



Microscopic Diagnosis of Malaria Parasitaemia in Pregnant Women

Amauche Martina Ngwu *

Department of Medical Laboratory Science, Faculty of Basic Medical Sciences,
Enugu State University of Science and Technology, Enugu, Nigeria

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* Corresponding author:

Amauche Martina Ngwu
e-mail: muchyscki@gmail.com

Abstract:

Malaria is the major cause of morbidity and mortality in the developing countries in which Nigeria is one of them. Malaria during pregnancy is a major cause of maternal death globally. Microscopic analysis of Giemsa stained blood film is still the most suitable method of malaria diagnosis in most developing countries. The aim of this study was to determine malaria parasites number per 100 thick film fields in a Giemsa stained blood film. This is a cross sectional study involving 90 pregnant women within the ages of 19-45 years. Thick and thin smears were prepared on clean glass slide using venous blood. The thick blood smears were stained with 3% Giemsa working solution diluted in pH 7.2 phosphate buffer and examined

for the presence of malaria parasites at 100 X oil immersion objective searching 100 fields in each thick smear. Blood smears were examined in the parasitology laboratory of Enugu State University of Science and Technology Teaching Hospital by an experienced microscopist. The level of parasitaemia was indicated in each positive case. The number of parasites in thick blood film was graded using the plus sign: + (1 to 10 parasites per 100 thick film fields); ++ (11 to 100 parasites per 100 thick film fields); +++ (1 to 10 parasites per one thick film field); ++++ (>10 parasites per one thick film field). Bar charts and pie charts were used in this study. Out of 90 pregnant women, 65 (72.2%) of the women had malaria parasite (MP). Out of the 65 pregnant women with MP 19 (21.1%) had one plus (1 to 10 parasites per 100 thick film fields) while 46 (51.1%) had two pluses (11 to 100 parasites per 100 thick film fields). Then 31 (67.4%) of those with two pluses were at age range of 26- 35 years, also 12 (26.1%) of them with two pluses were women that are less than 25 years. However, 25 (27.8%) had no malaria parasite (MP). The findings showed that higher number of the pregnant women had malaria parasites and higher number of individuals positive with malaria parasites had 11 to 100 parasites per 100 thick film fields. Also higher number of pregnant women that had 11 to 100 parasites per 100 thick film fields was at age range of 26-35 years.

Keywords: *parasites, smear, thick, film, fields, malaria.*

Introduction

Malaria is under control or no longer in existence in many parts of America, Europe and Asia, but in Africa due to so many factors such as increasing drug resistance, various mosquito vectors, decaying health systems, high cost of

laboratory equipments and reagents, unavailability of training and re-training of microscopist, poverty, illiteracy, insurgencies and attacks on health workers malaria infections have actually increased over the last 30 years (UNICEF and WHO, 2005; WHO, 2013). Malaria infection is common in Nigeria, with



nearly 100% of the population at risk of malaria. In Nigeria malaria is being transmitted year-round in the south to 3 months or less in the north (Okwa, 2003). The malaria species that is predominant in Nigeria is *Plasmodium falciparum*. Anopheles is a breed of mosquito and it was first reported and named by Johann Wilhelm Meigen in 1818. About 460 species are discovered, among them over 100 can cause human malaria, while about 30–40 passes parasites of the genus Plasmodium, that cause malaria in human's beings in endemic areas (Besansky et al., 1994). The vector for malaria is anopheline mosquito and disease causing pathogen is malaria parasite. In sub-Saharan African, *Anopheles gambiae sensu stricto* (s.s) is the most important vector of malaria followed by *Anopheles funestus* in some part of the Nigeria (Mouchet et al., 2008). Recent report on malaria showed that there were 247 million cases of malaria in 2021 compared to 245 million malaria cases reported in 2020. Also death rate from malaria as of 2021 was 619,000 compared to 625,000 in 2020. Half of malaria deaths worldwide were seen in Nigeria, the Democratic Republic of the Congo, United Republic of Tanzania and Niger (UNICEF and WHO, 2005). In Nigeria about 11% maternal deaths were caused by malaria. Also in Nigeria, 8.4% to 58.1% of complications in pregnancy are caused by malaria parasite (Amuta et al., 2014; Udomah et al., 2015). High prevalence rates of malaria in pregnancy have been reported in different parts of Nigeria, it ranges from 19.7% to 72.0% (Adefioye et al., 2007; Fana et al., 2015; Kagu et al., 2007; Uneke, 2008). Advanced compound microscopes were first seen in Europe around 1620 (Helden et al., 2010; William, 1996). High magnification simple microscope was developed by Antonie Van Leeuwenhoek in the 1670. Antonie Van Leeuwenhoek is regarded as the first recognized microscopist and microbiologist (Ford, 1992; Lane, 2015). Any scientist or technician who often uses a microscope for his or her laboratory work is known as microscopist. Microscopy of malaria enables the identification of different malaria causing parasites such as *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi* and also their different parasites stages like gametocytes, and the quantification of parasite density. In

