

**Original Article****Open Access*****Falciparum* malaria is associated with risk markers of type 2 diabetes mellitus in individuals with or without COVID-19 exposure**¹Adatsi, R., ²Pappoe, F., ³Bockarie, A. S., ⁴Derkyi-Kwarteng, L., ⁵Nsiah, P., ¹Weyori, E. W., ²Dankwa, K., ²Aniakwaa-Bonsu, E., ⁶Setorglo, J., and ^{*6}Acquah, S¹Public Health Reference Laboratory, Tamale Teaching Hospital, Tamale, Ghana²Department of Microbiology and Immunology, School of Medical Sciences, College of Health and Allied Sciences, University of Cape Coast, Ghana³Department of Internal Medicine and Therapeutics, School of Medical Sciences, College of Health and Allied Sciences, University of Cape Coast, Ghana⁴Department of Pathology, School of Medical Sciences, College of Health and Allied Sciences, University of Cape Coast, Ghana⁵Department of Chemical Pathology, School of Medical Sciences, College of Health and Allied Sciences, University of Cape Coast, Ghana⁶Department of Medical Biochemistry, School of Medical Sciences, College of Health and Allied Sciences, University of Cape Coast, Ghana*Correspondence to: sacquah@ucc.edu.gh**Abstract:****Background:** Scientific information on the impact of malaria on the risk of developing type 2 diabetes mellitus (T2DM) after recovery from the coronavirus disease 2019 (COVID-19) is limited in the Ghanaian context. The purpose of this study was to examine the association between selected risk markers of T2DM in *falciparum* malaria patients post-COVID-19 or not at a tertiary hospital in Ghana.**Methodology:** This was a descriptive cross-sectional comparative study of 38-recovered COVID-19 adult participants with malaria and 40 unexposed COVID-19 adults with malaria at the Tamale Teaching Hospital, Ghana. Demographic, anthropometric and levels of glucose, insulin, C-reactive protein and lipid profiles were measured in the two groups of participants under fasting conditions. Parasitaemia was assessed microscopically but insulin resistance and beta-cell function were assessed by the homeostatic model.**Results:** The COVID-19 exposed participants were older ($p=0.035$) with lower parasitaemia ($p=0.025$) but higher mean levels of insulin, insulin resistance, and beta-cell function compared with their unexposed counterparts ($p<0.05$). Parasitaemia correlated positively with a number of the measured indices of diabetogenic risk markers in the COVID-19 exposed group only, and predicted (Adjusted $R^2=0.751$; $p=0.031$) by beta-cell function, C-reactive protein and triglycerides with the model explaining about 75% of the observed variation. Parasitaemia could only be predicted (Adjusted $R^2=0.245$; $p=0.002$) by C-reactive protein with the model explaining just about a quarter of the observed variation in the COVID-19 unexposed group. Insulin resistance and sub-optimal beta-cell function were detected in both groups of participants.**Conclusion:** *Falciparum* malaria is associated with risk markers for development of T2DM irrespective of COVID-19 exposure. Insulin resistance, inflammation and sub-optimal beta-cell secretory function may drive the risk. The observed diabetogenic risk is higher in the recovered COVID-19 participants.**Keywords:** insulin resistance, *falciparum* malaria, type 2 diabetes mellitus, inflammation, COVID-19

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Copyright 2024 AJCEM Open Access. This article is licensed and distributed under the terms of the Creative Commons Attribution 4.0 International License <http://creativecommons.org/licenses/by/4.0/>, which permits unrestricted use, distribution and reproduction in any medium, provided credit is given to the original author(s) and the source. Editor-in-Chief: Prof. S. S. Taiwo**Le paludisme à *falciparum* est associé à des marqueurs de risque de diabète sucré de type 2 chez les individus avec ou sans exposition au COVID-19**¹Adatsi, R., ²Pappoe, F., ³Bockarie, A. S., ⁴Derkyi-Kwarteng, L., ⁵Nsiah, P., ¹Weyori, E. W., ²Dankwa, K., ²Aniakwaa-Bonsu, E., ⁶Setorglo, J., et ^{*6}Acquah, S¹Laboratoire de Référence en Santé Publique, Hôpital Universitaire de Tamale, Tamale, Ghana²Département de Microbiologie et d'Immunologie, École des Sciences Médicales, Collège de la Santé et des

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Résumé:

Contexte: Les informations scientifiques sur l'impact du paludisme sur le risque de développer un diabète sucré de type 2 (DT2) après la guérison de la maladie à coronavirus 2019 (COVID-19) sont limitées dans le contexte ghanéen. Le but de cette étude était d'examiner l'association entre certains marqueurs de risque de DT2 chez les patients atteints de paludisme à falciparum après le COVID-19 ou non dans un hôpital tertiaire du Ghana.

