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# NLRP3 Gene Polymorphisms and Association with Type 2 Diabetes mellitus and Malaria Co-morbidity in Yaounde, Cameroon

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**Abstract:** *Background:* Diabetes is increasingly prevalent in malaria endemic settings like Cameroon thus contributing to a double burden in the management of these inflammatory diseases. Studies have shown that NLRP3 inflammasome plays a key role in type-2 diabetes (T2DM) and malaria induced inflammation. However, the hypothesis that the Single Nucleotides Polymorphisms (rs10754558 and rs4612666) in the NLRP3 gene could be associated with T2DM and malaria comorbidity is relatively new. This study aimed at determining the association between NLRP3 rs10754558 and rs4612666 Single Nucleotide Polymorphisms with susceptibility to Type 2 Diabetes mellitus and malaria comorbidity in Yaoundé, Cameroon. *Methods:* A case-control study was performed on 100 conveniently collected blood samples, spotted on Whartman N$^{o}$ 3 filter paper from which DNA was extracted by the chelex-100 boiling method. Nested-PCR was used to confirm the presence of malaria and speciate *Plasmodium spp*. Genotyping of the NLRP3 gene SNPs was performed using Polymerase Chain Reaction and Restriction Fragment Length Polymorphism (PCR-RFLP). The Chi-square test ($X^2$) was used to establish associations. A P-value of <0.05 was considered significant. *Results:* The mean age of the study population was 55±12.38 years. Eighty-eight (88) participants were diagnosed with T2DM, whereof 7 (7.95%) were ascertained by nested-PCR to harbour malaria; *P. falciparum* being the dominant circulating species. The most predominant genotype and allele for rs10754558 and rs4612666, was the heterozygous genotype GC and wildtype allele G (52.00%, 69.00%), and the homozygous mutant genotype CC and

mutant allele C (63.00%, 76.50%) respectively. No statistical significance was found between the comorbid group and diabetes positive /malaria negative (D+M-) control group for the rs10754558 and rs4612666 SNPs. Statistical significance was found between the comorbid group and the diabetes negative/ malaria positive (D-M+) control group for the rs4612666 SNP. Individuals possessing the CC genotypes were 8 times more susceptible to diabetes and malaria comorbidity (OR=8.000, P=0.043), whereas individuals possessing the TC genotype were less susceptible (OR=0.079, P=0.030). *Conclusion:* An association was found between the NLRP3 rs4612666 SNP and susceptibility to Type 2 Diabetes mellitus and malaria comorbidity in our study.

**Keywords:** NLRP3, Gene Polymorphism, Type 2 Diabetes Mellitus, Malaria, Co-morbidity, Susceptibility

# 1. Introduction

The situation of diabetes and malaria in Africa and Cameroon is very disturbing especially with increasing incidence of non-infectious diseases like type 2 diabetes. It has been estimated that as the prevalence of diabetes keeps rising in malaria endemic areas, larger populations could be at high risk of malaria [1]. Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both [2]. Type 2 diabetes mellitus (T2DM) is characterized by inadequate production of insulin and inability of the body to respond fully to insulin [3]. In 2019, the International Diabetes Federation (IDF) estimated that 463 million in the world live with diabetes, representing 9.3% of adults between 20-79 years. The prevalence is predicted to increase to 700 million by 2045 if these trends continue. This is especially a concern in the Sub-Saharan Africa which had a prevalence of 19.4 million people in 2019 [4]. T2DM accounts for between 90% and 95% of diabetes, with increasing highest proportions in low and middle income countries (LMICS) [5]. Non-communicable diseases such as diabetes, obesity, hypertension, and cardiovascular disease, have increased globally, including malaria-endemic regions. Despite all measures to reduce the prevalence and incidence of malaria, it still remains a major Public health problem [6].

