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Malaria and Anaemia in Twin Pairs Aged Less than 7 Years from 8 Villages of the Western Region of Cote D'Ivoire

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Abstract

Background: *Anaemia is a critical public health issue affecting over one-quarter of the world's population in all income's countries. In Côte d'Ivoire, malaria kills four people every day including three children under the age of five in 2022. This research study aims to highlight the type of anaemia observed in twin pairs under 7 years of age infected with malaria in the western region of Côte d'Ivoire, in order to raise public awareness and recommend a sound public health strategy of care to alleviate this scourging problem.*

Methods: *A cohort of 65 male and female children which included 13 twin pairs aged 3 months to 6 years were recruited from March 2020 to May 2021 in 8 villages in the Tonkpi Region. The children provided venous blood samples for the diagnosis and characterization of anaemia (full blood count), while a Giemsa staining (GS) (thick and thin smears) and rapid diagnostic tests (RDTs) were used for the diagnosis of malaria. Univariate analysis (Chi-2 test ($\chi 2$) and P: (Probability) were used for comparison between groups. Significant test was considered at a threshold of 0.05.*

Results: *Of the 65 participants who completed the study, 34 (52.3% were female and 31 (47.7%) were male. Thirteen amongst them were a set of pair twins made of five sets of homozygotes and eight heterozygotes (10(38.5%) male and 16(61.5%) female). The Prevalence of the overall anaemia was 63.1% including 34.1% twins distributed as 46.4% mild, 51.2% moderate and 2.4% severe anaemia. The characterization of anaemia revealed that Normochromic microcytic anaemia was the predominant type (23.1%) followed by hypochromic microcytic anaemia (19,2%) in the twins. The prevalence of malaria was 62.2% and 75.4% based on GS and RDTs respectively in the overall population, with38.5% on GS and 80.8% on RDTs amongst the twins. The closed relation between Plasmodium infection and anaemia led to P. falciparum alone causing 57.1% of mild, 42.9% of moderate, and no severe case in the twin children.*

Conclusion: *Malaria infection was very prevalent among female (62.5%) aged [0-59M] and male (60.0%) aged]59 M - 6Y] twins' children. P. plasmodium was the most prevalent malaria parasites (90%) in both sex accounting for the high prevalence of mild and moderate normochromic microcytic anaemia (NMA) ob-*

served in the twins aged]59M - 6Y] and [0-59M] Espectively in the western region of Côte d'Ivoire.

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Introduction

Parasitic diseases have coexisted with mankind since the dawn of humanity and are still coexisting up until today. They are among the main causes of death and misery in the world today. These infections including malaria are endemic in various tropical countries where they affect mostly children leading to severe consequences such as acute or chronic anaemia with high morbidity and mortality rates worldwide [1-3]. In addition, the WHO report of 2025 estimated 282 million malaria cases in 2024 in the world. Although the WHO African Region accounted for 94% (265 million malaria) of cases globally, five (5) countries account for over half of all cases in the region (Nigeria (25.8%), the Democratic Republic of the Congo (13.3%), Uganda (5.0%), Ethiopia (4.7%) and Mozambique (3.9%) [3].

Anaemia is a serious global public health concern affecting almost a quarter of the world's population particularly young children. According to the World Health Organization's, countries of low, lower and middle income carry the highest burden of anaemia, mostly people living in the rural areas, or poor households and or people with no formal education [4]. In addition, it is estimated that 269 million (40%) of children aged 6 - 59 months globally are [5]. Furthermore, the WHO Regions of Africa together with South-East Asia are most affected with an estimated 103 million children affected by anaemia in Africa alone [5].

In Côte d`Ivoire, the recent World Bank report of the year 2020 on anaemia indicated a prevalence of 72.2% in children aged 6 to 59 months [6] while the WHO report of 2018 highlighted, the prevalence of malaria in 2017 to be estimated at 49% and 52% based on Giemsa staining (GS) of thick blood smear and rapid diagnostic testing (RDT), respectively. Furthermore, 8 557 000 malaria cases were observed in Cote d'Ivoire in 2024. The main objective of this community-based research was, to determine the prevalence and to characterize the type of anaemia observed in twin children aged below 7 years in the Western part of Côte d`Ivoire. Then, to highlight, draw the public attention on the seriousness of anaemia amongst children so that the government can institute a better strategy of care [5,7].

Methods

Study Area and Participants

This observational clinical research study was carried out from March 2020 to May 2021 in 8 villages in eight (8) villages of "TONKPI Region" (Man). Due to some difficulties to recruit twin pairs participants, the target sample size was limited at 65 children. And so, children aged 3 months to < 7 years were enrolled and provided blood samples for the diagnosis of anaemia and malaria. The 65 children belong to three (3) ethnical groups [Akan (Baoulé), Gour (Lobi & Sénoufo from Côte d'Ivoire), and Mandé (Yacouba) the predominant ethnic of the Tonkpi region. All study participants reside in rural area with similar life style patterns and their parents earn their living mostly as farmers, house wives or commercial traders.