Méthodologie: Il s'agissait d'une étude comparative transversale descriptive portant sur 38 participants adultes atteints de COVID-19 guéris et atteints de paludisme et 40 adultes COVID-19 non exposés atteints de paludisme à l'hôpital universitaire de Tamale, au Ghana. Les niveaux démographiques, anthropométriques et de glucose, d'insuline, de protéine c-réactive et le profil lipidique ont été mesurés dans les deux groupes de participants à jeun. La parasitaémie a été évaluée au microscope, mais la résistance à l'insuline et la fonction des cellules bêta ont été évaluées par le modèle homéostatique.

Résultats: Les participants exposés au COVID-19 étaient plus âgés ($p=0,035$) avec une parasitaémie plus faible ($p=0,025$) mais des niveaux moyens d'insuline, de résistance à l'insuline et de fonction des cellules bêta plus élevés que leurs homologues non exposés ($p<0,05$). La parasitaémie était corrélée positivement avec un certain nombre d'indices mesurés de marqueurs de risque diabétogène dans le groupe exposé au COVID-19 uniquement et prédite (R^2 ajusté=0,751; $p=0,031$) par la fonction des cellules bêta, la protéine C-réactive et les triglycérides avec le modèle expliquant environ 75% de la variation observée. La parasitaémie ne pouvait être prédite (R^2 ajusté=0,245; $p=0,002$) que par la protéine C-réactive, le modèle expliquant à peine environ un quart de la variation observée dans le groupe non exposé au COVID-19. Une résistance à l'insuline et une fonction sous-optimale des cellules bêta ont été détectées dans les deux groupes de participants.

Conclusion: Le paludisme à falciparum est associé à des marqueurs de risque de développement du DT2, quelle que soit l'exposition au COVID-19. La résistance à l'insuline, l'inflammation et la fonction sécrétoire sous-optimale des cellules bêta peuvent entraîner ce risque. Le risque diabétogène observé est plus élevé chez les participants guéris du COVID-19.

Mots-clés: Résistance à l'insuline, paludisme à falciparum, diabète sucré de type 2, inflammation, COVID-19

Introduction:

The coronavirus disease 2019 (COVID-19) which has caused a serious disruption in the global health system appears to be under control in a number of countries, especially, those in the sub-Saharan African region. In spite of the seeming success in curtailing the spread and eradication of COVID-19, notably, in sub-Saharan African countries, its potential long-term impact is yet to be unraveled. Developing countries in general continue to grapple with the coexistence of communicable and non-communicable disease conditions.

Generally, non-communicable diseases are chronic in nature whilst communicable ones, though acute, can have chronic sequelae depending on the specific agent of interest. Several evidence abound through comprehensive reviews, to demonstrate the susceptibility of diabetes patients to several infectious agents including parasites, viruses and bacteria (1-4). The role of such infectious agents in the pathogenesis of diabetes has been acknowledged (1,5).

Type 2 diabetes mellitus (T2DM) is a chronic metabolic non-communicable dis-

ease associated with dysregulation of those aspects of carbohydrate and lipid metabolism mediated by insulin. The condition is strongly promoted by obesity, westernized diet and sedentary lifestyle, which together, affect negatively, the biological role of insulin. Insulin resistance and beta-cell dysfunction have been implicated in the development of T2DM. Insulin resistance connotes diminished responsiveness of cells to insulin action.

Insulin is supposed to facilitate glucose uptake into cells for breakdown for energy generation or synthesis of glycogen and other macromolecules for storage. When cells develop resistance to these actions of insulin, glucose accumulation in blood is favoured. Insulin resistance can be caused by obesity, infection and several other factors that cause inflammation. Indeed, T2DM is known to develop through chronic inflammatory mechanisms suggesting that any inflammation-induced condition could have a probable link to T2DM.

COVID-19 is considered generally as an acute condition of inflammatory nature although its chronic potential as well as the severity of the disease cannot be overlooked

(6,7). Irrespective of the nature and severity of infection, inflammation is critical for driving the pathogenesis especially in cases of reinfection (8). Malaria is a disease caused predominantly by *Plasmodium falciparum* in our setting and remains the number one cause of morbidity in Ghana with multiple episodes over the life course of an individual. In severe cases, the disease has high potential for multiple organ damage just like COVID-19. Although the disease is in most cases mild to moderate in severity, and is generally treatable, its prevalence is higher than COVID-19 in our setting. In spite of this, COVID-19 is given a higher priority, due probably to its easy transmissibility and associated high global mortality in the adult population.

Several studies have considered malaria-COVID-19 co-infection in terms of prevalence, clinical profile and disease outcomes where coinfecting individuals seem to have an unfavorable prognosis compared to those with only COVID-19 infection (9-11). Other studies have reported associations between diabetes mellitus and infections (1-5). COVID-19 for instance, is thought to have a bidirectional relationship with diabetes whereby the infection promotes the development of diabetes in non-diabetic individuals and worsens glycaemic control in patients with diabetes (12).