Malaria is endemic in Africa and Cameroon. It is a vector-borne infection caused by a parasite *Plasmodium* sp. Five species are known to infect humans: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae* and *Plasmodium knowlesi* which is a zoonotic parasite, with *Plasmodium falciparum* being the most severe and fatal in Africa. Malaria is transmitted by the female *Anopheles* mosquito [7, 8]. Like diabetes mellitus, malaria continuous to remain a major public health problem despite efforts put in prevention and elimination. The WHO malaria report 2020 declares that in 2019 an estimated 229 million cases of malaria in 87 malaria endemic countries and 409000 deaths recorded with the most vulnerable group being children under the age of 5 and the African region accounted for 94% of all malaria deaths [9]. Diabetes and malaria are co-prevalent in most settings. Studies have shown that malaria parasitaemia was more common in people with diabetes and poor glycaemic control [10, 11]. Hyperglycaemia has also been shown to be associated with malaria [12]. T2DM and malaria are related to inflammatory processes which often lead to activation of protein complexes like the inflammasomes.

Inflammasomes are a family of large cytosolic multiprotein complexes that assemble in response to infection (pathogen-associated molecular patterns, PAMPs) or stress-associated stimuli (damage-associated molecular patterns, DAMPs) and lead to the activation of caspase-1-mediated innate immune responses [13]. Among the inflammasomes, the NLRP3 inflammasome is the most clinically implicated one and it is composed of NLRP3, the adaptor protein ASC (apoptosis-associated speck like protein containing a CARD) and caspase. Assembly of the inflammasome complex leads to activation of caspase-1 which triggers pyroptosis and the maturation and release of proinflammatory cytokines IL-1β and IL-18 [14]. While hemozoin and plasmodial DNA have been found to be potent activators of the NLRP3 inflammasome, plasmodial DNA is mostly detected in AIM2 inflammasome [15, 16].

The increasing multi-morbidities in the world and particularly the Low- and middle-income countries (LMICS) warrants the need to study diseases as clusters rather than isolate entities [17, 18]. New links between diseases could signal innovative prevention paths and generate knowledge which could also provide relevant evidence on treatment and management. There have been suggestions of a link between the NLRP3 inflammasome and insulin resistance, obesity, circulating immune markers, immunogenetic susceptibility, macrophage function, and chronic inflammation [19]. Inflammasome activity is critical for the control of invading pathogens but hyperactivation of the inflammasome may lead to tissue damage [14]. Altered function of the NLRP3 inflammasome or hyper production of inflammatory cytokines as a result of genetic variations, particularly gain-of- function genetic mutations have been shown to be linked to various inflammatory diseases, including obesity, insulin resistance, and T2DM [20–23]. Even though there is evidence about the increasing co-morbidity between diabetes and infectious diseases [18, 24], the concept that NLRP3 gene polymorphisms could be associated to diabetes and malaria co-morbidity is relatively new. Therefore, looking at unknown associations between non-communicable diseases like diabetes (with an increasing incidence in malaria endemic regions) and malaria seems relevant [17, 18]. Hence, the aim of this study was to investigate the SNPs (rs10754558 and rs4612666) of the NLRP3 gene and establish a possible

association with susceptibility to T2DM and malaria co-morbidity in Yaounde, Cameroon.

# 2. Materials and Methods

### *2.1. Study Setting and Location*

This study was carried out in Yaoundé, the capital of the Centre Region in Cameroon (3°51′ N 11°29′ E) the second largest city of Cameroon with a population of more than 4 million. It contains people from all works of life and people from all other 10 regions of the country. The city is located within the Congo-Guinean phytogeographic zone characterized by a typical equatorial climate with two rainy seasons extending from March to June and from September to November [25].

### *2.2. Study Population and Sampling*

A total of 100 participants were enrolled into the study. A detailed explanation of the study and its potential benefits was given to the participants who were then invited to participate in the study by using an information sheet. After the informed consent has been signed the participants were then enrolled in the study. An appointment was then fixed with the enrolled patients at the National Obesity Centre of the Yaounde Central Hospital. The patients were requested to fast for 8 hours over night prior to their day of appointment. Anthropometric parameters and fasting plasma glucose levels were gotten. A rapid diagnostic test (RDT), SD BIOLINE Malaria Ag *P. falciparum* was carried out by pricking the lateral surface of the ring fingers' tip and 5µl of capillary blood was placed in the round specimen well of the RDT. 4 ml of whole blood was collected in EDTA tubes from peripheral venepuncture of each participant. Part of the whole blood was used to prepare thick and thin films for malaria microscopy and the remainder centrifuged at 8000-rpm for 10 min, 50µl of the RBC pellets were spotted on Whatman 3MM® filter papers for DNA conservation and then stored in sealed dry bags containing silica gels for further molecular analysis.