Sociodemographic

Socio-demographic data including age, sex, height (to the nearest centimetre), weight (to the nearest 0.5 kg) and parent's profession were also collected using a questionnaire administered to each enrolled participant who signed a written informed consent or gave a fingerprint (illiterate participants).

Blood Collection

A total of 2 mL of venous blood was drawn in the morning into ethylenediaminetetraacetic acid (EDTA) treated vacutainer tubes from each of the 65 consented study participants. Blood samples were kept on ice until transported to the central laboratory at the Centre Hospitalier Regional of Man (CHR Man) for the haematology testing (FBC). Furthermore, approximately 10-20 µl of blood was collected from the children's pricked fingers for direct use in malaria RDTs.

Malaria Microscopy and Malaria Rapid Diagnostic Test

Malaria Microscopy

About 10 µl of the 2 ml of venous blood that was collected in the EDTA-treated tube was used to prepare GS thick and thin blood smear on a single slide for the count of malaria parasitemia [8,9]. The slides were analysed and quality controlled by well-trained and experienced laboratory technicians. Slides were considered positive when asexual forms and/or gametocytes of any Plasmodium species were observed on the blood film. Parasite density per microliter of blood was determined by the number of malarial parasites per 200 leukocytes on a thick blood film, assuming a white blood cell (WBC) count of 8000 leucocytes/µl of blood and then transformed into malaria parasite density by multiplying by a factor of 40. Malaria parasite density was classified as low (<5,000 parasites/µl of blood) or high (≥ 5,000 parasites/µl of blood) [8,9]. The GS (thick and thin blood smear) slides were scored as negative or positive. Positive scores were further classified into any of the plasmodium species e.g. Plasmodium falciparum, P. malariae, P. vivax, or P. ovale, P. knowlesi or as undetermined if the technician experienced difficulty clearly identifying the species.

Malaria Rapid Diagnostic Test

Approximately 10 to 20 µl of blood collected from the pricked finger of the child was tested in malaria RDT, making use of the plastic capillary tube provided in the RDT kit, as per manufacturer instructions (Humasis Malaria Pf/Pan Antigen Test; Humasis Co. Ltd, Humasis, South Korea). The result was scored as negative (i.e., no P. falciparum detected) or mixed (i.e., P. falciparum and other species of Plasmodium detected).

Haemoglobin Determination and Classification of Anaemia

The full blood count (FBC) was performed using URIT 3000 PLUS analyser, Urit Medical Electronics, China and the result printed and further analysed and characterized on the basis of the mean corpuscular haemoglobin (MCH) (normal value = 27 to 31 pictograms (pg) and mean corpuscular haemoglobin concentration (MCHC) (normal value = 33 - 36 grams per decilitre (g/dL) as Normochromic or Hypochromic and the mean cell volume (MCV) (normal value = 80 - 100 fl) as Normocytic, Microcytic or Macrocytic.

Anaemia was assessed and classified according to the age, sex of the participants and the concentration value of the haemoglobin (Hb) as per WHO guidelines [10]. Thus, children from 3 months to 6 years with an Hb level of less than 11 g/dL were considered as anaemic while those with an Hb level ≥11 g/dL were considered as normal (non-anaemic). Anaemia was also categorized as mild if the Hb level was less than 10 g/dL, moderate between 7 and 10 g/dL and severe below 7 g/dL and further characterized on the basis of the MCH and MCHC as Normochromic or Hypochromic and on the basis of the MCV as Normocytic, Microcytic or Macrocytic.

Data Management and Analysis

Data were entered into a database using the double-entry system in Epi-data version 3.1 (EpiData, Odense Denmark, 2004). Inconsistencies were cleaned and, after validation, the data were imported for the analysis to SPSS version 20 (IBM Corp; 2011) and STATA 18 software (STATA Corporation, College Station, Texas, USA). Chi-2 ($\chi 2$) test was used for comparison of proportions between groups. Univariate analysis (Chi-2 test ($\chi 2$) and P: (Probability) were used for comparison between groups. Significant test was considered at a threshold of 0.05.

Ethics Consideration

Ethical approval was sought at "Comité National d'Ethique des Sciences de la Vie et de la Santé (CNESVS) de la Côte d'Ivoire" and obtained (N/Ref: 024- 21/MSHP/CNESVS-km) before the start of the study. Further permission to conduct the study was obtained from each visited village chief`s. In general, the aims, the procedures, the potential risks and the benefits of the study were explained to the physicians, nurses, and assistant nurses of each Health Centre

involved and the villagers before the commencement of the study.

Since all the participants were minors, consent from one of the biological parent or legal representative was sought and obtained before any study procedure was completed. Only voluntary consented participants were included in the study. Treatment was made available free of charge to all sick participants. Those who required further assistance were referred to the local government health centre for assistance. Participants were further informed that their information will be anonymized (confidential) using a coding system instead of their names.

Results

Characteristics of the Study Populations

A total of 65 children from 8 villages were enrolled in the study, of whom 31 (47.7%) were male and 34 (52.3%) were female. In addition, there was thirteen (13) set of twin pairs of which 10(38.5%) were male and 16 (61.5%) females. Five sets (5) were Homozygotes and eight (8) were Heterozygotes (Table 1).