However, not much has been done to evaluate the probable effect of malaria on risk markers for T2DM development in individuals who have recovered from COVID-19 compared with their counterparts without COVID-19 exposure. This information is important for appropriate preventive measures to address the probable long-term impact of COVID-19 in malaria-endemic regions of the globe as far as diabetes development is concerned. Therefore, the current study was designed to examine the effect of malaria on selected markers of T2DM risk in COVID-19 exposed individuals compared with their non-exposed counterparts at a tertiary facility where malaria treatment is uncommon.

Materials and method:

Study site:

The study was conducted at Tamale, in Ghana's Northern Region. The laboratory evaluation was done at the Public Health Reference Laboratory (PHRL) of the Tamale Teaching Hospital (TTH). TTH is a tertiary health facility serving as the major referral hospital for the Upper East, Upper West, Oti, North-East and Savanna regions and parts of the Bono East and Brong-Ahafo regions.

In Ghana, malaria is commonly treated at the primary and secondary health

facilities but not the tertiary health facilities. The PHRL of the TTH is accredited by the International Organization for Standardization (ISO: 9001) and currently serves as the national reference laboratory for bacterial meningitis in Ghana. PHRL is currently the major testing site for COVID-19 for the five Northern Regions of Ghana.

The Northern Regions have a total area of 26,524 square kilometres and a population of 2,320,939, with Tamale metropolis having a population of 374,744 and a total area of 454 square kilometres, according to the 2021 Population and Housing Census (13). Agriculture accounts for more than 85% of all economic activities in the region. Savanna and grassland are the main types of vegetation, with baobab trees scattered throughout. The area has a generally dry climate with just one rainy season that lasts from May to October. The range of yearly rainfall observed is between 750 mm and 1050 mm. High temperatures occur around the end of the dry season, which begins in November and run through to March or April. The Northern Region is bounded on the north by the North-East Region, on the east by the international boundary separating Ghana and Togo, on the south by the Oti Region, and on the west by the Savannah Region. The capital of the Northern Region is Tamale.

Study design and participants:

The study was a descriptive cross-sectional comparative design conducted among malaria patients within the Tamale metropolis who had recovered from confirmed COVID-19 in comparison with their counterpart without any history of COVID-19. The COVID-19 exposed participants had recovered completely from the disease at least six months prior infection with the *Plasmodium* parasite. On the other hand, the comparative group had malaria but no history of COVID-19.

Data from the PHRL suggest a total of 9,658 suspected cases of COVID-19 in the Tamale metropolis but only 561 tested positive in 2021 resulting in a positivity rate of 5.8%. With a metropolitan population of 374,744 according to the 2021 Population and Housing Census of the Ghana Statistical Service (13), the prevalence of COVID-19 will be 0.15%. Although malaria is endemic in the region, patients with malaria are rarely managed at the TTH facility because of its tertiary care role in the health system.

In this study, only 78 individuals could be confirmed for malaria after inviting 280 individuals to participate in the study (Fig 1) through a simple random process over a 6-month period (February – July, 2023). Being a preliminary study, the flat

'rule of thumb' principle was employed, where 35 participants per study group was anticipated.

Sample and data collection:

Designed questionnaires were used for the collection of socio-demographic information and clinical data of participants. Specific information included age, history of COVID-19 exposure and other health conditions necessary for the assessment of the inclusion and exclusion criteria.

Ten milliliters of venous blood were obtained from each participant through a routine venipuncture technique under fasting conditions. The blood was aliquoted appropriately into serum separator tubes (4 ml) and fluoride tubes (4 mls) for the respective preparation of serum and plasma for measurement of indices. The remaining 2 ml whole blood was then used for the preparation of thick and thin films for malaria microscopy.

Malaria parasite, blood pressure and anthropometric measurements:

Malaria parasitaemia was determined microscopically using a standard thick blood film under oil immersion procedure by an experienced microscopist. Thin blood film procedure was used for species identification. COVID-19 exposure was determined by real-

time polymerase chain reaction and serological tests protocols approved at the time by the Ghana Health Service in line with acceptable World Health Organisation protocol for COVID-19 diagnosis and surveillance.

Blood pressure was measured by an experienced nurse with a standard mercury sphygmomanometer on the right arm of participants in sitting position after resting for at least five minutes. The average of three measurements at 5 minutes apart per measurement was recorded as the blood pressure for the participant. Meanwhile, weight was measured to the nearest 0.1 kg with height to the nearest 0.1 cm. Body mass index was computed as the ratio of weight in kilogramme to the square of the height in metre (kg/m²). Weight and height were measured in light clothing without footwear.

Waist circumference was measured in centimetres with an inflexible tape measure at the midpoint between the lower margin of the last rib and the top of the iliac crest (14). In terms of hip circumference, it was measured around the widest portion of the buttocks. Waist-to-hip ratio was then computed by dividing waist circumference by the hip circumference. Waist-to-height ratio was computed by dividing the waist by the height.