*Calculation of Parasite Density*

Parasite density was calculated by multiplying the number of parasites counted per microscopic field, and dividing it by the number of white blood cell count of each. If on counting 200 WBC, the number of parasites is not up to 100, the count was continued till 500 WBC and calculations made to get the exact parasitaemia of the patient. Results were reported in (p/ul). At least 10 fields were explored before confirming a slide to be negative. Two microscopists viewed the slides and a third confirmed them for quality control. Parasitaemia was calculated using the formula below.

$$\text{Parasites per µl} = \frac{\text{Number of parasites counted} \times 8000}{\text{Number of WBCs counted}}$$

### *2.3. DNA Extraction*

Total DNA was extracted from dried blood spot on filter papers using Chelex boiling method as described by Plowe et al [26]. This method is based on the disruption of the cell membrane using saponin. Proteins and other molecules are washed out by Phosphate Buffered Saline (1XPBS) and when heated, chelex which is a cationic resin, chelates all cations (with highest affinity for divalent metal ions and transition metal), and leaves the negatively charged DNA in solution [27], which was later stored at $20^{O}$C until molecular analysis.

### *2.4. Genotyping*

#### *2.4.1. Malaria Parasite Speciation by Polymerase Chain Reaction (PCR)*

Amplification was done by Nested PCR, adapted from a previously described protocol by Georges *et al,* [28]. The primer sequence for the gene fragments targeted are shown in (Table 1). Amplification of target gene fragments was performed using a T3 Thermocycler (Biometra, UK). All PCR reactions were performed in a total master mix of 20 µl consisting of Nuclease free water, 10 µl One Taq® Hot Start 2X Master Mix with standard buffer (New England Biolabs, MA, USA.), 0.4 µM of each primer and 2 µl of DNA extract. Cycling conditions for the outer PCR was 95°C for 5 min; 25× [94°C for 1 min; 58°C for 1 min; and 72°C for 2 min]; 72°C for 5 min and 95°C for 5 min; 30× [94°C for 1 min; 58°C for 1 min; and 72°C for 2 min]; 72°C for 5 min for the inner PCR. The amplicons after amplification if not immediately used was stored at 4°C. PCR Products were run on a 2% agarose stained with Ethidium bromide and visualized under UV light.

#### *2.4.2. Genotyping for NLRP3 Gene Polymorphisms*

Genotyping Single Nucleotide Polymorphisms (SNPs); rs10754558 and rs4612666 in the NLRP3 gene was done using the Polymerase Chain Reaction and Restriction Fragment Length Polymorphism (PCR–RFLP) technique, adapted from a previously described protocol by Bai *et al.,* [23]. The sequence of primers of gene fragments targeted were as follows; for rs10754558, (UTR-F):5'-TGCTTAAGGCCATTAATTGTG3';(UTR-R):5'CTCCACC ATGGACAAGGAAG-3': for rs4612666, (IN7-F):5'-CAGGACAATGACAGCATCGGGTGT-TGAT-3'; (IN7-R):5'-GCTGCCATAAAATTTCAACATAA-3'. Each PCR was carried out in a total master mix of 20 µl consisting of Nuclease free water, 10 µl One Taq® Hot Start 2X Master Mix with standard buffer (New England Biolabs, MA, USA.), 0.5 µM of each primer and 2 µl of DNA extract. Amplification of target gene fragments was performed using a T3 Thermocycler (Biometra, UK). The PCR protocol was as follows: pre-denaturation (95°C for 3mins), denaturation (95°C for 30secs), annealing (56.1°C for 30secs), elongation (72°C for 60secs), amplified for 35 cycles and a final elongation of 72°C for 7mins to terminate all reactions. The amplicons after amplification if not immediately used was stored at 4°C. Digestion for NLRP3 (rs10754558 and rs4612666) was done with BpiI [Genotypes, GG: 260bp; GC: 260bp, 158bp, 102bp; CC: 158bp, 102bp] and MboI [Genotypes, TT: 261bp; TC: 261bp, 236bp, 25bp; CC: 236bp,

25bp] restriction enzymes respectively, according to a previously described protocol by Bia *et al*. [23]. All products were run on a 2% agarose stained with Ethidium bromide and visualized under UV light.

