***Original Research Article***

**Assessment of malaria parasite density in asymptomatic adult with type 2 diabetes**

**Abstract**

**Background**: The importance of infection in the regulation of blood glucose is complex, but independent association of infectious agents such as plasmodia with increased glucose level through insulin resistance has been demonstrated in type 2 diabetes. The aim of this study was to evaluate malaria parasite density (MPD) of asymptomatic type 2 diabetes mellitus patients.

**Place and Duration of Study:** Haematology unit and Diabetes Outpatient Clinic both of Enugu State University Teaching Hospital, between July and November 2024.

**Methodology:** The study population consisted of seventy (70) type 2 diabetes mellitus patients and thirty (30) apparently healthy subjects. Haemoglobin (HGB), packed cell volume (PCV) and red blood cell count (RBC) were measured by automated haematology analyzer, Erythrocyte sedimentation rate (ESR) by westergren method, fasting blood glucose (FBG) by glucose oxidase method and thick and thin blood film were determined.

**Results:** Among the 70 type 2 diabetes mellitus patients, 53 had mild parasitemia (*MPD* < 2000 parasites/μl), 17 had moderate parasitemia (*MPD*: 2000 to < 10,000 parasites/μl) and none had severe parasitemia (*MPD* ≥ 10,000 parasites/μl). Malaria parasite density (1346.61 *+/-* 968.50 MPD/µL) and erythrocyte sedimentation rate (35.75 *+/-* 22.82 mm/hr) were significantly higher in type 2 diabetes mellitus patients compared to apparently healthy control subjects (525.16 *+/-* 216.20 MPD/µL; 17.51 *+/-* 5.26 mm/hr) (P<0.05). Packed cell volume (36.88 *+/-* 6.62 %) were significantly lower in type 2 diabetes mellitus patients compared to apparently healthy control subjects (39.93 *+/-* 3.90 %) (p= 0.02). There was a significant negative correlation between malaria parasite density (MPD) and fasting blood glucose (*P*=0.035, *r*=0.253).

**Conclusion:** This study found that in Enugu, Nigeria, type 2 diabetes mellitus patients had mild parasitaemia, decreased PCV and that MPD had significant correlation with fasting blood glucose.

Keywords: malaria, parasites, density, diabetes, mellitus, asymptomatic

1. **Introduction**

In spite of the reduction in global malarial cases, malaria still affects millions of people globally. In 2021, 247 million cases of malaria were reported globally, with 619,000 deaths [1]. It has also been reported that out of this number, 194.4 million cases and 407,000 deaths happened in the African region, making malaria the leading cause of death in African. Malaria infection is caused by the *Plasmodium* parasite with over 100 species diagnosed [2]. Out of this 100 species, only five of them, *Plasmodium falciparum, Plasmodium malariae, Plasmodium vivax, Plasmodium ovale* and *Plasmodium knowlesi* are transmitted to humans [2]. The malaria parasites have a complex lifecycle which requires the female anopheles mosquito and the human host [3]. Sporozoites are produced in mosquito, transmitted upon infective bite to the human host. In the humans, the sporozoites attack the liver and develop further into infectious merozoites which then mixed with the red blood cells upon release into the blood stream. In the red blood cells, multiplication continues and the multiplication is responsible for the clinical presentation of malarial disease. Out of the five *Plasmodial* species that cause human malaria, *Plasmodium falciparum* is responsible for more than 95% of the global malaria burden [4]. Diabetes is a known metabolic disorder characterized by fasting hyperglycaemia and the forming of chronic vascular complications. The incidence of type 2 diabetes mellitus has tripled in the past 15 years in association with malaria especially in Africa. The current report on type 2 diabetes mellitus has established it as a powerful cardiovascular risk factor with yearly mean mortality rate of 5.4% [5-7]. Glucose being almost a general energy source for all kinds of animals, the *Plasmodium* parasite hardly survives when deprived of glucose [8]. The reliance of the *Plasmodium* parasite on glucose for survival makes it possible to see reduced glucose level in individuals infected with malaria compared to controls as normally observed in critical cases of the disease [9].The incidence of patients having lower glucose level to the extent of developing clinical hypoglycaemia has been attributed to factors like impairment of gluconeogenesis, malnutrition, glucose consumption by parasite, starvation and hyperinsulinemia [10]. It has been reported widely that type 2 diabetes mellitus patients, are known to be more endangered to malaria and present a higher parasitaemia than controls [11, 12]. High susceptibility to *Plasmodium falciparum* infection seen in diabetics could be associated to immune defect of diabetics compared with non diabetic controls subjects. Malaria being one of the notable diseases has long been linked with inflammation [13]. In fact, inflammation is shown to have been supporting the clinical signs and symptoms associated with malaria [14]. The inflammatory activities is moderated by many markers like tumor necrosis factor alpha (TNFa), interleukins (IL), C-reactive protein (CRP) and interferon gamma (IFNg) [15-19]. Again, malaria is strongly associated to insulin resistance mediated by inflammation [17, 20]. As a matter of fact, insulin resistance is a fundamental risk factor for the development of type 2 diabetes mellitus [21, 22]. Generally, inflammatory markers like C-reactive protein, selected interleukins, tumor necrosis factor alpha and interferon gamma which have been connected in the development of insulin resistance are also implicated in the pathogenesis of malaria [9, 18]. The malaria parasite density gives information on the seriousness of malaria infection and on the response to treatment. Parasite counts are done for *plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale* asexual stages. Except there is request, gametocytes are not included in the count, but their presence is always recorded. Most parasite counts are done on thick blood films. But if thick film is not available or it is spoiled, a thin film count can be used. A thin film count is also carried out when there are more than 100 parasites in each field of the thick film, which corresponds to more than 80 000 parasites/μL [23, 24]. Because there is established link between malaria and type 2 diabetes, this study therefore assessed malaria parasite density and some blood parameters in asymptomatic adults with type 2 diabetes.