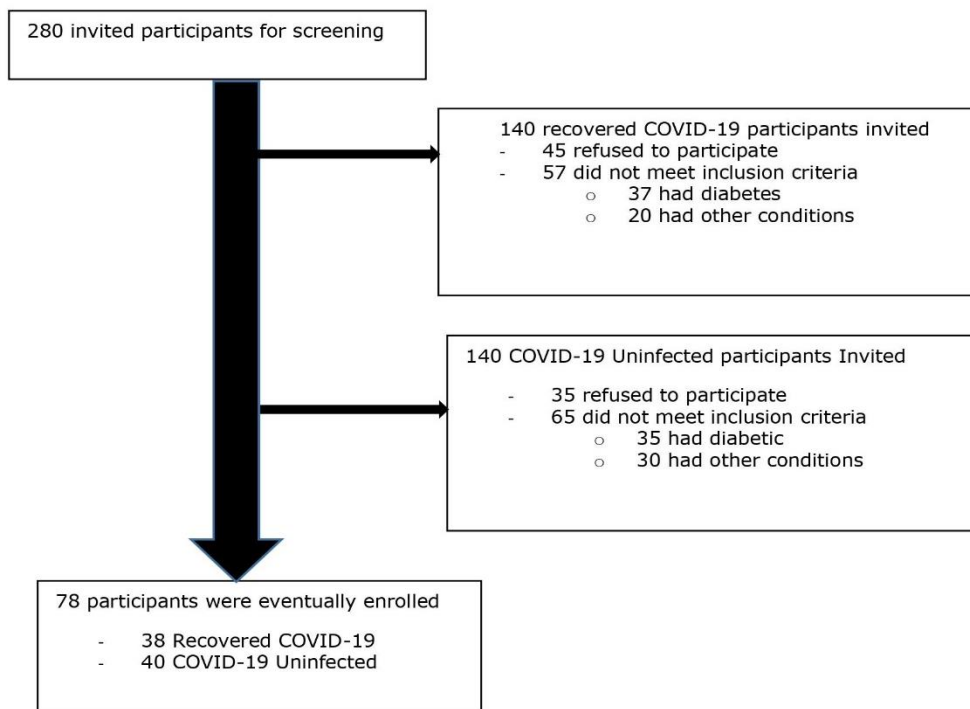


Fig 1: Flow chart for recruitment of participants

Estimation of fasting laboratory indices:

All measurements were done after samples and reagents were brought to room temperature. Blood glucose was measured by the glucose-oxidase method using a standard procedure with the Mindray BS240 automated chemistry analyser (Mindray Diagnostics, Nanshan Shenzhen, China). Additionally, lipid profile was measured by a standard method with the same autoanalyser.

Serum insulin level was determined by a commercial immunoturbidimetric test kit (Kamiya Biomedicals Company, K-ASSAY Seattle, USA). The assay is based on the principle that immune complexes formed in solution between sample insulin and human insulin-specific antibody coated on latex particles, scatter light proportional to insulin concentration in samples. In this specific case, reagents and samples were brought to room temperature. Exactly 16 μ L of serum was aspirated into 175 μ L of reagent 1 (buffer reagent) and 65 μ L of reagent 2 (Latex suspension) in a multi-point end point platform for measurement at 578 nm main and 800 nm sub reactions in the programmed autoanalyser. The concentration of insulin in sample was determined from a calibration curve prepared by plotting absorbance against standard concentration of insulin in accordance with instructions from the kits manufacturer. Insulin resistance was then calculated by the homeostatic model assessment formulae developed by Matthews et al., (15) for insulin resistance, $HOMA-IR = (\text{glucose} \times \text{insulin}) / 22.5$ and beta-cell function, $HOMA-B = 20 \times \text{fasting insulin } (\mu\text{IU/ml}) / \text{fasting glucose (mmol/ml)} - 3.5$ (15).

Ethical approval:

The study was approved by the Tamale Teaching Hospital Ethical Review Committee (THERC/24/02/23/01). All protocols followed were in accordance with the ethical requirements of the Ghana Health Service and the World Medical Association declaration of Helsinki (16). A written informed consent was obtained from each study participant.

Data analysis:

The Statistical Package for the Social Sciences (SPSS Inc., Chicago, USA) version 17.0 software was used for the data analysis. Data were log transformed for improved nor-

mality of distribution and presented as mean \pm standard deviation. Mean levels of indices between COVID-19 exposed and non-exposed groups were compared with independent sample *t*-test. Bivariate correlation was used to examine linear relationship among measured indices in each study group followed by multiple stepwise linear regression analyses to identify predictors of indices of interest. In all analyses, $p < 0.05$ was applied for the determination of statistical significance.