***Table 1.*** *Primers sequences for Malaria Parasite Speciation.*

| Species | Primer Identity | Sequence (5'→3') | Amplicon Size (bp) |
|---|---|---|---|
| *Plasmodium spp.* | rPLU1 | TCAAAGATTAAGCCATGCAAGTGA | 1600-1700 |
| | rPLU5 | CCTGTTGTTGCCTTAAACTTC | |
| *P. falciparum* | rFAL1 | TTAAACTGGTTTGGGAAAACCAAATATATT | 206 |
| | rFAL2 | ACACAATGAACTCAATCATGACTACCCGTC | |
| *P. malariae* | rMAL1 | ATAACATAGTTGTACGTTAAGAATAACCGC | 145 |
| | rMAL2 | AAAATTCCCATGCATAAAAAATTATACAAA | |
| *P. ovale* | rOVA1 | ATCTCTTTTGCTATTTTTTAGTATTGGAGA | 226 |
| | rOVA2 | ATCTAAGAATTTCACCTCTGACATCTG | |
| *P. vivax* | rVIV1 | CGCTTCTAGCTTAATCCACATAACTGATAC | 121 |
| | rVIV2 | ACTTCCAAGCCGAAGCAAAGAAAGTCCTTA | |

P: Plasmodium; *spp*: species; r: ribosomal

### *2.5. Statistical Analysis*

Data from this study were transcribed from laboratory worksheet records unto Microsoft Excel, version 2016. Descriptive statistics, percentage rates, and frequencies were used to describe the socio- demographic and clinical data. Allele frequencies were calculated using the Hardy-Weinberg formula. Data were analysed using the IBM SPSS biostatistics version 20.0 software (SPSS, Chicago, IL). Chi Square test ($X^2$ test) was used to establish associations between variables. Where the number of expected observations was less than 5, the Fisher's test was used. The Odds ratio was evaluated using a confidence interval of 95% A $p < 0.05$ was considered significant in all comparisons.

### *2.6. Ethical Consideration*

This study received ethical approval from the Regional Ethics Committee for Human Health Research (CRECRHH) under the ethical clearance document number CE $N^0$0065/CRECRHC/2018. Prior to participant enrolment, written and signed consent from each participant was obtained. The potential risks and benefits risks and benefits as well as data privacy and confidentiality were explained to all participants. Only those who signed the informed consent form were included in the study.

## 3. Results

### *3.1. Distribution of Demographic and Clinical Characteristics of the Study Participants*

Out of the 100 participants enrolled in this study, 62% (62/100) were women and 38% (38/100) were men. The mean age was 55±12.38 years, the mean BMI was 29.90, the mean Temperature 36.80°C. This is summarized in (Table 2) below. 88 participants were confirmed to be diabetic after fasting plasma glucose (FPG) and glycated haemoglobin (HbA1C) measurements.

### *3.2. The Frequency of Malaria in the Study Population*

The frequency of malaria infection established by RDT, microscopy and nested PCR were 17% (17/100), 4% (4/100) and 12% (12/100) respectively. Malaria was diagnosed among the diabetic patients using RDT, Microscopy and PCR. The frequency of malaria amongst the 88 diabetic patients using PCR, light microscopy and HRP2-RDT were as follows (7/88, 7.95%), (1/88, 1.14%) and (5/88, 5.68%) respectively. The circulating malaria species among diabetic patients detected by PCR as mono infection were *Plasmodium falciparum* (6/7, 85.71%) and *Plasmodium malariae* (1/7, 14.29%). No mixed infection was seen. *Plasmodium ovale* and *Plasmodium vivax* were not detected.

***Table 2.*** *Demographic distribution and clinical characteristics of the study participants.*

| Variable | | Population N=100 |
|---|---|---|
| Age (Mean±SD) | | 54.86±12.38 |
| Gender | Male | 38 |
| | Female | 62 |
| BMI (Mean±SD) | Obese | 37.11±11.81 |
| | Overweight | 27.98±1.42 |
| | Normal | 22.55±2.02 |
| Temperature (Mean±SD) | | 36.76±0.66 |
| SBP (Mean±SD) | High | 153.27±18.27 |
| | Normal | 131.71±2.39 |
| | Low | 115.75±9.75 |
| DBP (Mean±SD) | High | 95.08±6.87 |
| | Normal | 82.36±1.76 |
| | Low | 70.78±6.87 |
| Pulse (Mean±SD) | | 92.43±92.75 |

