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Impact of Nutritional Parameters and Socio-demographic Factors in twin pairs Diagnosed with Anaemia in Western Region of Côte D'ivoire

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ABSTRACT

Background: Anaemia is a critical public health problem affecting all incomes country. Globally, 39.8% of children aged 6-59 months are affected of which the WHO African Region accounts for 60.2% of that prevalence. Even though the global prevalence of anaemia in children below five has slowly decreased over the years as per WHO, in Côte d`Ivoire, 72% of the children aged 6-59 months were anaemic in 2019 and the prevalence remains high. In terms of malnutrition, the national prevalence is 6.8%. This research study aims to highlight the nutritional and socio-demographic parameters observed in twin pairs diagnosed with anaemia in the Western part of Côte d`Ivoire, in order to raise public awareness and recommend baselines for future public health strategy of care concerning children of the same age group.

Methods: We recruited 65 male and female children, aged 3 months to 6 years in 8 villages of the Tonkpi Region from March 2020 to May 2021. A questionnaire including anthropometry was used to screen for malnutrition and venous blood samples with a FBC to diagnose and characterize anaemia. Univariate analysis (Chi-2 test (χ2) and P: (Probability) were used for comparison between groups while multivariate analysis was used for the risk factors. Significant test was considered at a threshold of 0.05.

Results: From 65 children who completed the study, 52.3% were female *versus* 47.7% male. Thirteen amongst them were a set of pair twins (10(38.5%) male and 16(61.5%) female). Five sets were homozygotes and eight heterozygotes. Parents were predominantly either housewives 30 (46.2%) or farmers 25 (38.5%). Overall prevalence of anaemia was 41(63.1%) including 14 (34.1%) twins of which 19 (46.4%) was mild, 21(51.2%) moderate and 1(2.4%) severe. Twin pairs expressed equal proportion of mild and moderate anaemia (26.9%) with no severe case observed. Normochromic microcytic anaemia was the predominant form (23.1%) followed by hypochromic microcytic anaemia (19,2%). The high majority of twins 22 (84.6%) were on family meals and 4 (15.4%) on mixed feeding. Malnourished twin pairs aged 0-59 months were sixteen (61.5%) of which 13(81.2%) had moderate acute malnutrition and 3(18.8%) severe acute malnutrition. There was no significant difference related to the intensity of the malnutrition in terms of age group (0-59 months: 7(26.9%) *versus* 59 months to 6 years: 9(34.6%).

Conclusion: Normochromic microcytic was the predominant (23.1%) form of anaemia amongst the twins with 84.6% of them being on family meals, 15.4% on mixed feeding and none on breastfeeding regiment. Twin pairs children aged 0-59 months were almost equally affected (anaemic) as those of 59 months to 6 years' (12 (46.2%) *vs* 14 (53.9%).

Keywords: Anaemia; Nutrition; Socio-demographic; Prevalence; Characterization of anaemia; Children under 7 years; Côte d'Ivoire.

INTRODUCTION

Anaemia is defined as a reduction in the haemoglobin level in the peripheral blood below the normal threshold set for a particular population. Very commonly in the subtropical regions, malnutrition and co-infections are the main causes of morbidities and death according to age and region. Globally, malnutrition remains a serious public health issue linked to either the deficiencies/excesses of nutrient intake or an imbalance of essential nutrients or an impaired nutrient utilization[1]. And so, when using anthropometric parameters such as the weight, the height and the age of a child, one is capable to categorize or identify whether that child is stunted, wasted, and underweight; or simply by determining the micronutrient deficiency of that child[2]. In this regards, a good marker for a recurring or chronic under-nutrition is the low height-for-age z score which is indicative of a stunting condition while a wasting condition is defined as the low weight-for-height z score that quite often refers to an acute or severe loss of weight[2]. On the other hand, underweight which is defined as a low weight-for-age z score can as well be used to classify both a wasted or stunted child. Lastly, impaired nutrients refer to the shortage of elemental vitamins (vitamin A and folate) and minerals (iron, zinc, and iodine) that are necessary to secure an optimum developmental growth of a child during pregnancy and lactation, and may indicate socio-economic challenges due to poverty and food insecurity[1-2]. Each of these conditions constitute very serious burdens that can last for the individuals and their families, for the communities and for the respective countries. Indeed, it is estimated that almost half of children under 5 years' old deaths are linked to under-nutrition and approximately 2 billion people worldwide are affected by micronutrient deficiency[1]. Furthermore, in low- and middle-income countries a high rate of infectious disease is another direct cause of malnutrition[3-4].

