Malaria among febrile neonates attending the neonatology unit of the Bamenda Regional Hospital

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Abstract
Malaria remains the leading cause of infant's mortality in malaria endemic countries like Cameroon. Due to the presence of passively acquired maternal antibodies, malaria in neonates was thought to be scarce. Consequently routine malaria checks are mostly not considered for febrile neonates. Nonetheless findings from malaria endemic areas have proven that malaria in neonates is not uncommon. This study is therefore designed to evaluate malaria among febrile neonates attending the neonatology unit of the Bamenda Regional Hospital. A structured questionnaire and laboratory diagnostic test methods were used for data collection. Maternal sociodemographic data and malaria predisposing factors for neonates with and without malaria were determined using sums and percentages of mean. Regression analysis was used to determine the effects of age of mother, parity, mother's educational level, sex of neonate and malaria predisposing factors on malaria parasitaemia. Chi-square (and Fisher’s exact) test was used to calculate sensitivity (%), specificity (%), predictive values (%), likelihood ratios, odd ratios, relative risk and attributable risk. A total of 189 neonates were included in the study out of which 33 (17.46%) tested malaria positive. 54.55% (18/33) of the malaria positive neonates were ˃3–4 weeks old, while only 09.09% (03/33) were within 0–1 week of age. Neonates who presented with both signs of "fever + refusal to feed" were found to be most likely (P = 0.0188) to test malaria positive. Based on these findings routine malaria test is recommended especially for all neonates who presents with both signs of "fever + refusal to feed", in malaria endemic regions.

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1. Introduction
Malaria is known to be the leading cause of infant's morbidity and mortality in malaria hyper endemic regions (Le, 2000; Sacarlal et al., 2009). However severe malaria in neonates is thought to be rare because their red blood corpuscles possess a great amount of fetal hemoglobin. These cells are relatively resistant to the penetration of malarial parasites (Riley et al., 2001; Vidyashankar et al., 2003). For the first 3–6 months of life, infants seem to be fairly protected from severe complications of malaria infection (Riley et al., 2001; Vidyashankar et al., 2003). Because this type of protection is gotten from passively acquired maternal antibodies (Riley et al., 2001), there is absence of readily available standardized guidelines to manage neonatal malaria (Mohan et al., 2014; Odinaka et al., 2015). Despite the presence of passively acquired maternal antibodies, previous studies have reported malaria incidence in infants born in endemic regions to vary from 0 to 33% (Lehner and Andrews, 1988; Quinn...
et al., 1982). Therefore the need for epidemiological data in order to determine neonatal malaria burden in endemic regions was emphasized (Alao et al., 2013).

However the incidence of severe neonatal malaria is not also uncommon and may be mistaken for neonatal sepsis, due to similar clinical manifestations (Odinaka et al., 2015). There are also occurrences of congenital malaria in endemic regions (Mukhtar et al., 2006) although it was believed to occur at low rates (Olupot-Olupot et al., 2018). In a study where up to 77.4% of neonates had fever as the only clinical presentation, 17.4% had congenital malaria while 24.8% suffered from acquired neonatal malaria. Although congenital malaria may not require treatment because it is often asymptomatic and spontaneously clears, neonates with unexplained fever and refusal to feed should be tested for malaria (Falade et al., 2007). There was a significant occurrence of fever in babies with neonatal malaria and fever had been reported as the typical sign of neonatal malaria (Fischer, 1997; Ojukwu et al., 2004; Runsewe-Abiodun et al., 2006). Studies in other malaria endemic regions have recommended that malaria parasite checks be included as part of routine workup for all neonates with fever (Eberechukwu and Oluwajenyo, 2017; Mohan et al., 2014; Runsewe-Abiodun et al., 2006). This routine workup for neonates is yet to be implemented in the current study area. Therefore, this study was designed to evaluate malaria among febrile neonates attending the Bamenda Regional Hospital.