SD= Standard deviation, BMI=Body mass index, DBP=Diastolic blood pressure, SBP=Systolic blood pressure

### *3.3. Genotype and Allele Frequency of NLRP3 rs10754558 and rs4612666 SNP*

The rs10754558 single nucleotide polymorphism revealed the distribution of GG (4, 40.00%), GC (5, 50.00%) and CC (1, 10.00%) for the comorbid group (D+M+) and GG (33, 42.31%), GC (42, 53.85%) and CC (3, 3.85%) in the diabetes positive and malaria negative (D+M-) control group (Table 3). A distribution of GG (7, 58.33%), GC (5, 41.67%) and CC (0, 0.00%) was observed in the diabetes negative and malaria positive (D-M+) control group (Table 4). Also, the rs4612666 single nucleotide polymorphism revealed the distribution of

TT (1, 10.00%), GC (1, 10.00%) and CC (8, 80.00%) for the comorbid group (D+M+) and TT (8, 10.26%), TC (19, 24.36%) and CC (51, 65.38%) in the diabetes positive and malaria negative (D+M-) control group (Table 3). A distribution of TT (1, 8.33%), TC (7, 58.33%) and CC (4, 33.33%) was observed in the diabetes negative and malaria positive (D-M+) control group (Table 4).

### *3.4. Association Between NLRP3 Gene Polymorphisms and Susceptibility to Diabetes and Malaria Comorbidity*

Results from the association analysis showed no statistical significance between the comorbid group and diabetes positive /malaria negative (D+M-) control group for the rs10754558 and rs4612666 SNPs. Individuals possessing the homozygous mutant genotype for both polymorphisms showed a reduced risk of developing diabetes/malaria comorbidity (OR=0.360, P=0.388; OR=0.472, P=0.487 respectively) (Table 3). Statistical significance was found between the comorbid group and the diabetes negative/ malaria positive (D-M+) control group for the rs4612666 SNP. Individuals possessing the CC genotypes were 8 times more susceptible to diabetes and comorbidity (OR=8.000, P=0.043) where as individuals possessing the TC genotype were less susceptible (OR=0.079, P=0.030). The presence of the Wildtype allele T was found to be a risk factor for the development of diabetes and malaria comorbidity (OR=8.000, P=0.043) (Table 4).

***Table 3.*** *Association between rs10754558 and rs4612666 SNPs and susceptibility to diabetes and malaria co- morbidity (Case=D+M+, Control=D+M-).*

| NLRP3 | Case (D+M+) N=10 | Control (D+M-) N=78 | OR | 95%CI | P value |
|---|---|---|---|---|---|
| rs10754558 | | | | | |
| GG | 4 (40.00%) | 33 (42.31%) | 1.100 | 0.287-4.212 | 1.000 |
| GC | 5 (50.00%) | 42 (53.85%) | 1.167 | 0.313-4.355 | 1.000 |
| CC | 1 (10.00%) | 3 (3.85%) | 0.360 | 0.034-3.844 | 0.388 |
| Allele | | | | | |
| G | 13 (65%) | 108 (69.23%) | 4.222 | 0.347-51.332 | 0.307 |
| C | 7 (35%) | 48 (30.77%) | 1.100 | 0.287-4.211 | 1.000 |
| rs4612666 | | | | | |
| TT | 1 (10.00%) | 8 (10.26%) | 1.029 | 0.115-9.206 | 1.000 |
| TC | 1 (10.00%) | 19 (24.36%) | 2.898 | 0.345-24.382 | 0.444 |
| CC | 8(80.00%) | 51(65.38%) | 0.472 | 0.094-2.382 | 0.487 |
| Allele | | | | | |
| T | 3 (15.00%) | 35 (22.44%) | 0.472 | 0.094-2.382 | 0.487 |
| C | 17(85.00%) | 121 (77.56%) | 0.972 | 0.109-8.702 | 1.000 |

SNP= Single Nucleotide Polymorphism, OR= Odds Ratio, level, CI= confidence interval, P-value=statistical significance level