Elsewhere and most particularly in West-Africa, malnutrition is very prevalent in 0-59 months old children as reported in studies conducted in various countries like Nigeria where the prevalence of underweight children was 22% in 2018[5], in The Gambia, 10.6% in 2018[6], in Liberia, 11.4% in 2020[7], in Senegal, 71.27% in 2019[8], in South Africa, 6.8% in 2018[9], and in Democratic Republic of the Congo (DRC), 43% in 2018[10].

In Côte d'Ivoire, malnutrition has an enormous weight on the development of key sectors such as education, health and economy with the national prevalence at 6.8% and severe acute malnutrition at 1.9%[11]. Parasitic infections such as malaria are endemic leading to severe consequences such as anaemia with high morbidity and mortality rates, especially in children[12]. Furthermore, anaemia is endemic like Malaria and rages with varying prevalence from one region to another, depending on age and sex. The recent WHO report of the year 2020 indicated that the prevalence of anaemia was 72.2% in children aged 6 to 59 months and 51% in women of childbearing age (women aged 15 to 49) in Côte d`Ivoire[13].

Hence, it seems appropriate, even essential, to conduct research to highlight these morbidities, which represent some public health problems for a better prevention and cure. This paper reports findings on the nutritional status and socio-demographic parameters observed in twin pairs diagnosed with anaemia and secondly characterize the type of anaemia observed in the Western part of Côte d`Ivoire, in order to recommend better strategy of care.

METHODS

Study area and participants

This observational clinical research study was carried out from March 2020 to May 2021 in eight (8) villages of "TONKPI Region" (Man). A sample of 65 participants amongst which thirteen sets of twins' pairs was obtained. Children aged 3 months to less than 7 years were enrolled and provided blood samples for the diagnosis and characterization of the anaemia and a questionnaire detailing socio-demographic, nutritional, and anthropometric

information was administered to inaugurate the nutritional health status (Supplemental Table).

Socio-demographic

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 through the same questionnaire administered to each enrolled participant who signed a written informed consent or gave a fingerprint (illiterate participants).

Nutrition information

Nutritional data including the type of diet and its component as well as the frequency of the eaten food/diet were also collected through the same administered questionnaire.

The type of diet was categorized as exclusive breastfeeding if the child is only breastfeeding, mixed feeding when in addition to the breast milk the mother is given the family food or complementary or supplementary food, and exclusive family food when the child is weaned and eating only the family food.

The component refers to the content of the diet while the frequency refers to how many meals the child takes per day.

Anthropometric data

Anthropometric information was obtained using the Weight-for-height z-score (WH-Z score) for the children aged between 0-59 months (≤ 240 weeks) and Body Mass Index-for-age z-score (BMI-Z score) for the children aged between 59 months to 6 years [241-336] Weeks through the same administered questionnaire.

Anthropometric indices were calculated using either the BMI (weight/height^2) or the reference medians recommended by the World Health Organization (WHO) and classified according to standard deviation units (z-scores), based on the WHO criteria[14]. The indicators studied were weight-for-height Z scores (WHZ) and BMI z-score. The WH-z score was calculated by expressing the Height in cm divided by the Weight in kg. Was considered as normal a WH-Z score between -1 and -1.5, Moderate acute malnutrition <−2 standard deviations (SD) and ≥−3 SD of the median, Severe acute malnutrition <− 3 SD of the median, Overweight age >2 SD and ≤3 SD of the median, and Obese >3 (SD) of the median. Similarly, the BMI z-score was calculated by expressing the Weight in Kilograms (kg) divided by the square of Height in meter (BMI = kg/m^2). Was considered as normal BMI = 18.5 - 24.9; malnourished 9 - 18.4 (moderate = 16 -18.4; severe = 9-15.9), and Overweight = 24.9 – 30, and Obese = > 30.