2. Materials and methods

2.1. Study area

This study was carried out at the neonatology unit of the Bamenda Regional Hospital (BRH) which serves as a referral hospital for most satellite health facilities in the North West Region. This hospital is in the North West Region of Cameroon, Mezam Division, precisely in the Mezam I Sub Division. The Mezam Division has five health districts with a total population of 575,312 inhabitants made up of diverse cultural and religious backgrounds, educational level and social status (Egbe et al., 2016). Bamenda is also situated at the height of 1258 m above the sea level. There are two seasons in Bamenda, the dry and the rainy seasons, with a balance rainfall per year being 2064 mm (and 172 mm per month).

The maternity unit of the BRH has a fully functioning labour room and maternity ward. This unit is taken care of by two obstetricians, three general practitioners, 12 midwives and nurses. In this unit, the average number of births per month is 320. The neonatology unit has three sections; a nursery for preterm/low birth weight, a ward for term neonates and a ward for infants two to three months. It averages about 900 admissions per year. This unit is equipped with eight incubators and 30 cots. The neonatology unit is taken care of by one pediatrician, one general practitioner and eight nurses.

2.2. Study participants

This study was carried out within the period of three months from February 2018 to April 2018, at the neonatology unit of the Bamenda Regional Hospital. The study population was all febrile neonates consulting at the Bamenda Regional Hospital during the study period. The inclusion criteria was all neonates (≤ 28 days) consulting with specific signs of fever and/or refusal to feed and whose mothers accept to enroll them into the study. Signed informed assents were gotten from the mothers who accepted to enroll their neonates into the study. All neonates presenting with fever and who met the eligibility criteria, were recruited consecutively into the study until the minimum required sample size was obtained.

The sample size calculation was done using the following formula:

$$N = \frac{z^2pq}{d^2}$$

Where:

$$z^2 = (1.96)^2$$

p (previous prevalence of neonatal malaria) = 14.4% (0.144) (Mvilongo et al., 2018).

q = (1−0.144).

N = minimum sample size (189)

The ethical clearance for this study was gotten from the Ethical Review Committee of the University of Bamenda.

2.3. Data collection

A structured questionnaire was used to collect data on both maternal and neonatal characteristics. The maternal characteristics included maternal age, parity, educational level, use of insecticide treated bed nets and the presence of malaria risk factors (standing water, hips of dirt, bushes and gutters) around home. Neonatal characteristics included neonatal age, sex and specific sign. The laboratory form was designed to record result of the malaria microscopy test, which was either negative or positive. Parasitaemia was quantified and recorded as follows: <1000 parasites/μL of blood (%), 1000–5000 parasite/μL of blood or >5000 parasite/μL of blood.

2.4. Sample collection

Capillary blood was collected from the neonates and blood films (thick and thin) were prepared within a period of 30 min, following the techniques recommended by Cheesbrough et al., 2006 (Cheesbrough, 2006).
2.5. Microscopy test method

The prepared blood films were processed and stained with 3% Giemsa staining technique (Cheesbrough, 2006). Duplicate slides were independently examined by two experienced microscopists and a third experienced microscopist confirmed the results with discrepancies. Parasite density per microlitre of blood was estimated following the methods in a previous study (Abeku et al., 2008; Sumbele et al., 2014).

2.6. Data analysis

Baseline descriptive statistics of maternal sociodemographic data and malaria predisposing factors for neonates with and without malaria were determined using sums and percentages of mean. Regression analysis was used to determine the effects of the independent variables on the presence of malaria parasitaemia in the neonates. The independent variables under consideration are: age of mother, parity, mother’s educational level, sex of neonate and malaria predisposing factors (presence of gutters, bushes, standing water and hips of dirt around homes). The dependent variable is malaria parasitaemia. Regression analysis was done using GraphPad Prism version 8.2.1. A four-fold ($2 \times 2$) contingency table displaying the frequency distribution for each specific sign (fever, refusal to feed and fever plus refusal to feed) presented by the neonate was entered into GraphPad Prism version 8.2.1. In each of the four cells, the contingency table had frequencies for the absence and presence of the specific sign, for both the positive and negative malaria cases. The Chi-square (and Fisher’s exact) test was used to calculate sensitivity (%), specificity (%), predictive values (%), likelihood ratios, odd ratios, relative risk and attributable risk. The Fisher’s exact test was used to compute the $P$ value at 95% confidence interval.