***Table 4.*** *Association between rs10754558 and rs4612666 SNPs susceptibility to diabetes and malaria co- morbidity (Case=D+M+, Control=D-M+).*

| NLRP3 | Case (D+M+) N=10 | Control (D-M+) N=12 | OR | 95%CI | P value |
|---|---|---|---|---|---|
| rs10754558 | | | | | |
| GG | 4(40.00%) | 7(58.33%) | 0.476 | 0.086-2.628 | 0.669 |
| GC | 5(50.00%) | 5(41.67%) | 0.714 | 0.132-3.868 | 1.000 |
| CC | 1(10.00%) | 0(0.00%) | - | - | 0.454 |
| Allele | | | | | |
| G | 13(65.00%) | 19(79.17%) | - | - | 0.454 |
| C | 7(35.00%) | 5(20.83%) | 2.100 | 0.381-11.589 | 0.669 |
| rs4612666 | | | | | |
| TT | 1(10.00%) | 1(8.33%) | 1.222 | 0.066-22.402 | 1.000 |
| TC | 1(10.00%) | 7(58.33%) | 0.079 | 0.008-0.843 | 0.030* |
| CC | 8(80.00%) | 4(33.33%) | 8.000 | 1.126-56.795 | 0.043* |
| Allele | | | | | |
| T | 3(15.00%) | 9(37.50%) | 8.000 | 1.127-56.795 | 0.043* |
| C | 17(85.00%) | 15(62.50%) | 0.818 | 0.045-14.996 | 1.000 |

SNP= Single Nucleotide Polymorphism, OR= Odds Ratio, level, CI= confidence interval, P-value=statistical significance level.

## 4. Discussion

Multiple genetic alterations have influenced inflammatory diseases contributing to disease susceptibility. NLRP3 gene encoding protein, which belongs to cytoplasmic NLR receptor, is an important component of inflammasome. SNPs of NLRP3 greatly influence pathogenic challenges and lead to disease outcome, such as type 1 diabetes, primary gouty arthritis, cardiovascular diseases, and malignant tumours [29–32]. Variations of NLRP3 gene are prone to promoting systemic inflammation. Type 2 diabetes mellitus is a public health problem in Cameroon and is estimated that the prevalence of diabetes is 6%. So far, the identified factors correlated with type 2 diabetes mellitus are environment, ethnicity, family history and genetic mutation. Studies have revealed that occurrence of type 2 diabetes mellitus is closely associated with oxidative stress and chronic inflammation [33]. NLRP3 inflammasome has also been implicated in the pathogenesis of a number of complex diseases, including metabolic disorders

such as type 2 diabetes, Large Artery Atherosclerotic Ischemic Strokes and Micro embolic Signals, and in infectious diseases such as malaria [15, 23, 34, 35]. Malaria remains a public health threat in endemic regions like Cameroon with an incidence of 25.9%. Diabetes and malaria have a link as they are associated with altered immunity and inflammation. Inflammatory responses due to repeated malaria infections may lead to prolong physiological changes that increase susceptibility to diabetes [36]. A recent study has reported that the NLRP3 inflammasome SNPs are associated to type 2 diabetes [23].

In this study we found the G allele and the GC genotype of rs10754558 SNP to be the most prevalent with frequencies of 69.00% (69/100) and 52% (52/100) respectively. These results were similar to that reported by in 2016 by Bai *et al* [23]. These could be due to variety of NLRP3 activators in the diabetic condition and the uncontrolled urbanization and major changes in lifestyle which has led to reduced physical activity and unhealthy diets, and thus contribute to development of mutations within the population. These findings were contrary to that reported by Cheng *et al*, who found the C allele to be the most prevalent allele [34]. These differences could be attributed to ethnicity and environmental conditions. Also, the C allele and the CC genotype of rs4612666 SNP, were the most prevalent with frequencies of 76.50% (76.5/100) and 63.00% (63/100) respectively. These results are in line with that reported by Cheng *et al*, who found the C allele to be the most prevalent allele [34]. These similarities could be due to activation of NLRP3 by similar DAMPs like potassium efflux in inflammatory diseases. The mechanism of NLRP3 activation have been proposed to be involved in several molecular and cellular events, including K+ efflux, Ca2+ signalling, mitochondrial dysfunction, and reactive oxygen species (ROS) production [37]. Findings from this study were contrary to that reported by Bai *et al* who found the TC genotype to be the most prevalent with a frequency of 43.37% [23]. The differences in the results may be attributed to ethnicity and environmental factors.