Table 1: Distribution of the study participants and set of twin's pairs per village and sex (N = 65) in Western region of Côte d'Ivoire.

Department	Sous-Prefecture	Villages	Sex		Enrolled Participants n (%)	Type of twin		Sex		
			Male	Female		Homozygote	Heterozygote	Male	Female	Enrolled Participants n (%)
BIANKOUMA	Kpata	Kpata	5 (7,7)	5 (7,7)	10 (15,4)	0	2	2	2	4 (15.4)
MAN	Gbangbegouiné-Yati	Kouitongouiné 2	10 (19.4)	10 (19.4)	20 (30,8)	1	3	3	5	8 (30.7)
		Douelé-Dimba	4 (6.2)	1 (1.5)	5 (7,7)	1	0	2	0	2 (7.7)
		Tiakeupleu	4 (6.2)	5 (7,7)	9 (13.9)	0	1	1	1	2 (7.7)
		Guiapleu	4 (6.2)	5 (7,7)	9 (13.9)	1	0	0	2	2 (7.7)
Total	4	8	31 (47.7)	34 (52.3)	65 (100)	5	8	10 (38.5)	16 (61.5)	26 (100)

Prevalence of Anaemic Participants

Overall, there were 41(63.1%) participants enrolled in the study that were anaemic and displayed predominantly a moderate anaemia 21(51.2%) followed by mild anaemia 19 (46.4%) and severe one 1(2.4%) (Table 2). Amongst these anaemic children, 14(34.1%) were twin pairs and accounted for 57.1% of mild, 42.9% of moderate anaemia; with no severe case (Table 2).

It was further noted in the overall study population that the severity of anaemia varied by age group (children of [0-59M] months old: 64.4% versus the children of]59M - 6Y] years old: 36.6%). The younger children were at a higher risk of moderate (85.7%) and severe anaemia (100%) compare to the older children (14.3% vs. 0% respectively). In addition, the older children had the higher prevalence of mild (63.2%) anaemia (Table 2). The same patterns were also observed amongst the twin pairs children where the majority of anaemic cases were moderate (83.3%) in the younger children, while the older children had the highest cases of mild (62.5%) anaemia. No severe case was observed. These observations indicated that the severity of anaemia did not affect the number of gestations i.e. being singletons or twins. Details are summarized in Table 2.

Table 2: Severity of anaemia by sex and age amongst twin pairs in the Western region of Côte d'Ivoire.

Anaemic participants	Sex		Severity of anaemia study population			Twins	Severity of anaemia in Twins pairs		
Age group	Male	Female	Mild	Moderate	Severe	Twins	Mild	Moderate	Severe
[0-59M]	4 (40.0)	10 (62.5)	7 (36.8)	18 (85.7)	1 (100)	12 (46.2)	3 (37.5)	5 (83.3)	0
]59M - 6Y]	6 (60.0)	6 (37.5)	12 (63.2)	3 (14.3)	0	14 (53.9)	5 (62.5)	1 (1.7)	0
Total	10 (32.3)	16 (47.1)	19 (46.4)	21 (51.2)	1 (2.4)	26	8 (57.1)	6 (42.9)	0

Distribution of the type of anaemia according to sex and age

There was no significant variation in the prevalence of each type of anaemia by sex (48,8% male versus 51.2% female). Both male and female and the different age groups (younger children: 66.7% versus older children: 57.7%) were almost equally affected. The only noted variation was the high prevalence of normochromic microcytic anaemia (NMA) at a rate of 23.1% in the twin pairs children while the hypochromic microcytic anaemia (HMA) at a prevalence of 36.9% was the predominant one in the overall study population (Table 3). Nevertheless, both HMA and NMA were the predominant type of anaemia either in the twin pairs or in the overall study population. No case of Macrocytic anaemia was detected in the study (Table 3).

Table 3: Characterization and Prevalence of anaemia by sex and age, in the Western region of Côte d'Ivoire.

Type of Anaemia	Anaemic Participants	Twin	Male	Female	$\chi 2$	P	[0 - 59 M]	]59 M - 6 Y]	$\chi 2$	P
HMA	24 (36.9)	5 (19,2)	11 (35.5)	13 (38.2)	0.053	0.82	18 (46.2)	6 (23.1)	4	0.1
NMA	10 (15.4)	6 (23.1)	4 (12.9)	6 (17.6)	0.28	0.6	5 (12.8)	5 (19.2)	0	0.5
NNA	5 (7.7)	2 (7.7)	4 (12.9)	1 (2.94)	2.266	0.13	2 (5.1)	3 (11.5)	1	0.3
HNA	2 (3.1)	1 (3.8)	1 (3.2)	1 (2.9)	0.004	0.95	1 (2.6)	1 (3.9)	0	0.8
MA	0	0	0	0	-	-	0	0		
Total	**41 (63.1)**	**14 (34.1)**	**20 (48.8)**	**21 (51.2)**			**26 (66.7)**	**15 (57.7)**		

HMA: hypochromic microcytic anaemia, NMA: normochromic microcytic anaemia, NNA: normochromic normocytic anaemia, HNA: hypochromic normocytic anaemia, MA: macrocytic anaemia