3. Results

All the registered positive cases were infected with *Plasmodium falciparum* parasitaemia. The total prevalence of malaria parasitaemia among the neonates was 17.46% (33/189). The 3–4 weeks age group had the highest percentage (54.55%) of malaria positive neonates meanwhile the 0–1 and 1–<2 weeks age groups each had the least (09.09%). Most (54.55%) of the positive cases had <1000 parasite/$\mu L$ of blood (Table 1).

Most (50.79%) of the mothers were within the 30 to <40 years age group. This age group had the highest percentage (63.64%) of mothers with malaria positive neonates. Most (63.64%) of the mothers with malaria positive neonates were University graduates. The male to female ratio among the neonates was 1.35:1 and up to 81.84% of the malaria positive cases were males. Fever was generally the most common (61.90%) specific sign. Among the malaria positive neonates, 54.55% were those who had fever meanwhile 36.36% had fever + refusal to feed as specific signs. Bushes and gutters around the house, and the lack of use of insecticide treated mosquito bed nets were the most prevalent (54.55% each) predisposing factors among the women with malaria positive neonates (Table 2).

Only one of the tested independent variables had significant effects on neonatal malaria in this study. Increase in mother’s educational level from primary to high school and to University graduate, resulted to 2.877 unit increase in parasite/$\mu L$ of blood ($P = 0.0045$) in neonatal malaria (Table 3).

3.1. Discussion and interpretation of results

In this study, 17.46% (33/189) of the neonates tested positive for malaria parasitaemia. Although malaria in neonate was thought to be scarce (Odinaka et al., 2015; Riley et al., 2001; Vidyashankar et al., 2003), studies have repeatedly demonstrated otherwise. A 2-year research review in Nigeria reported that 24.8% of neonates suffered from neonatal malaria (Runsewe-Abiodun et al., 2006). Meanwhile a later study equally carried out in Nigeria reported the prevalence of neonatal malaria to be 24.7% (Okoli et al., 2013). Neonatal malaria in the current study is relatively low compared to the above mentioned previous studies which were carried out in the years 2006 and 2013 (Okoli et al., 2013; Runsewe-Abiodun et al., 2006). This decrease could be attributed to the global reduction in cases of malaria infections, especially with the implementation of malaria control measures (Organization WH, 2015). Effective implementation of malaria control measures have been shown to previously reduce neonatal malaria. In a 5 year study, the incidence of neonatal malaria declined by 12% after 3 years of implementation of malaria control measures (Organization WH, 2015).

From the study, malaria incidence was highest (54.55%) among the older neonates (3–4 weeks) as compared to the younger ones (Table 1). Neonates in the 0–1 and 1–2 weeks age groups were least infected. Although it may be difficult to differentiate between acquired and neonatal malaria (D’Alessandro et al., 2012; Mohan et al., 2014), it is most probable that malaria at the age of 3–4 weeks and even 2–3 weeks is acquired. Moreover, congenital malaria has been reported to either clear spontaneously or clinically manifest only during the first week of life (Falade et al., 2007). In the current study, up to 90.91% (30/33) of the neonates with malaria were more than a week old. Therefore, malaria was probably mostly acquired. This finding agrees with those of a study carried out in the Gambia. A higher incidence of malaria was equally reported among neonates who were more than a week old (Obu and Ibe, 2011). In another study 6.9% of neonates had congenital malaria while 24.7% had neonatal malaria (Okoli et al., 2013). This also indicates that acquired neonatal malaria was more common. Contrary to the current study, a previous study reported a higher prevalence (75%) of congenital malaria and a lower prevalence (25%) of acquired malaria (Runsewe-Abiodun et al., 2006). These discrepancies may have resulted from variations in the cases of malaria in pregnancies in the different age groups.
Table 1
Distribution of malaria parasitaemia levels according to age of neonates.

