite density was observed in the second trimester so as to anaemia in the same trimester. Dual infection, therefore, makes life difficult to the mother due to higher frequency.

The study also indicates that the prevalence of malaria in pregnancy was higher in the youngest (65.5%) and the oldest (34.5) age group with significant association with anaemia that shows higher prevalence higher in younger age group of (16-30) years (50.9%) by PCV and (47.3%) by Hb, by (31-45) years is (12.7%) and (21.8%), respectively. This is in agreement with the findings of previous studies (Movkenhaupt *et al.*, 2000; Wakibara *et al.*, 1997; Ven *et al.*, 2000; Marcella *et al.*, 2003 and Jenavine *et al.*, 2015). This observation could be attributed to the facts that the youngest age groups are immunologically naïve to malaria infection since they are having malaria infection in pregnancy for the first time (primagravidae). Age and weaker immune status may have also played a role in the increased incidence of malaria in women within the oldest age group thereby predisposing them to malaria infection. Lander *et al.*, (2012) also

recorded high prevalence of malarial infection and anaemia among younger study subjects. They express an opinion that the prevalence reduces as women advance in age due to acquired immunity. According to the earlier studies, the cause of anaemia could be associated with many risk factors which include hook worm infestation, lack of iron intake and malarial parasitaemia. (Jenavine *et al.*, 2015; Brookis *et al.*, 2008; Aikawa 1988). The prevalence of malaria was seen across all gestational ages. Results obtained include all primagravidy and multigravidy unlike that of (Gajida *et al.*, 2011) which was restricted only to primagravidy and was conducted in the same state.

Meanwhile, pregnant women are susceptible to developing hypoglycaemia and based on our findings, higher positive cases were obtained in the second trimester (61.8%) followed by (23.6%) in the third trimester. This clearly indicates that hypoglycaemia is an excellent indicator of malaria parasite infection in pregnant mothers. This is in line with many literatures (Tasawar *et al.*, 2003; Usaid, 2007; WHO, 2012). In spite of malarial infection, the severity of infection was found to be moderate. Hypoglycemia and anemia have been assessed in Thailand and Ethiopia by Nosten and Newman (2003). Interestingly, none of these patients developed anaemia and hypoglycaemia after specific antimalarial treatment. The limitation of this research was that we could not follow these women to investigate/ report the outcome after undergoing the antimalarial treatment.

CONCLUSION

The prevalence of malaria parasite is high among pregnant women living with anemia and hypoglycemia. Younger maternal age carries a higher risk of infection. Also, the second trimester carries the highest risk of infection. Additionally, primagravidae are at the highest risk of malarial infection and serious complications.

Recommendations

Based on the results obtained from this study, the following are recommended:-

1. There is a need to include screening for malaria and blood glucose level in addition to routing PCV/ Hb checkup during antenatal
2. Malaria, anaemia and Hypoglycaemia pregnant patients should be followed-up after treatment to ascertain the success of management.
3. Relevant studies should be carried out regularly in Kano State in order to secure information on the prevalence of malaria infection in pregnancy.

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