Results:

The study involved a total of 78 malaria patients made up of 38 COVID-19 exposed and 40 COVID-19 unexposed individuals. The COVID-19 exposed malaria patients were confirmed to have recovered completely from COVID-19 by the approved protocol at least six months before the commencement of the study while the unexposed group had no history of COVID-19 infection. Both groups had no history of diabetes or other health conditions known to influence any of the measured indices. The COVID-19 exposed participants were older ($p = 0.035$) with lower parasitaemia ($p = 0.025$), but higher mean levels of HOMAIR ($p = 0.049$), total cholesterol ($p = 0.042$) and triglycerides ($p = 0.001$) compared with their unexposed counterparts (Table 1).

Pearson bivariate correlational test revealed that parasitaemia correlated positively ($p < 0.05$) with insulin, insulin resistance, beta-cell function, fasting blood glucose, C-reactive protein, age, triglyceride and blood pressure in malaria patients with prior exposure to COVID-19. However, in participants without prior COVID-19 exposure, parasitaemia correlated positively ($r = 0.525$; $p = 0.001$) with C-reactive protein only (Table 2).

Low-density lipoprotein cholesterol (LDLc) correlated ($p < 0.05$) positively with insulin resistance, beta-cell function and fasting blood glucose in the COVID-19 unexposed participants only with no such observations in the group with prior exposure to COVID-19. Generally, the pattern of correlation was positive in both groups but the strength of the correlation differed between the two groups and the specific parameters that correlated also differed between the two groups for a number of the measured indices (Table 2).

Table 1: Comparison of mean levels of indices between COVID-19 exposed and COVID-19 unexposed patients with *falciparum* malaria in Tamale Teaching Hospital, Ghana

Parameter	COVID-19 exposed (n = 38)	COVID-19 unexposed (n = 40)	t	p value
Age (years)	35.23 ± 1.42	29.28 ± 1.40	2.156	0.035*
Parasites (μL^{-1})	112.00 ± 2.00	168.00 ± 2.00	-2.304	0.025*
Insulin (mIU/L)	26.17 ± 2.02	19.51 ± 1.74	1.868	0.066
HOMAIR	5.39 ± 2.40	3.70 ± 1.86	2.006	0.049*
HOMAB (%)	108.86 ± 1.78	91.20 ± 1.70	1.277	0.206
FBG (mmol/L)	4.63 ± 1.24	4.27 ± 1.13	1.967	0.054
TCHOL (mmol/L)	3.18 ± 1.42	2.57 ± 1.57	2.077	0.042*
LDL (mmol/L)	2.78 ± 0.81	3.40 ± 0.96	1.013	0.315
HDL (mmol/L)	1.48 ± 1.48	1.35 ± 1.49	0.96	0.341
CRP	1.39 ± 2.70	0.95 ± 1.70	1.996	0.05
Triglyceride (mmol/L)	1.40 ± 1.45	0.96 ± 1.54	3.645	0.001*
Body mass index (kg/m ²)	24.30 ± 1.08	23.83 ± 1.19	0.544	0.588
Waist-to-hip ratio	0.90 ± 1.02	0.88 ± 1.06	1.621	0.11
Waist-to-height ratio	0.45 ± 1.02	0.44 ± 1.19	0.612	0.543
Waist circumference (cm)	74.79 ± 1.13	71.34 ± 1.24	1.031	0.307
Systolic BP (mmHg)	126.14 ± 1.08	126.76 ± 1.12	-0.196	0.845
Diastolic BP (mmHg)	78.77 ± 1.12	80.34 ± 1.15	-0.602	0.549

* = statistically significant at $p < 0.05$; LDL=low-density lipoprotein cholesterol; HDL=high-density lipoprotein cholesterol; BP = blood pressure; CRP = C-reactive protein; TCHOL = total cholesterol; FBG = fasting blood glucose; HOMAIR = homeostatic model of insulin resistance; HOMAB = homeostatic mode of beta cell function

In subsequent stepwise multiple linear regression analyses controlling for appropriate confounders, parasitaemia was predicted (Adjusted $R^2=0.751$; $p=0.031$) jointly by the levels of beta-cell function, C-reactive protein and triglycerides, with the model explaining about 75% of the observed variation in participants with prior exposure to COVID-19 (Table 3). However, in the control COVID-19 unexposed group, parasitaemia could only be predicted (Adjusted $R^2=0.245$; $p=0.002$) by C-reactive protein with the model explaining just about a quarter of the observed variation of parasitaemia in that study group (Table 4).

Interestingly, in both study groups, variation in insulin level could be fully explained by fasting glucose and insulin resistance levels (Adjusted $R^2 = 1$, $p < 0.001$ for both groups, Tables 3 & 4). Similarly, levels of fasting blood glucose, insulin resistance and beta cell function could generally be predicted to perfection or almost perfection by the same set of independent variables in both study groups (Adjusted R^2 range = 0.911-1.0, $p < 0.001$, Tables 3 & 4). Total cholesterol level was however, predicted by age (Adjusted $R^2 = 0.439$, $p < 0.001$, Table 3) only in

the COVID-19 exposed group with the model being able to explain just about 40% of the observed variation. Systolic and diastolic blood pressures partially predicted each other to a similar extent in both groups but the final models included different parameters in each of the study groups.