1. **Methods**

The study was conducted at Haematology unit and Diabetes Outpatient Clinic both of the Enugu State University Teaching Hospital, Enugu State, Nigeria from July to November, 2024.

**2.1 Study design**

This was a case control study involved type 2 diabetes mellitus patients as test subjects and apparently healthy non-diabetic adult as controls.

**2.2 Inclusion and exclusion criteria**

Diabetic patients who gave consent, with confirmed cases of type 2 diabetes mellitus in the last one year, were recruited as test subjects, while apparently healthy non diabetic subject who gave consent were recruited as controls. Individuals who did not give consent and those with chronic system or organ illness and on malaria medication were excluded from the study.

**2.3 Selection of subjects**

A total of one hundred (100) subjects were enrolled into the study. Seventy (70) individuals with glucose oxidase confirmed diabetes aged 40-60 years, and thirty (30) apparently healthy individuals without diabetes were recruited into the study.

**2.4 Sample collection**

Five milliliters (5ml) of peripheral blood was collected asceptically from the subjects by venipuncture into ethylenediaminetetraacetic acid (EDTA) and fluoride oxalate sample containers. An aliquot of the sample from the syringe was used to prepare thick and thin blood films on an appropriately labeled clean grease-free slide. The EDTA samples were used for haemoglobin estimation, packed cell volume and erythrocyte sedimentation rate (ESR). The samples in fluoride oxalate containers were spun at 5000 rpm for 5 min. Plasma were collected and stored at −20 °C in appropriate sample vials for assay of fasting blood sugar.

**2.5 Laboratory methods**

**2.5.1 Identification of malaria parasite by Giemsa staining technique**

Giemsa staining procedure was used for identification and quantification of malaria parasites in thick films. A positive smear of malaria parasite was used with each new batch of working Giemsa stain to ensure proper staining results are attained. Thick and thin blood films were allowed to air-dry and thin blood films fixed with absolute alcohol. The blood films were stained using 10% of the Giemsa stock stain for 10 min [25].

**2.5.2 Calculation of malaria parasite density**

Malaria parasite densities were recorded as a ratio of parasites to white blood cells in thick smears. *Plasmodium* parasites were counted against 200 white blood cells (WBCs) on the thick film. A total of 500 WBCs were counted where less than nine (9) parasites were counted after counting against 200 WBC. Malaria parasite densities (parasite/μl of whole blood) were then calculated as follows: (number of parasites counted/WBC counted) × WBC count per μl of blood.