Nigeria, microscopy is commonly used method of choice for investigation of malaria treatment failure. Giemsa is the widely used stain for malaria microscopy and the examination is done by both thin and thick film (Giemsa, 1904; Shapiro & Mandy, 2007). The aim of this study was to determine malaria parasites number per 100 thick film fields in a Giemsa stained blood film.

Materials and Methods

This cross sectional study was conducted at antenatal unit and parasitology laboratory of Enugu State University of Science and Technology Teaching Hospital (ESUTTH) Enugu, Nigeria. The study was carried out between the month of June and September 2022. Ninety (90) pregnant women between the ages of 19 and 45 years were recruited for the study. They were attending antenatal at antenatal care unit of the Enugu State University of Science and Technology Teaching Hospital. The approval for this study was given by the Research Ethics Committee of Enugu State University of Science and Technology Teaching Hospital (ESUTH) Enugu. Written informed consent was obtained from each pregnant woman. Those who were unable to sign inform consent form was excluded. In each subject, 2.5ml of venous blood samples were obtained into Ethylene Diamine Tetra acetic Acid (EDTA) bottle. Thick smears were prepared on clean glass slide using venous blood. The blood smears were stained with 3% Giemsa working solution diluted in pH 7.2 phosphate buffer and examined for the presence of malaria parasites at 100 X oil immersion objective searching 100 fields in each thick smear. The malaria parasites seen were graded using the following plus sign + (1 to 10 parasites per 100 thick film fields); ++ (11 to 100 parasites per 100 thick film fields); +++ (1 to 10 parasites per one thick film field); ++++ (>10 parasites per one thick film field). Bar charts and pie charts were used. Bar chart compares different sets of data among different groups easily, while pie chart allows the viewer to see a data comparison at a glance, allowing

them to do an immediate analysis and quickly understand details.

Laboratory Method

Frosted end glass slides were used. The glass slides were washed, dried and wrapped before the preparation of blood film. Giemsa stock solution was prepared by placing 50 methanol-cleaned glass beads into an amber bottle. Three point eight grams of Giemsa stain powder was weighed on an analytical balance and poured into the bottle that contained beads through a funnel. Also 100mL of methanol was gently poured to ensure that all the dry stain was washed into the bottle. The screw cap on the bottle was tightened and shaken in circular motion for 3 minutes. Two hundred and fifty milliliters of glycerol were added to the mixture and shaken again for 5 minutes. The remaining 150 mL of methanol was added to the mixture through the funnel in order to ensure that the last of the methanol washes the last of the glycerol from the funnel into the stain mixture. The screw cap on the bottle was tightened and shaken continuously for 3 minutes on the first day. The shaking was done for period of 3 minutes every day for seven days. Giemsa stock solution, batch number, name of the person who prepared the stock, date of preparation and date of expiration were clearly labeled on the bottle. The screw cap on the bottle was tightened again to prevent absorption of water vapor from the air, and the bottle was stored in a cool place away from direct sunlight.