Prevalence of Anaemic Participants with Malaria Infection

Prevalence of Malaria (Rapid Diagnostic Test) By Sex and Age

Of the 65 participants that were enrolled in the study, 49 (75.4%) were diagnosed using the RDT testing tool. Amongst those, 25 (38.5%) were mono-infection caused by P. falciparum, 24 (36.9%) mixed species infections, and 16 (24.6%) were tested negative (Table 4). Both male (n =23, 46.9%) and female (n = 26, 53.1%) were almost equally infected (data not shown), with younger children (n = 28, 71.8%) being infected less often than older children (n = 21, 80.8%). Mono-infection with P. falciparum (n = 25, 38.5%) and co-infection with P. mixed (n = 24, 36.9%) were equally predominant (Table 4).

With regard to the twin pairs children, 21 (80.8%) of them were diagnosed with the RDT testing of which male children (n = 9, 42.9%) were slightly infected than female (n = 12, 57.1%) (data not shown). Also, both age groups were almost equally affected by malaria plasmodium ([0-59M]: 11, 78.6% versus]59M - 6Y]: 10, 83.4%) (Table 4). On the other hand, our data revealed that mono-infection with P. falciparum (n = 13, 50.0%) were slightly predominant compare to the co-infection with P. mixed (n = 8, 30.8%) (Table 4).

Table 4: Prevalence Of Malaria by Rdt In Twin Pairs and Study Population According to Sex and Age, Western Côte D'ivoire.

	Sex		Diagnosis of malaria in study population RDT*			Total	Sex		Diagnosis of malaria in Twins RDT*			Total
Age Group	Male	Female	Pf	P mixed (Pf / Pm)	Neg.		Male	Female	Pf	P mixed (Pf / Pm)	Neg.	
[0-59M]	18 (58.1)	21 (61.8)	16 (41.0)	12 (30.8)	11 (28.2)	28 (71.8)	4 (40.0)	10 (62.5)	8 (57.2)	3 (21.4)	3 (21.4)	11 (78.6)
]59 M - 6 Y]	13 (41.9)	13 (38.2)	9 (34.6)	12 (46.2)	5 (19.2)	21 (80.8)	6 (60.0)	6 (37.5)	5 (41.7)	5 (41.7)	2 (16.6)	10 (83.4)
Total	31	34	25 (38.5)	24 (36.9)	16 (24.6)	49 (75.4)	10 (32.3)	16 (47.1)	13 (50.0)	8 (30.8)	5 (19.2)	21 (80.8)

RDT = Rapid diagnostic test, M = Month, Y = Year, Pf = Plasmodium falciparum, Pm = Plasmodium malariae, Neg. = Negative, (%): Prevalence.

Prevalence of Malaria (Giemsa Staining-Thick and Thin Blood Smear Test) By Sex and Age

The GS thick and thin blood smear (Microscopic) on one slide was completed for only 37 (56.9%) participants. Out of the 37 slides examined, 23 (62.2%) were positive and 14 (37.8%) were negative (Table 5).

Table 5: Prevalence Of Malaria by Blood Smear (Gs) In Study Population and In Twin Pairs According to Sex and Age, Western Côte D'ivoire.

	Sex		Diagnosis of malaria in study population (GS*)			Total	Sex		Diagnosis of malaria in Twins (GS)			Total
Age Group	Male	Female	Pf	P mixed (Pf / Pm)	Pm		Male	Female	Pf	P mixed (Pf / Pm)	Pm	
[0-59M]	18 (58.1)	21 (61.8)	13 (72.2)	3 (100)	2 (100)	**18 (78.3)**	4 (40.0)	10 (62.5)	5 (55.6)	0	1 (100)	**6 (60.0)**
]59 M - 6 Y]	13 (41.9)	13 (38.2)	5 (27.8)	0	0	**5 (21.7)**	6 (60.0)	6 (37.5)	4 (44.4)	0	0	**4 (40.0)**
Total	31	34	18 (78.3)	3 (13.0)	2 (8.7)	23 (62.2)	10 (32.3)	16 (47.1)	9 (90.0)	0	1 (10.0)	10

GS= Giemsa Staining, M = Month, Y = Year, Pf = Plasmodium falciparum, Pm = Plasmodium malariae, (%): Prevalence.

There was no variation in terms of sex given the fact that 11 male participants (47.8%) and 12 female participants (52.2%) were affected (Table 6).

Table 6: Prevalence Selon Le Sexe Et L'age Des Cas De Paludisme Par L'examen De Gs, Western Cote D'ivoire.

	Enrolled	Infected study pop. *	Infected Twin pairs	% (IC à 95 %)	χ2	P-value	MPD (IC à 95 %)	P-value
Sex								
Male	31	11	4	36.4 (19.2-54.6)			11882.6 (2876,4-20888,7)	
Female	34	12	6	50.0 (19.7-53.5)	0.003	0.987	8959.8 (5522.4-23442.0)	0.124
Age Group								
[0-59M]	39	18	6	33.3 (30.1-62.8)			10636.3 (790.2-20482.4)	
]59 M - 6 Y]	26	5	4	80.0 (6.6-39.4)	4.946	0.026	9354.4 (9169.6-27878.4)	0.655
Total		23		38.5 (5.9-23.9)			10357.7 (2340.9-18374.4)	

Thick smear, M = Month, Pop. = population, χ2: Chi 2 test, P: Probability, %: Prevalence, CI: Confidence Interval, MPD: Mean Parasite Density.