Recently, studies have shown that malaria infection is significantly associated with poor glycaemic control and hyperglycaemia [11, 12]. Malaria infection has been shown to stimulate the promotion of inflammatory cytokines like Il-1β, due to induction of systemic inflammation [15, 38] and the activation of NLRP3 inflammasome [40]. Consequently, these inflammatory markers could be a cause or direct consequence of hyperglycaemia or diabetes [41]. Inflammatory responses to repeated infections with malaria parasites may lead to sustained physiological changes that increase susceptibility to diabetes [36].

In this study, we found that although there was an increased risk (OR=2.056, CI=0.207-20.461, P value=0.461) to the susceptibility of patients to co-morbidity with the CC genotype of rs10754558, there was no significant difference in the distribution of this genotype in the co-morbid vs D+M-. Likewise, in the co-morbid vs D-M+ there was no significant difference in the distribution of the C allele of rs10754558 (OR=2.100, CI=0.380- 11.588, P-value=0.395) and the GC genotype (OR=1.400, CI=0.259-7.582, P- value=1.000). These findings were similar to that reported by Cheng *et al* and not in line with that reported by Bai *et al,* who found the rs10754558 to be associated with development of type 2 Diabetes mellitus [23, 34]. This difference may be attributed to the variation in the ethnic origins, lifestyles and environmental factors of the study populations. Individuals possessing the CC genotypes of rs4612666 were 8 times more susceptible to Type 2 diabetes and malaria co-morbidity with a significant difference observed in the distribution of this genotype in the co-morbid vs D-M+ groups (OR=8.000, P=0.043). These results were similar to that reported by Cheng *et al* in 2018 [34]. These similarities could be due to mechanisms such as potassium efflux, extracellular ATP, translocation of lysosomal proteolytic contents, and cholesterol crystals deposits which are essential intermediate steps in activating NLRP3 inflammasome. Bai *et al*, reported no significant association between NLRP3 rs4612666 and type 2 diabetes mellitus in the Chinese population [23]. This difference could be attributed to the combined activation of NLRP3 inflammasome by DAMPs from diabetes and PAMPs in malaria infection like ROS, extracellular ATP, urate, hemozoin, glycosylphosphatidylinositol (GPI) and infected red blood cells. Abnormal activation of the NLRP3 inflammasome have been shown to contribute to inflammatory diseases including intestinal cancer, and autoinflammatory diseases such as keratitis/conjunctivitis [41, 42].

Further results from this study showed that, individuals possessing the TC genotype of rs4612666 were less susceptible to Type 2 Diabetes mellitus and malaria comorbidity, with a significant difference in the distribution of this genotype in the co-morbid vs D-M+ groups (OR=0.079, P=0.030). These results are not similar to that reported by Cheng *et al,* in which the TC genotype was not associated to large atherosclerotic stroke and micro embolic signals [34]. Ethnic differences may be a plausible explanation for the lack of association.

This study has a relative strength in shedding light on a link between NLRP3 genetic polymorphisms in the development of T2DM and malaria co-morbidity. Also, the possibility of a gene-gene or SNP-SNP interactions between polymorphisms may have a role in the pathogenesis of T2DM and malaria co-morbidity.

# 5. Conclusion

In summary, this study showed that the NLRP3 rs4612666 single nucleotide polymorphism was associated with susceptibility to Type 2 Diabetes mellitus and malaria comorbidity, and the T allele might be a risk factor for Type 2 Diabetes mellitus and malaria comorbidity. However, the limitation of the relatively small sample size should be noted. Thus, further studies with larger sample sizes should be conducted to confirm this conclusion.

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## Authors Contributions

WFM, JCM, AMN, MCVF, CFT contributed to the design of the study. ES, IMKW, JPKC, LNN coordinated the study. IMKW, MGF, REF, CHD, CNNT, PTNN supervised the sample collection. MCVF, CFT, RBL, JPKC, WOTN performed the molecular analysis. CFT, AHEM, WOTN, MCVF performed data analysis. MCVF, CFT drafted the manuscript. All authors contributed in the revision of the manuscript and approved the final version of the manuscript prior to submission.

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## Conflicts of Interest

The authors declare no conflicts of Interest.

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