Water buffered to pH 7.2 was prepared by weighing 0.7 g of potassium dihydrogen phosphate (KH_2PO_4) onto a two-pan trip balance. Wooden spatula was used to place KH_2PO_4 onto the filter paper in the left-hand pan until it reached 0.7 g. The weighed KH_2PO_4 was transferred to the glass beaker. One hundred and fifty milliliters of water were added and stirred with a clean spatula until all the salt dissolved. One gram of disodium hydrogen phosphate (Na_2HPO_4) was added to the solution in the beaker and stirred with a clean spatula until all the salt dissolved. When all the salts have dissolved, the solution was transferred

into a conical flask, and top up to the 1-Litre mark with water. The buffered water was poured into an amber glass bottle. Buffered water, pH 7.2, name of the person who prepared it, date of preparation and date of expiration were clearly labeled on the amber bottle.

Fresh 3% Giemsa working solution was prepared from stock solution by placing 97 mL of previously prepared buffered water, pH 7.2 into a clean measuring cylinder. The Giemsa stock solution was filtered through paper (Whatman #1) and transfer to a 25mL container. A pipette was used to measure 3mL of Giemsa stock solution. The Giemsa working solution was prepared just before staining the blood film(s), and used within 15 minutes of preparation (UNICEF and WHO, 2005).

Statistical Analysis

Bar charts and pie charts were used. Bar chart compares different sets of data among different groups easily, while pie chart allows the viewer to see a data comparison at a glance, allowing them to do an immediate analysis and quickly understand details.

Results

A total number of 90 pregnant women attending antenatal at antenatal care unit were studied. Results of Giemsa stained thick blood smear are as follows fig 1, fig 2, fig 3 and fig 4.

Out of 90 pregnant women studied, 65 (72.2%) had malaria parasite, while 25 (27.8%) had no malaria parasite (MP) (Fig 1).

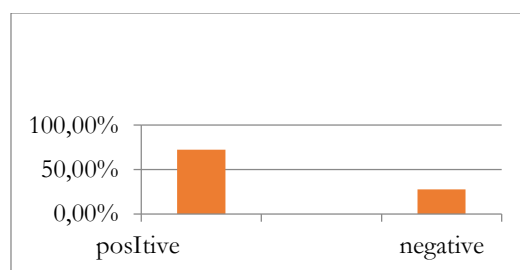


Figure 1. Detection of Malaria Parasite

Out of the 65 pregnant women with MP, 19 (21.1%) had one plus (1 to 10 parasites per 100 thick film fields) while 46 (51.1%) had two pluses (11 to 100 parasites per 100 thick film fields). However, none of them had three pluses (+++) (1 to 10 parasites per one thick film field) and four pluses (++++) (>10 parasites per one thick film field) (Fig 2).