Discussion:

Diabetes mellitus is postulated to exert a bidirectional relationship with COVID-19. This implies that DM patients have poorer COVID-19 outcome on the one hand and the risk of developing DM is increased by COVID-19 at the other end. This bidirectional relationship poses a challenge to elucidation of the role of other infectious agents in the development of DM in individuals who have recovered from COVID-19. However, in malaria-endemic regions of the globe, malaria remains a major threat to health before, during and after the COVID-19 pandemic (17). This comparative cross-sectional observational study sought to examine the probable role of malaria in the evolution of T2DM post COVID-19 by comparing levels of selected markers of insulin resistance, beta-cell function,

Table 2: Bivariate correlations of measured parameters in COVID-19 exposed and COVID-19 unexposed participants

Parameter	COVID-19 exposed participants		Parameter	COVID-19 unexposed participants	
	Correlation coefficient	p value		Correlation coefficient	p value
Parasitaemia & Insulin	0.81	<0.001	Parasitaemia & C- reactive protein	0.525	0.001
Parasitaemia & Insulin resistance	0.781	<0.001	Insulin & Age	0.503	0.002
Parasitaemia & beta cell function	0.817	<0.001	Insulin & fasting blood glucose	0.489	0.003
Parasitaemia fasting glucose & blood	0.543	0.002	Insulin & Diastolic blood pressure	0.476	0.003
Parasitaemia & Age	0.437	0.003	Insulin resistance & Age	0.498	0.002
Parasitaemia & Triglyceride	0.436	0.021	Insulin resistance & Fasting blood glucose	0.628	<0.001
Parasitaemia & Systolic blood pressure	0.433	0.021	Insulin resistance & LDL	0.49	0.002
Parasitaemia & Diastolic blood pressure	0.41	0.03	Insulin resistance & Diastolic blood pressure	0.488	0.003
Parasitaemia & C- reactive protein	0.772	<0.001	Beta cell function & Diastolic blood pressure	0.424	0.01
Insulin & Age	0.594	0.001	Beta cell function & LDL	0.431	0.009
Insulin & fasting blood glucose	0.771	<0.001	Beta cell function & Age	0.516	0.001
Insulin & Systolic blood pressure	0.42	0.026	Fasting blood glucose & LDL	0.368	0.027
Insulin & Diastolic blood pressure	0.475	0.011	Fasting blood glucose & Diastolic blood pressure	0.331	0.048
Insulin & C-reactive protein	0.705	<0.001			
Insulin resistance & Age	0.615	<0.001			
Insulin resistance & Fasting blood glucose	0.86	<0.001	Age & LDL	0.42	0.011
Insulin resistance & Systolic blood pressure	0.452	0.016	Age & Systolic blood pressure	0.363	0.029
Insulin resistance & Diastolic blood pressure	0.5	0.007	Age & Diastolic blood pressure	0.593	<0.001
Insulin resistance & C-reactive protein	0.698	<0.001	Total cholesterol & C-reactive protein	0.376	0.024
Beta cell function & Age	0.532	0.004	LDL & Diastolic blood pressure	0.472	0.009
Beta cell function & Fasting blood glucose	0.592	0.001			
Beta cell function & Diastolic blood pressure	0.411	0.03			
Beta cell function & C-reactive protein	0.681	<0.001			
Age & Fasting blood glucose	0.575	0.001			
Age & C-reactive protein	0.375	0.049			
Systolic blood pressure & C-reactive protein	0.452	0.016			

inflammation and lipid metabolism in malaria patients with prior exposure to COVID-19 to their counterparts without COVID-19 exposure in the Tamale metropolis of Ghana.

Our results show that, the COVID-19 exposed individuals were older with higher levels of insulin resistance, total cholesterol and triglycerides but lower level of parasitaemia than their counterparts without prior exposure to COVID-19. Although COVID-19 virtually affects all ages, available data points to a higher proportion of the affected in the

adult age group (18,19). This is to be expected when viewed from the perspective of immunosenescence with the effectiveness of immunological responses to various challenges decreasing with age (20). Therefore, the higher age of COVID-19 exposed participants compared with their unexposed counterparts in the current study is consistent with findings of previous reports (18,19). It suggests that the younger COVID-19 unexposed group could mount a more effective immunological response needed for COVID-19 prevention.