The prevalence of malaria was higher among younger children [0-59M]: 78.3% than the oldest ones]59 M - 6 Y]: 21.7%) in the overall study population (Table 5).

In terms of the mean parasite density, the study revealed that the younger children had a higher level of parasitemia (10 636.3/µl of blood) compared to the older children (9354.4/µl of blood) (Table 6). Microscopic analysis indicated the presence of two Plasmodium species identified as P. falciparum and P. malariae. Overall, 18 children were infected by P. falciparum (78.3%), 2 by P. malariae (8.7%), and 3 by a mixture i.e. co-infection of P. falciparum and P. malariae (13.0%) among the examined slides of the infected persons (Table 5).

Amongst the ten (10) twin pairs who were infected with the plasmodia parasites and diagnosed positive with the GS testing tool, 9(90.0%) had P. falciparum and 1(10.0%) P. malariae. No co-infection was observed (Table 5). Similarly, twin children aged [0-59M] months were the most affected with the malaria

plasmodium compared to those aged]59M - 6Y] years (60.0% versus 40.0%) (Table 5). Once again, P. falciparum was the prevalent species (00.0%) infecting twin children in the study area.

It is important to emphasize with regard to the malaria diagnosis that the RDT testing was carried out for each of the 65 enrolled children, whereas the GS thick and thin blood smear was carried out for only 37 participants due to a lack of adequate quantity of blood samples. In total, 31 children in the younger age group [0-59M] and 6 children in the older age group]59M - 6Y] had thick and thin blood smears.

Severity of anaemia in children infected with malaria by sex and age

With regard to the severity of the anaemia due to P. falciparum and P. malariae infections in the overall study population, our study findings showed 46.4% mild, 51.2% moderate, and 2.4% severe amongst the 41 anaemic children. Similarly, out of the 14 anaemic twin pairs, 8 (57.1%) had mild and 6 (42.9%) moderate anaemia. No severe case was observed (Table 2).

The RDT testing results of the 63.1% overall anaemic children that displayed 46.4% mild, 51.2% moderate and 2.4% severe anaemia were infected with 38.5% of P. falciparum and 36.9% co-infection (Table 2 and 4).

On the other hand, 53.8% of anaemic twin pairs that displayed 57.1% mild, 42.9% moderate anaemia with no severe case were infected with 50.0% of P. falciparum and 30.8% mixed infection (Table 2 and 4). In addition, amongst the children aged [0-59M] months the majority (n = 16, 41%) was infected with P. falciparum, same as in the twin pairs of the same age group (n = 8, 57.2%). Whereas in the children aged]59M- 6Y] year old, the mixed infection (P. falciparum & P malariae) was the most prevalent (n = 12, 46.2%). In contrast, mono-infection of P. falciparum and mixed infection of P. falciparum & P malariae was equally prevalent (n = 5, 41.7%) in the twin pairs children of the same age group (Table 4).

We further observed based on the GS testing results in the overall study population that, amongst the malaria infected children aged [0-59M] months, 13 (72.2%) including 5 (55.6%) twin pairs were affected with P. falciparum, 2 (100%) children including 1 (100%) twin pair were affected with P malariae. And another, 3 (100%) children (no twin) were co-infected with P. falciparum & P malariae (Table 5). In the]59M-6Y] years old children, only P. falciparum infection (27.8%) was observed amongst the 5 infected children of which 4 were twin pairs (n = 4, 80.0%). Neither a co-infection nor P malariae mono-infection were observed in that age group regarding the overall study population and the twin pairs children (Table 5).

In terms of the severity of the anaemia, the study showed that malaria infected children displayed predominantly moderate anaemia (n = 21, 51.2%) in the overall study population while in the twin pairs the mild anaemia was the most prevalent (n = 8, 57.1%) form (Table 2). In addition, children aged [0-59M] months from the overall study population showed a prevalence of 100%, 85.7% and 36.8% for severe, moderate and mild anaemia respectively; whereas the twin pairs of the same age group showed a prevalence of 83.3% and 37.5% for moderate and mild anaemia respectively. There was no severe anaemia among them (Table 2). On the other hand, the older children,]59M- 6Y] years old from both the overall study and the twin pairs population revealed the predominant form of the anaemia to be mild at a rate of 63.2% and 62.5%. respectively. No severe case was also observed in the same age group of both study populations (Table 2).

Association (Univariate And Multivariate) By Sex and Age Analysis

In terms of the association between the Plasmodium species and other variables, such as type of anaemia, sex, age, and severity of the anaemia, the univariate logistic regression results showed no statistically significant variation between malaria infection, sex and, age group. Furthermore, normochromic microcytic and hypochromic microcytic anaemia although the most predominant types of anaemia in twin pairs children and general study population respectively, there was also not statistically significant variation in the type of anaemia and Plasmodium falciparum was the most prevalent Plasmodium species irrespective of the children age (Table 5). Multivariate logistic regression analysis could not be done due to the fact that the size of the sample was not representative enough (small).