| Age/weeks | <1000 parasite/μL of blood (%) | 1000–5000 parasite/μL of blood (%) | Total (%) |
|-----------|-------------------------------|-------------------------------------|-----------|
| 0–1 week  | 03 (16.67)                    | 00 (00)                             | 03 (09.09) |
| 1–<2 weeks| 00 (00)                       | 03 (20.00)                          | 03 (09.09) |
| 2–<3 weeks| 03 (16.67)                    | 06 (40.00)                          | 09 (27.27) |
| 3–4 weeks | 12 (66.67)                    | 06 (40.00)                          | 18 (54.55) |
| Total (%) | 18(54.55)                     | 15 (45.45)                          | 33        |

Table 2
Distribution of malaria test results in neonates according to the variables under consideration.

| Variables | Positive (%) | Negative (%) | Total (%) |
|-----------|--------------|--------------|-----------|
| Age range of mother (years) |              |              |           |
| 10 to <20 | 03 (09.09)   | 12 (07.69)   | 15 (07.94) |
| 20 to <30 | 09 (27.27)   | 66 (42.31)   | 75 (39.68) |
| 30 to <40 | 21 (63.64)   | 75 (48.08)   | 96 (50.79) |
| ≥40       | 00 (00)      | 03 (01.92)   | 03 (01.59) |
| Number of children/mother |              |              |           |
| Primipare | 24 (72.73)   | 87 (55.77)   | 111 (58.73) |
| Multipar (4 to 6) | 09 (27.27)   | 66 (42.31)   | 75 (39.68) |
| Grand multipar (≥ 6) | 00 (00)      | 03 (01.92)   | 03 (01.59) |
| Educational level of mother |              |              |           |
| Primary School | 03 (09.09)   | 15 (09.62)   | 18 (09.52) |
| High School | 09 (27.27)   | 66 (42.31)   | 75 (39.68) |
| University Graduate | 21 (63.64)   | 75 (48.08)   | 96 (50.79) |
| Sex of Neonate |              |              |           |
| Male      | 27 (81.81)   | 81 (51.92)   | 108 (57.14) |
| Female    | 06 (18.18)   | 75 (48.08)   | 81 (42.86) |
| Specific sign |              |              |           |
| fever     | 18 (54.55)   | 99 (63.46)   | 117 (61.90) |
| refusal feed | 03 (09.09)   | 30 (19.23)   | 33 (17.46) |
| refusal feed + fever | 12 (36.36)   | 27 (17.31)   | 39 (20.63) |
| Malaria predisposing factors |              |              |           |
| Standing water around homes | 15 (45.45)   | 66 (42.31)   | 81 (42.86) |
| No standing water around homes | 18 (54.55)   | 90 (57.69)   | 108 (57.14) |
| Bushes around homes | 18 (54.55)   | 90 (57.69)   | 108 (57.14) |
| No bushes around homes | 15 (45.45)   | 66 (42.31)   | 81 (42.86) |
| Hips of dirt around homes | 12 (36.36)   | 60 (38.46)   | 72 (38.10) |
| No hips of dirt around homes | 21 (63.64)   | 96 (61.54)   | 117 (61.90) |
| Gutters around homes | 18 (54.55)   | 78 (50.00)   | 96 (50.79) |
| No gutters around homes | 15 (45.45)   | 78 (50.00)   | 93 (49.21) |
| Use of ITNs | 15 (45.45)   | 99 (63.46)   | 114 (60.32) |
| No use of ITNs | 18 (54.55)   | 57 (36.54)   | 75 (39.68) |
| Total (%) | 33 (17.46)   | 156 (82.52)  | 189       |