Table 3: Multiple linear regression analyses for predictors of measured indices in COVID-19 exposed participants

Parameter	Predictor(s)	Unstandardized coefficients Beta (std error)	Standardized coefficients Beta	Adjusted R ²	p-value
Parasitaemia	Constant	1.618 (0.086)			
	HOMAB	0.606 (0.189)	0.45	0.621	<0.001
	CRP	0.309 (0.105)	0.4	0.703	0.013
	TG	0.494 (0.214)	0.249	0.751	0.031
Insulin	Constant	3.114 (0.000)			
	HOMAIR	1.000 (0.000)	1.251	0.974	<0.001
	FBG	-1.000 (0.000)	-0.307	1.000	<0.001
HOMAIR	Constant	-3.114 (0.000)			
	Insulin	1.000 (0.000)	0.799	0.974	<0.001
	FBG	1.000 (0.000)	0.245	1.000	<0.001
HOMAB	Constant	2.915 (0.006)			
	Insulin	1.033 (0.002)	1.27	0.934	<0.001
	FBG	-1.041 (0.006)	-0.393	1.000	<0.001
FBG	Constant	3.114 (0.000)			
	HOMAIR	1.000 (0.000)	4.078	0.727	<0.001
	Insulin	-1.000 (0.000)	-3.259	1.000	<0.001
TCHOL	Constant	-1.332 (0.534)			
	Age	0.757 (0.149)	0.725	0.439	<0.001
Systolic BP	Constant	2.937 (0.367)			
	DBP	0.464 (0.081)	0.673	0.551	<0.001
	WHR	1.188 (0.302)	0.366	0.67	0.005
Diastolic BP	Constant	-1.392 (0.853)			
	SBP	1.165 (0.175)	0.805	0.55	<0.001
	TCHOL	0.11 (0.038)	0.346	0.657	0.009

HOMAIR = homeostatic model of insulin resistance; HOMAB = homeostatic mode of beta cell function; FBG = fasting blood glucose; DBP = diastolic blood pressure; SBP = systolic blood pressure; TCHOL = total cholesterol; TG = triglyceride; CRP = C-reactive protein.

Table 4: Multiple linear regression analyses for predictors of measured indices in the COVID-19 unexposed participants

Dependent variable	Independent variable(s)	Unstandardized coefficients beta (std error)	Standardized coefficients beta	Adjusted R ²	p value
Parasitaemia	Constant	5.171 (0.099)			
	CRP	0.633 (0.185)	0.518	0.245	0.002
Insulin	Constant	3.114 (0.000)			
	HOMAIR	1.000 (0.000)	1.123	0.97	<0.001
	FBG	-1.000 (0.000)	-0.219	1.000	<0.001
HOMAIR	Constant	-3.114 (0.000)			
	Insulin	1.000 (0.000)	0.891	0.97	<0.001
	FBG	1.000 (0.000)	0.195	1.000	<0.001
HOMAB	Constant	3.085 (0.331)			
	Insulin	1.007 (0.057)	1.052	0.866	<0.001
	FBG	-1.075 (0.259)	-0.246	0.911	<0.001
FBS	Constant	3.114 (0.000)			
	HOMAIR	1.000 (0.000)	5.118	0.373	<0.001
	Insulin	1.000 (0.000)	-4.559	1.000	<0.001
Systolic BP	Constant	1.774 (0.349)			
	DBP	0.763 (0.086)	0.947	0.588	<0.001
	TCHOL	-0.071 (0.027)	-0.271	0.671	0.005
	HOMAB	-0.047 (0.023)	-0.218	0.702	0.048
Diastolic BP	Constant	-0.238 (0.503)			
	SBP	0.713 (0.127)	0.575	0.588	<0.001
	WC	0.467 (0.09)	0.703	0.745	<0.001
	BMI	-0.259 (0.108)	-0.331	0.779	0.023

HOMAIR = homeostatic model of insulin resistance; HOMAB = homeostatic mode of beta cell function; FBS = fasting blood sugar; DSB = diastolic blood pressure; SBP = systolic blood pressure; TCHOL = total cholesterol; BMI = body mass index; WC = waist circumference; CRP = C-reactive protein.

However, both groups of participants succumbed to the malarial disease, implying that, no group could mount an adequate immunological response to prevent the establishment of the disease in relation to malaria.

Indeed, parasitaemia correlated positively with almost every evaluated risk index of T2DM development in the COVID-19 exposed group as far as this study is concerned but in the COVID-19 unexposed group, only C-reactive protein correlated positively with parasitaemia. This observation points to the probable critical role of malaria in promoting risk of T2DM in individuals who have recovered from COVID-19 compared with their counterparts who did not get infected with the COVID-19 disease. This finding is further buttressed by the results of the stepwise multiple linear regression analyses that revealed C-reactive protein, triglycerides and beta-cell function as predictors for parasitaemia in the COVID-19 exposed group with the final model accounting for about 75% of the observed variation in parasitaemia. This is in sharp contrast to the linear regression analysis results of the COVID-19 unexposed group that showed only C-reactive protein as a predictor of parasitaemia with the model explaining just 24.5% of the observed variation in parasite levels. The higher number of predictors of parasitaemia coupled with the superior nature of the model in accounting for the variation in parasitaemia for the COVID-19 exposed group suggests that the risk of developing T2DM in this group is higher than in their unexposed counterparts.