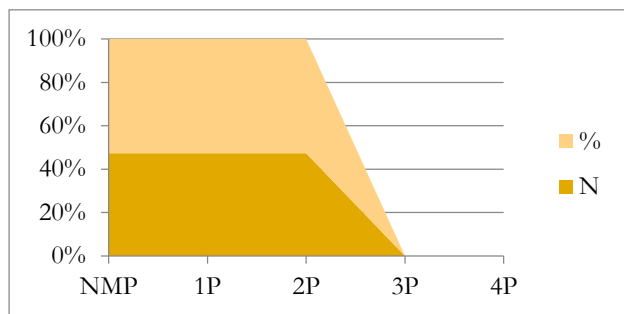


Figure 2. Plus Distribution

Abbreviations: NMP= No malaria parasite, p=plus

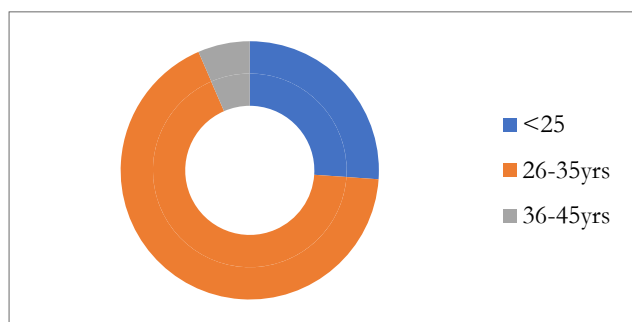


Figure 3. Age Distribution of One Plus

Abbreviations: Abbreviation: yrs= years

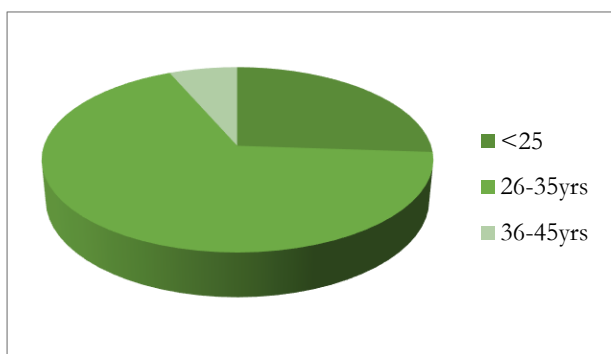


Figure 4. Age Distribution of Two Pluses

Out of 19 that had one plus, 3 (15.8%) were less than 25 years, 11 (57.9%) were between the age of 26 and 35 years, while 5 (26.3%) were 36-45 years (Fig 3).

Out of 46 that had two pluses, 12 (26.1%) were less than 25 years, 31 (67.4%) were between the age of 26 and 35 years, while 3 (6.5%) were between the age of 36-45 years (fig 4).

Discussion

In Nigeria microscopic diagnosis of malaria infection using Giemsa stain is still widely used in health facilities. This study is the first study to show microscopic evaluation of malaria parasite using Giemsa stain and plus sign in Enugu State University of Science and Technology Teaching Hospital. This study need to be considered by Enugu State Ministry of health for future study and also to improve antenatal care system. In 2021 African region had 95% of all malaria cases and 96% of death. Out of that 95%, Nigeria accounted for 31.3% (WHO, 2018). Early diagnosis of malaria still remains number one option to reduce diseases and prevent deaths. Also parasite-based diagnostic testing using microscopy is strongly recommended by World Health Organization (WHO). The essence of diagnostic testing is because it enables health care providers to promptly differentiate between malaria and non-malaria fevers (WHO, 2015). In this study the results showed malaria prevalence of 72.2%. This result shows that malaria is still a major public health concern in Enugu. The result is consistent with a previous report (Ali et al., 2019) showing that malaria is endemic in the study area. Again the prevalence rate of 72.2 % in this study is extremely high compared to previous report from other part of Nigeria. Previous studies in Nigeria reported prevalence rate of 24.5% in Eastern Nigeria, 13.7% in the West (Lagos state) and 27.3% in the North (Sokoto State) (Anumudu et al., 2006; Faruk and Adeoye, 2010; Nwele and Nwaorgu, 2017). Also the high prevalence rate of 72.2% in this study is similar to previous report in Nigeria that ranged from 41.7%-86.7% (Dawaki et al., 2016; Odikamnoru et al; 2017). The malaria prevalence of 72.2% seen in this study may be due to

microscopic (gold standard) examination method used.

Conclusion

This study showed the highest prevalence among middle age group (26-35 years) in the individuals with one plus. Again the study showed the highest prevalence among middle age group (26-35 years) in the individuals with two pluses. The result differs from previous reports that showed highest prevalence among young age group (Mohammed et al., 2019; Perkins et al., 1997). The high prevalence of malaria in this study may be due to women involvement in household responsibilities, such as cooking dinner outdoors, waking up early in the morning to prepare children for school and fetching water especially in Enugu metropolis where there is scarcity of water and not sleeping under insecticide treated net (ITN). In conclusion, malaria is still a public health concern in pregnancy, especially in the Enugu metropolis. Also timely diagnosis to differentiate malaria from non-malaria fever is important in other to commence the appropriate treatment.

Conflict of interests

Author declare that no competing interests exist.

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