Table 3
Summary results for regression analysis, with malaria in neonates as the dependent variable.

| Variable | t | P value | 95% confidence interval |
|----------|---|---------|------------------------|
| Intercept | 2.668 | 0.0083** | −0.9366 to −0.1401 |
| Age (weeks) | 0.5435 | 0.5875 | −0.00580 to 0.03739 |
| Sex | 1.936 | 0.0545 | −0.001881 to 0.1951 |
| Number of children by mother | 1.237 | 0.2177 | −0.003853 to 0.01240 |
| Mother's age | 1.038 | 0.3009 | −0.03833 to 0.01240 |
| Mother's educational level | 2.877 | 0.0045** | 0.03631 to 0.1950 |
| Standing water by home | 1.681 | 0.0946 | −0.1834 to 0.01469 |
| Bushes by home | 0.7881 | 0.4317 | −0.1374 to 0.05896 |
| Hip of dirt by home | 0.8871 | 0.3762 | −0.1493 to 0.05669 |
| Gutters by home | 1.396 | 0.1644 | −0.02949 to 0.1722 |
| Use of ITNs | 0.8269 | 0.4094 | −0.1431 to 0.05861 |

** Significant P-value. The calculated sensitivity (%), specificity (%), PPV (%) and NPV (%) and LR were only significant (P = 0.0188) for “Fever + refusal to feed” (Table 4).
In this study, malaria parasitaemia was generally not severe as up to 54.55% (18/33) of the infected neonates had <1000 parasites/μL of blood, meanwhile 45.45% (15/33) had between 1000 and 5000 parasites/μL of blood. There were no cases with more than 5000 parasites/μL of blood. The absence of higher parasite density could be attributed to the presence of passively acquired maternal antibodies (Riley et al., 2001; Vidyashankar et al., 2003). This is in line with studies which have equally reported low malaria parasite density in neonates. For example a study which examined the growing incidence of neonatal malaria reported parasite density ranging from 47 to 1019/μL of blood (Makhtar, 2007). Another study on the epidemiology of congenital malaria reported an even lower parasite density of 8 to 200/μL of blood (Falade et al., 2007). The presence of a higher parasite density (1000–5000 parasites/μL of blood) in this study further suggests the presence of acquired neonatal malaria, since congenital malaria is known to be often mild and asymptomatic (Falade et al., 2007). Moreover the relatively high parasite density in this study is a risk factor for severe parasitaemia if left untreated. Also, the absence of prompt malaria diagnosis could facilitate the degeneration of mild parasitaemia to severe parasitaemia. Moreover it may be rapidly fatal if severe neonatal malaria is mistaken for neonatal sepsis, since both illnesses present with similar clinical manifestations (Mukhtar et al., 2006; Odinaka et al., 2015).

The mother's age, parity and neonate's sex were all shown to have no significant effect on malaria in the neonates in this study. Malaria predisposing factors considered in this study are; use of ITNs, standing water, bushes, hips of dirt and gutters around the house. None of these predisposing factors significantly contribute to the presence of malaria in these neonates. These predisposing factors have been shown to significantly contribute to malaria existence in adults (Azunie, 2017; Essendi et al., 2019; Guerra et al., 2018; Musoke et al., 2018). The passively acquired immunity in the neonates may have been responsible for the protection in this current study. However increase in the mother's educational level was shown to significantly contribute to the presence of malaria in the neonates. About 50.79% of the mothers included in this study are University graduates while only about 09.52% of them are at the primary school level. In the study area, the high school and University graduates are mostly workers in the private sector which sometimes do not offer maternity leave. The lack of proper attention and maternal care for their neonates is a possible risk factor for malaria. Moreover most of the private sector workers do not request for maternity leave due to high levels of unemployment and job insecurity.