Discussion

This research article reports the findings of an observational community-based research study conducted in twin pairs aged less than 7 years from 8 villages of the Western region of Côte d'Ivoire. The overall prevalence of anaemia amongst the five sets of homozygotes and eight heterozygotes' twins revealed that almost half of the children (n = 14, 53.8%) enrolled were anaemic. Both sexes were involved although the female kids (n = 10, 71.4%) were more affected than male ones (n = 4, 28.6%).

In addition, mild anaemia was more prevalent among the]59M - 6Y] age group children (62.5%) while moderate anaemia was the predominant one in the [0-59M] months old (83.3%). In the overall study population, the prevalence of anaemia was (n = 41, 63.1%) of which moderate anaemia accounted for 51.2%, mild anaemia 46.4% and severe one 2.4%. These results are consistent with Starck et al., study conducted in 23 low to middle-income countries (LMICs) or that of Bezie et al., in sub-Saharan Africa amongst children aged 6-59 months' old, or Ehouman and collaborators study previously reported in Côte d'Ivoire [2, 11, 12]. Our findings are far from the WHO's target goal initially set target for 2025 and later adjusted to 2030 [13].

Indeed, according to Starck et al., most children (62.9%) suffered from at least mild anaemia and the prevalence was higher in plasmodium infected children (82.4%) [11]. Bezie and collaborators reported 61.9% a prevalence of anaemia in children aged 6–59 months in 27 countries from the sub-Saharan Africa (SSA) without characterizing it [12]. Furthermore, in Mozambique, Aly and collaborators reported in their study in 2024, a prevalence of 83% of anaemia in children aged 6 –59 months, of which 12% were severely, 48% moderately and 22.4% mildly anaemic. Anaemic female participants' prevalence was 49% compared to 51% males, which is the reverse scenario in our twin pair's population. In addition, children with moderate to severe anaemia were the youngest (p = 0.02) [14]. In our study, although no severe case was observed amongst the twin pairs, 2.4% severe case was observed in the general study population among the youngest population. This observation remains consistent with our findings.

On the other hand, in Guinea Conakry, Kuotu and collaborators studies conducted also in children aged 6 to 59 months in 2023 reported a prevalence of 68.3% of anaemia made of 41.1%, moderate anaemia, followed by severe anaemia at 28.6% and mild anaemia at 28.2% in the general population. However, the anaemia was not characterized [15]. The prevalence of severe anaemia was higher than our data with the exception of mild and moderate anaemic cases beside the fact these kids were receiving fortified diets (iron supplementation (86%) and a fortified diet (86%) [15].

In Burkina Faso, as study by Starck et al., in children under 5 years conducted in a population of 17 599 children reported 83.2% anaemia discriminated into 31.1% moderate, 42.8% mild and 9.2% severe anaemia. Anaemic male (84.5%) and female (81.8%) were almost equally affected [16]. Although, the anaemia was not characterized; the overall prevalence's of the anaemia and severe cases were higher compared to our study findings. Nonetheless, the distribution pattern of the anaemia by sex was online with our general population study data.

It was further observed in our study that the younger twin pairs children aged [0-59M] months were more affected with the anaemia than older children aged the]59M - 6Y] years.

This may be explained by various factors; first due to the gestational numbers (multiple gestations) is source of iron deficiency and anaemia. Indeed, studies have revealed that iron deficiency and anaemia are prevalent in women with multiple gestations under normal pregnancy evolution [15, 17] as well as when coupled to nutritional issues [18]. Indeed, Ru et al., reported in their clinical trial study that out of the 83 women carrying twins, triplets, or quadruplets, 37% delivered with anaemia [17]. On the other hand, Ehouman et al., previously reported that weaned and mixed-fed twin pairs were at higher risk of mild (84.2%) and moderate (81.0%) anaemia in their study [18].

This was due to the fact that their respective mothers were of low-socioeconomic incomes mostly housewives or farmers and or retailers with no formal education. Furthermore, that all parents were living in a rural area where a proper feeding of a normal household is a daily challenge [18]. In contrast, twin pairs children aged]59M-6Y] years old exhibited the

highest prevalence of mild anaemia (62.5%) and the lowest prevalence of moderate (16.7%) with no severe anaemia (0.0%).

This may be explained by the fact that older twin pairs children might have been spending most of their time playing outside or running errands in places where they may grab additional foods like legumes, nuts and seeds, and fruits and vegetables since they all live in rural areas, where their diet is rich and accessible in the above-mentioned vegetables and legumes [18]. These extra micronutrients may provide these children with a healthy iron balance, and reduce their absolute deficiency in iron as previously described by Gupta et al. in 2016, and the Physicians Committee for Responsible Medicine (PCRM)'s Guideline in 2022 [19,20].