Additionally, this observation points to a probable heightened future burden of T2DM in malaria-endemic regions of the globe because of the COVID-19 pandemic. This is because multiple bouts of malaria are highly possible in one's lifetime (21), especially for individuals residing in malaria-endemic regions of the globe (22). Therefore, with multiple episodes of malaria, the secretory function of the beta-cell can be unduly heightened, beyond sustainable level and result in eventual exhaustion and the consequent decline in insulin levels to favour hyperglycaemia as observed in diabetes (23,24).

Although direct evidence of beta-cell failure through exhaustion is non-existent in the current study, a critical examination of the HOMAB values points to some hypersecretory activity in the COVID-19 exposed group but a relatively reduced beta-cell secretory function for the COVID-19 unexposed group in spite of the statistically comparable HOMAB values of the two study groups. The relatively reduced secretory function of beta cells in the COVID-19 unexposed participants may suggest reduced beta-cell mass due probably to inflammation-induced apoptosis (25). This increases the risk

of those participants to the development of T2DM in the future when metabolic demand on the beta cells increases through obesity, multiple bouts of malaria and other environmental factors. Thus, in both groups of participants, the risk of T2DM development in future through beta-cell failure will increase if metabolic load and bouts of malaria increase (23,24).

Indeed, in both groups of participants, insulin resistance was clearly established during malaria in support of earlier findings except that the degree of insulin resistance observed for COVID-19 exposed group was significantly higher than their COVID-19 unexposed counterparts (5,26). This observation of a clear establishment of insulin resistance in the current study further points in the direction of increased risk for future development of T2DM. A study on Sprague-Dawley rats to examine the nature of *Plasmodium berghei*-induced insulin resistance demonstrated that effective clearance of the infectious agent resulted in a reduced insulin resistance (27). However, in that study, a second episode of infection, at a relatively lower parasitaemia, induced insulin resistance comparable to the level of the first episode (27).

In humans, several episodes of malaria are possible in a lifetime, implying that at every episode of malaria, comparable level of insulin resistance could be established at a rather relatively reduced parasite level than the previous episode to a point that even at asymptomatic level of infection, insulin resistance could be present. This is a challenge because such individuals may remain undetected for treatment due to their milder symptoms and therefore continue to maintain their insulin resistant and low-grade inflammatory state for adequate duration to pave way for possible development of T2DM under favourable conditions such as increased metabolic demand caused by nutrients overload (23, 24).

Dyslipidaemias of various components of the lipid profile have been associated with T2DM development or its complications in a number of studies (28-30). The components of lipid profile include total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and triglycerides, and abnormal levels of any of these components poses a risk to cardiovascular health (28-30). In the current study, dyslipidaemia was not detected in the participants, probably due to the relatively younger age compared to previous studies (28-30), coupled with the relatively small sample size. However, the total cholesterol and triglycerides components of the lipid profile in this study differed between the two groups; with the COVID-19 exposed group demonstrating significantly higher

levels compared to their unexposed counterparts although the levels fell within the normal range. The normal levels of the components of lipid profile could easily be considered as indicative of low risk to the development of T2DM. However, a recent large sample prospective longitudinal study in the Netherlands (31), has demonstrated that even in healthy individuals without metabolic syndrome, raised triglycerides levels within the normal range increased the risk of development of T2DM. Considering that, the participants in the current study appear younger, the observed normal lipid profile could still represent a critical risk to T2DM development in the future in the context of the findings by Szili-Torok et al., (31) because of the likelihood of increase in the triglyceride component of the lipid profile as one ages. Therefore, the older COVID-19 exposed group with higher triglycerides level appear more vulnerable to developing T2DM than their counterparts without prior exposure to COVID-19 in future if triglyceride levels rise within the normal range with time in line with the postulation of Szili-Torok et al (31). Above all, the association of total cholesterol with blood pressure in the study participants further supports this view of increased risk of lipid-driven future development of T2DM in the current study.

Our study is not without limitations. Firstly, being a cross-sectional study, causality could not be established. Secondly, the sample size appears relatively small making it difficult for undue generalization of findings. However, the current sample size is enough for statistical analysis and extrapolation of findings to populations that are very similar in characteristics to those of the current study under similar disease conditions, for a proof-of-concept study of this nature. Thus, our evidence of malaria association with increased diabetogenic risk in clinically exposed COVID-19 participants compared with their unexposed counterparts, is a worthy baseline information with implications for the evolution of T2DM in malaria-endemic regions of the globe.

Conclusion:

Falciparum malaria is associated with increased risk for development of T2DM irrespective of COVID-19 exposure through the association of inflammation with insulin resistance and beta-cell secretory function.

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Contributions of authors:

SA, LDK, FP, KD, ASB, PN, and JS contributed to conception, design and the conduct of the study; RA and EWW were responsible for sample selection, data collection and laboratory analysis; SA, LDK, FP, KD, ASB, PN, JS, EAB, RA and EWW were involved in data analysis and drafting of manuscript; SA, LDK, FP, KD, ASB, EAB, PN, and JS revised the manuscript for important intellectual contents. All authors read and approved the final manuscript.

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