“Fever”, “refusal to feed” and “fever + refusal to feed”, were the specific signs presented by the neonates in this study. Only “fever + refusal to feed” had significant sensitivity, specificity, likelihood ratio, odds ratio, relative risks, attributable risks and negative and positive predictive values. This mean that “fever + refusal to feed” tested positive for malaria diagnosis of the neonates in this study. There was a high specificity (82.69%) and NPV (86%) for “fever + refusal to feed”. This also mean that neonates who did not present with both “fever + refusal to feed”, were most likely to test malaria negative. The likelihood ratio (2.101), odds ratio (0.42), relative risk (0.4727) and attributable risk (0.045449) of “fever + refusal to feed” were also high. This further confirms the fact that neonates with both “fever + refusal to feed” as specific signs have a far higher chance of testing malaria positive. The likelihood ratio, sensitivity, odds ratio, relative risks and attributable risks for “fever” alone were generally higher than for “refusal to feed” alone. This generally mean that neonates with only “fever” as the specific sign were more likely to test malaria positive than neonates who only refused to feed.

### Table 4
Sensitivity, specificity, likelihood ratio, positive and negative predictive values for diagnosis of malaria with the specific signs.

| Variable                        | Sensitivity (%) (95% CI) | Specificity (%) | PPV (%) | NPV (%) | LR      | P - value |
|---------------------------------|--------------------------|-----------------|---------|---------|---------|-----------|
| Fever                           | 54.55 (37.99 to 70.16)   | 36.54 (29.39 to 44.33) | 15.38 (9.99 to 23.01) | 79.17 (68.43 to 86.95) | 0.8595   | 0.4303    |
| Refusal to feed                 | 0.6909 (0.3260 to 1.528) | 0.7385 (0.4092 to 1.368) | 0.054449 |         |         |           |
| Fever + Refusal to feed         | 0.42 (0.1280 to 1.334)   | 0.4277 (0.1571 to 1.300) | 0.01014 |         |         | 0.2110    |

* Significant P-value. “Fever + refusal to feed” is the specific sign with the highest odds ratio; 2.730, relative risks; 2.198 and attributable risks; 0.1677 (Table 5).

### Table 5
The odds ratio, relative risk and attributable risk of the specific signs by neonates, for diagnosis of malaria.

| Variable                        | Odds Ratio (%) (95% CI) | Relative risks (%) | Attributable risks (%) | P - value |
|---------------------------------|--------------------------|-------------------|------------------------|-----------|
| Fever                           | 0.6909 (0.3260 to 1.528) | 0.7385 (0.4092 to 1.368) | 0.054449 |         |         | 0.4303    |
| Refusal to feed                 | 0.42 (0.1280 to 1.334)   | 0.4277 (0.1571 to 1.300) | 0.01014 |         |         | 0.2110    |
| Fever + refusal to feed         | 2.730 (1.144 to 5.993)   | 2.198 (1.172 to 3.954) | 0.0167 |         |         | 0.0188*   |

* The odds ratio, relative risk and attributable risk for diagnosis of malaria with the specific signs.
4. Conclusion

Malaria was mostly common in older neonates, suggesting a higher prevalence of acquired malaria as opposed to congenital malaria. “Fever”, “refusal to feed” and “fever + refusal to feed” were the three specific signs presented by the neonates at consultation. Results show that neonates who presented with both “fever + refusal to feed” were most likely to test malaria positive. Neonates who only refused to feed were the least likely to test malaria positive. Therefore routine malaria tests are recommended for all neonates who present with both signs of “fever + refusal to feed”. At least three months maternity leave should be granted to all employed women who put to birth, in order to ensure adequate maternal care.

Data Availability and Funding Statement

All the data for this study can be gotten from the authors, on request. This study was funded by the authors.

Declaration of Competing Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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