In addition, the younger children aged [0-59M] months exhibited the highest prevalence in terms of anaemia intensity whether twin pairs (moderate: 83.3% and severe: 0%) or not (moderate: 85.7% and severe: 100%) in our study. In fact, maternal anaemia during pregnancy is intertwined with childhood anaemia and have both maternal and foetal consequences. This highest prevalence's may be explained by the fact that iron is a major factor in anaemia in the first 46 months of a new born baby life. And the baby may suffer from reduced iron store problem from birth up to infancy if that iron demand is reduced during the rapid growth period. Indeed, the interval from conception of the fetus to two (2) years of life is a critical window when nutritional needs must be met. If these needs are not met, children may experience morbidity or mortality [15, 21-23].

Another finding in this study was that Normochromic microcytic anaemia (NMA) had the highest prevalence rate (23.1%) in the twin pairs children. Similarly, hypochromic microcytic anaemia (HMA) was the predominant one (36.9%) in the overall study population on the basis the MCH, MCHC, and MCV characterization. Indeed, iron deficiency is the most common cause of microcytic and hypochromic either absolute (decreased stores of iron) or functional (inadequate mobilization of the iron stores beside it adequacy) and is the principal aetiology of anaemia which threats significantly the outcomes of life quality and health [24,25].

In addition, Iron deficiency anaemia is classically described as a microcytic anaemia and appears when the body's iron demand is not met by iron absorption from the diet. In most cases, microcytosis is the result of impaired haemoglobin synthesis. Disorders of iron metabolism and protoporphyrin and heme synthesis, as well as impaired globin synthesis, lead to defective haemoglobin production and to the generation of microcytosis and microcytic anaemia [26, 27]. In Côte d'Ivoire, Franziska and colleagues reported a prevalence of 50% iron deficiency anaemia in 2001 same as other researchers [28-31]. Elsewhere, other studies have also reported Iron deficiency anaemia [32-34].

Infectious diseases such as malaria is the most important parasitic disease of human kind. Together with iron deficiency they are the leading factors contributing to the global anaemia disease burden. In this study, plasmodium infected twin children were diagnosed in 80.8% and 76.9% by RDT and GS tools respectively versus 75.4% and 62.2% in the overall study group by RDT and GS as well. In Ghana, Dormechele et al., reported a prevalence of 61.5% of malaria over 5 years' cross-sectional hospital study (2012-2016) among children under-five in 2020 using RDT testing tool.

There was no significant variation in the sex (male: 49.8% versus female: 50.2%) [35]. Similar studies conducted in West Africa, first by Starck et al., in Burkina Faso reported 44% cases (vary from 17.4 - 65.2%) of acute malaria that was defined as positive for both RDT and microscopy testing [11]. This prevalence was lower than ours (Twin pairs: 47.6% and General population: 46.9%). Secondly, in Nigeria, a study by Asiya and Muhammad in 2025 reported a malaria prevalence of 81.5% using RDT testing tool which is higher than our result [36].

In Asiya and Muhammad, study's more male was infected (83%) compared to the female counterparts (80%) with the youngest subjects being the most (85.9%) affected. In terms of sex, the reverse case of scenario was observed in our data where more female (Twin pairs: 77.8% and General population: 84.2%) were infected than male (Twin pairs: 60.0% and General population: 83.3%). However, in terms of age group their finding is in conformity with our one. Indeed, younger children aged [0-59M] months

old were the most infected (Twins pairs: 71.4% and General population: 83.8%) compared to the older children,]59 M - 6 Y].

Elsewhere in Africa, Rugiranka and collaborators observed in children less than five years of age in Malawi, a malaria prevalence of 37.2% using an RDT as diagnostic tool. Also, there was no gender variation as well (male: 36.5% vs. female: 35.8%). In Mozambique, Aly et al., reported 39% of malaria cases by RDTs (female: 40% vs Male: 38%) [14, 37]. In both studies, the reported prevalence was lower compare to our findings.

In contrast to the high malaria prevalence most often reported in the sub-Saharan countries, Gena et al., in 2025 reported 8.5% overall prevalence of malaria of which 4.7% was diagnosed by Microscopy and 6.7% by RDT in Ethiopia [38]. A much lower prevalence compared to our study findings where on the basis of the GS testing (General population: 62.2% and Twin pairs: 76.9%) and by RDT (General population: 75.4% and Twin pairs: 80.8%). Our high prevalence is echoed in several studies report in most West African countries [2, 35, 36] and elsewhere [14, 37].

Gena and collaborators further reported among malaria positive children, a prevalence of 56.3% attributed to P. falciparum and 2.1% of P. vivax. Our study identified 78.3% P. falciparum, 13.0% mixed infections made of P. falciparum & P. malariae and 8.7% P. malariae while no P. vivax was identified, instead a P. malariae. The infection rate was almost equal between genders (44.4% males and 55.6% females) which is in conformity with our findings. Numerous studies have reported genders prevalence lower than what we observed in our study (Dormechele et al., Gena et al., and Rugiranka et al.,) although the overall gender pattern/trend indicates that the female sex are slightly infected more often than male [35, 37, 38].