Malaria parasite densities for all individuals were calculated using assumed WBC counts of 8.0 × 109/L of blood, determined by the World Health Organization (WHO) to be used comfortably in facilities which do not have the equipment to determine patients’ absolute full blood count value. Malaria parasite densities of infected subjects were grouped into three categories: mild, moderate and severe. Those categorized with severe malaria had a parasite density of ≥ 10,000 parasites/μl, those with moderate malaria had a parasite density of 2000 to < 10,000 parasites/μl; while mild malaria had a parasite density of < 2000 parasites/μl [25]

**2.5.3 Determination of erythrocyte sedimentation rate (ESR)**

The erythrocyte sedimentation rate is a simple non-specific screening test that indirectly measures the presence of inflammation in the body.

Principle

When anticoagulated whole blood is allowed to stand in a narrow vertical tube for a period of time, the red blood cells, under the influence of gravity, settle out from the plasma. The rate at which they settle is measured as the number of millimeters (mm) of clear plasma present at the top of the column after one hour (mm/hr) [26].

Procedure

Two (2ml) of venous blood was collected into tube containing 0 .5 ml of sodium citrate. It was stored for 2 hours at room temperature. The blood was drawn into a Westergren tube to the 200 mm mark. The tube was placed in a rack in a strictly vertical position for 1 hour at room temperature, at which time the distance from the lowest point of the surface meniscus to the upper limit of the red cell sediment was measured. The distance of fall of erythrocytes, expressed as millimeters in 1 hour, was the ESR [26].

**2.5.4 Determination of fasting blood sugar**

Glucose oxidase is one of the most generally used enzymes for glucose detection because it reduces oxygen to hydrogen peroxide (H2O2) while also oxidizing glucose to gluconic acid [27]. Glucose is oxidized to gluconic acid, whereas oxygen is simultaneously reduced to hydrogen peroxide by the enzyme glucose oxidase. Hydrogen peroxide is then split to form water and nascent oxygen by the enzyme peroxidase. The nascent oxygen reacts with 4-aminoantipyrine, and in the presence of phenol, this reaction produces quinoneimine, which is a colored compound that can be analyzed using colorimetric analysis. The intensity of the color produced correlates directly with the concentration of glucose in the sample. Colorimetric analysis is performed at 505 nm and compared to the standard, which is treated similarly [28].

**Statistical analysis**

The Student *t*-test was used to compare the mean difference between groups. Pearson’s correlation coefficient was used to determine associations between variables. Statistical significance was set at probability, *P* < 0.05. All data obtained were analyzed using Statistical Package for the Social Sciences (SPSS) version 25.0 software.

1. **Result**

Table 1 shows mean ± SD of MPD and some haematological parameters of the patients and controls. The MPD of patients (1346 ± 968.50) were significantly higher compared to control (525.16 ± 416.20) (p<0.001). The ESR of patients (35.75 ± 22.82) were significantly higher compared to control (17.51 ± 10.26) (p=0.003). While the PCV of patients (36.88 ± 6.62) were significantly lower compared to control (39.93 ± 3.90) (p=0.020). Table 2 shows a comparison of malaria parasite density, HGB, PCV, RBC and ESR in male and female subjects with type 2 diabetes. The result shows no significant difference (p>0.05). Table 3 shows a comparison of malaria parasite density, HGB, PCV and RBC based on FBG level of the patients. The result shows no significant difference (p>0.05). Table 4 shows correlation of malaria parasite density against HGB, PCV, ESR, and FBG. Malaria parasite density correlated negatively with fasting blood sugar (r=-0.253, p=0.035). Figure 1 showed the range of parasite density, 53 (75.7%) had mild parasitemia (*MPD* < 2000 parasites/μl), 17 (24.3%) had moderate parasitemia (*MPD*: 2000 to < 10,000 parasites/μl) and none had severe parasitemia (*MPD* ≥ 10,000 parasites/μl).

 **Table 1 MPD, HGB, PCV, RBC and ESR of test and control**

|  |
| --- |
| Parameters Test (N=70) control (N=30) p-value  |
|  MPD/µL 1346.61± 968.50 525.16±416.20 <0.001\* HGB g/dl 12.31 ± 2.19 13.26 ± 1.29 0.994 PCV % 36.88 ± 6.62 39.93± 3.90 0.020\* RBC 1012/L 4.22 ± 0.67 4.58 ± 0.45 0.941ESR mm/hr 35.75± 22.82 17.51 ±10.26 0.003\*  |