These finding corroborate the patterns of our data as well with the exception of Rugiranka et al., study. The fact that females often showed slightly higher infection rates than males, is not clear but presumably attributable to the fact that in rural settings, males most commonly spend their time outside running errands or playing while female tends to stay with their mothers in closed doors with much mosquitoes' bites exposure [2, 36]. The high prevalence of malaria in our study findings may be explained by the inadequate use of protective practices such insecticide-nets (ITNs) and indoor residual spraying (IRS) or because our study covered both the dry and the rainy season as reported elsewhere [2, 36, 39].

Another finding in this study relates to the mean parasite density. Indeed, our findings/study data revealed that the younger children had a higher level of parasitemia (10 636.3 /µl of blood) compared to the older children (9354.4 /µl of blood). This may be explained by the maturity of the immune system of the children aged]59 M - 6 Y] years compared with the youngest children [0-59M], with an immature immune system relying mainly on the mother`s transmitted immune protection (maternal antibodies) [40-43]. Furthermore, these high variations in the parasitemia may also be attributed to various other factors, such as the geographic locations, the socio-demographics, the genetic variations among the populations, the utilization of malaria preventive measures, or the nutritional and immunity status of the infants themselves as reported in Marete et al., study [44].

It is also observed in our study that most children aged [0-59M] months old were infected by P. falciparum in both twin pairs (57.2%) and overall study population (41.0%) while a combination of malaria parasites P. falciparum & P malariae (co-infection) was the most prevalent (46.2%) in children aged]59M- 6Y] year old (46.2%) using RDT whereas P. falciparum was the most prevalent malaria parasite (72.2%) identified when using GS testing as a diagnostic tool. Beside P. falciparum, other malaria parasites have also been reported in West Africa.

Indeed, a recent study by Berzosa and collaborators in Equatorial Guinea 2024 reported 69.2%, 87.2%, and 97% of P. falciparum by RDT, GS, PCR respectively. Furthermore, 0.1% of P. vivax was identified by PCR, 1.4 % and 0.4 % P. malariae by GS and PCR while 0.3% and 0.6% of P. ovale by GS and PCR respectively [45]. Similarly, a study by Garba et al., in 8 West African countries (Benin, Burkina Faso, Côte d'Ivoire, Ghana, Nigeria, Mali, Niger, and Senegal) using molecular techniques revealed 93.5% P. falciparum, 80.6% P. malariae, 77.4% P. ovale, and 35.5% P. vivax circulating malaria species.

Amongst these 8 countries only 3 (Burkina Faso, Côte d'Ivoire, and Ghana) do not have all 4 strains currently in circulation (P. falciparum, P. ovale, and P. malariae) while the remaining 5 have all 4 strains (P. falciparum, P. ovale, P. malariae, and P. vivax) in circulation (Benin, Ghana, Nigeria, Mali, Niger, and Senegal) [46]. Elsewhere, in sub-Saharan Africa, Gena et al., reported beside P. falciparum a 2.1% of P. vivax in Ethiopia [38]. In Cameroon, Tchuenkam et al., reported in 2025 a malaria prevalence of 54.8% of which 49.9% were P. falciparum, 4% P. ovale, and 0.9% of P. malariae. Co-infections (3.5%) were also common (P. falciparum & P. ovale then P. falciparum & P. malariae) [47].

The observation of the circulation of such species in the overall population of respective African countries and elsewhere are in line with our findings although in our study no molecular testing was done and could explained why our prevalence's were low. The occurrence of these species and the reported rates in combination with the co-infection rates should draw attention to the fact that West Africa is becoming a potential hub for previously non-encountered malaria species with a potential of mutations.

Our study is influenced by some limitations; firstly, all twin pairs children had a rapid test (RDT), but no corresponding microscopy test (GS) result. Secondly, the size of the study population including the set of twin pairs we worked with was a relatively small sample size of participants. Thirdly, our limited budget did not allow us measure the serum ferritin levels to assess the iron status or the reticulocyte count for confirming if the anaemia is regenerative or not. Lastly, we did not assess WHO signs and symptoms of malaria, such as hyperglycaemia, acidosis, and hyperparasitemia and the presence of other parasites except plasmodium parasites that may have co-infected the same participant causing the observed anaemia in our study group. The acquired knowledge when carrying this study should help further investigations to determine which additional diagnostic testing is need to identify the multifactorial causes of febrile illnesses accomplished in other countries.

Conclusion

Childhood anaemia is a severe public health problem for children aged 7 years in Côte d'Ivoire. This study finding revealed/underscore several factors identified as significant predictors of anaemia severity following malaria infestation. This includes child age, sex, and gestation. The type of anaemia displayed by malaria infected children was predominantly moderate (51.2%) in the overall studied population and mild in the twin pairs (57.1%). The majority of young children [0-59M] were infected by mono-infection of P. falciparum either in the twin pairs or overall study population while in the older children]59M-6Y] mixed-infection of P. falciparum & P. malariae were the most predominant. further studies are need investigated the growing prevalence of malaria parasites co-infection in the country and address the risks that it may poses.

Author Contributions

MAE contributed to the conception of the study, data analysis and interpretation, drafting of the manuscript, and agreed to be accountable for all aspects of the work. WCPE was involved in the data capturing, analysis and review of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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