**Table 2 shows mean ± SD of MPD and some haematological parameters of the male and female patients**

|  |
| --- |
| Parameters Male (N=30) female (N=40) p-value  |
|  MPD/µL 1496.43 ± 991.31 1234.25 ± 947.94 0.508 HGB g/dl 12.31 ± 2.27 12.31 ± 2.17 1.000 PCV % 36.93 ± 6.81 36.85 ± 6.56 0.999 RBC 1012/L 4.22 ± 0.71 4.22 ± 0.65 0.999FBG mmol/L 7.30 ± 1.05 6.44 ± 0.90 0.665 ESR mm/hr 34.07± 20.29 36.10 ± 16.89 0.689  |

**Table 3 shows mean ± SD of MPD, HGB, PCV and RBC in prediabetes stage (5.6-5.9 mmol/L) and above 7.0mmol/L**

|  |
| --- |
|  5.6-6.9 mmol/L (N=30) 7.0 mmol/L or higher (N=31) p-value  |
|  MPD/µL 1385.66 ± 974.92 1203.00 ± 932.52 0.736 HGB g/dl 12.45 ± 1.72 12.05 ± 2.54 0.749 PCV % 37.34 ± 5.17 36.09 ± 7.63 0.736 RBC 1012/L 4.29 ± 0.39 4.17 ± 0.86 0.754  |

**Table 4 shows correlation of malaria parasite density against HGB, PCV, ESR, and FBS**

|  |
| --- |
|  variable MPD/µL  r p n HGB g/dl -0.074 0.542 70 PCV % -0.072 0.556 70  RBC 1012/L 0.003 0.982 70  ESR mm/hr -0.047 0.700 70 FBG mmol/L -0.253 0.035\* 70  |

Fig 1: Range of parasite density

Mod =moderate

Discussion

Malaria is an ailment caused by the Plasmodium parasite. The ailment happened when a susceptible host of the parasite presents the vital clinical signs and symptoms of the disease. The parasite density gives information on the seriousness of the infection and on the response to treatment. Parasite counts are carried out for *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale* asexual stages. Gametocytes are counted only when requested, but their presence is always noted. Parasite counts are done on thick blood films or on thin blood film when thick film is damaged or not available [25]. Several studies had reported that type 2 diabetes patients are at risk of malaria and they also express a higher parasitaemia than non-diabetic controls [11, 29]. Consistent with previous studies, this study shows higher levels of parasite density in type 2 diabetes mellitus patients than in non-diabetic controls. This may be partly due to immune weakness of diabetics compared with non-diabetic controls [30]. Parasite count is essential in *P. falciparum* infection cases which are undoubtedly dangerous. Parasitemia density for positive malaria blood film must be determined because the clinician needs to know the seriousness of the disease and case response for treatment. Diagnosis of asymptomatically infected individuals has been the utmost challenge given that persons living in areas of high infectivity often carry parasitaemia at densities lower than the detection limits of available field diagnostics, such as microscopy and rapid diagnostic tests [31]. A study in western Kenya reported that 68% of asymptomatically infected adults (> 21 years old) had sub-microscopic parasitaemia [32] which is in line with the result of the present study that observed mild parasitaemia (MPD<2000 parasites/µL) in 75% of patients. Fasting blood glucose is a routine test used to screen for prediabetes and diabetes. Elevated fasting blood glucose is a usual sign of insulin resistance and it can suggest prediabetes and diabetes. In this study, MPD was found to be significantly correlated with fasting blood glucose. Danquah *et al*., [29] in their study of adult diabetes and non-diabetes in the Cape Coast metropolis of the Central region of Ghana, reported overt insulin resistance due to *falciparum* *malaria.* In addition, Wyss *et al.,* [33] and On’kin *et al.,* [34] reported a significant relation between insulin resistance and malaria infection. The finding of the current studysuggests that asymptomatic malaria can potentially compromise the management of type 2 diabetes mellitus and can even cause anaemia of unknown origin. In the current study low PCV and high ESR was observed. The low PCV and high ESR seen in this study may be due to chronic hydrolysis of erythrocytes by malaria parasites in the infected patients. For that reason, the authors recommend the incorporation of asymptomatic malaria screening into the management of Enugu and at large Nigeria patients with type 2 diabetes mellitus especially among those with signs of anaemia.

**Conclusion**

This current study found that in Enugu, Nigeria, type 2 diabetes mellitus patients had mild parasitaemia, decreased PCV and that MPD had significant correlation with fasting blood glucose.

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