Blood collection

2 mL of venous blood were drawn in the morning in 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 (CHR) of Man for the haematology testing (FBC).

Haemoglobin determination and classification of anaemia

The full blood count (FBC) was performed using URIT 3000 PLUS analyser, Urit Medical Electronics, China and the printed result was 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 [15]. 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. Risk factors were assessed using multivariate logistic regression analysis. P-values less than 0.05 were considered significant.

Ethical 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 parents or legal representatives 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. Out of this total, 34 (52.3%) were female and 31 (47.7%) were male. In addition, there were thirteen (13) sets of twin pairs of which 10(38.5%) were male and 16 (61.5%) female. These 13 sets were constituted of five (5) sets of Homozygotes and eight (8) sets of Heterozygotes babies (Table 1).

Table 1: Distribution of the study participants and set of twins pairs per village and sex (N = 65) in TONKPI Region Côte d'Ivoire, from March 2020 to May 2021.

Department	Sous-Prefecture	Villages	Sex		Examined Participants n (%)	Type of twin		Sex		Examined Participants n (%)
			Male	Female		Homozygote	Heterozygote	Male	Female	
BIANKOUMA	Kpata	Kpata	5 (7,7)	5 (7,7)	10 (15,4)	0	2	2	2	4 (15.4)
MAN	Gbangbégouiné-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)
	Man	Bantégouin	1 (1.5)	2 (3.1)	3 (4,6)	1	0	0	2	2 (7.7)
		Lamapleu	1 (1.5)	3 (4.7)	4 (6.2)	1	1	1	3	4 (15.4)
	Sangouiné	Tiapleu	2 (3.0	3 (4.7)	5 (7.7)	0	1	1	1	2 (7.7)
Total	4	8	31 (47.7)	34 (52.3)	65 (100)	5	8	10 (38.5)	16 (61.5)	26 (100)

Socio-demographic of participants

The overall study participants (65 children) belong to three (3) ethnic groups [Akan (Baoulé), Gour (Lobi & Sénoufo from Côte d'Ivoire), and Mandé (Yacouba) the predominant ethnic group of the Tonkpi region. We noted that 54 (83%) of the

participants were Yacouba followed by the Sénoufo 5 (7.7%), Baoulé 4 (6.2%) and the Lobi 2 (3.1%). The majority of the twins 22 (84.6%) were Yacouba followed by the Baoulé and the Lobi with an equal proportion 2 (7.7%) (Table 2).

Table 2: Prevalence of twins and parental occupation, in the western region of Côte d'Ivoire, from March 2020 to May 2021.

Ethnic group	Tested	Twins	Non-twin	Profession	Tested	Twins	Non-twin
Baoulé	4 (6.2)	2 (7.7)	2 (5.1)	Retailer	6 (9.2)	4 (15.4)	2 (5.1)
Lobi of CI	2 (3.1)	0	2 (5.1)	Hairstylist	4 (6.1)	4 (15.4)	0
Sénoufo of CI	5 (7.7)	2 (7.7)	3 (7.7)	House wife	30 (46.2)	12 (46.1)	18 (27.7)
Yacouba	54 (83.0)	22 (84.6)	32 (82.1)	Farmer	25 (38.5)	6 (23.1)	19 (48.7)
Total	65 (100)	26 (100)	39 (100)	Total	65 (100)	26 (100)	39 (100)

CI: Côte d'Ivoire

In terms of the profession of the parents, the majority of them were either house wives 30 (46.2%) or farmers 25 (38.5%). Amongst the twins, 12 (46.1%) parents were housewives while 6 (23.1%) were farmers.

All study participants reside in rural areas with similar lifestyle patterns and their parents earn their living mostly as farmers, house wives or commercial traders.

Anthropometric data

The study participants' age was stratified into 2 groups: a) 0-59 months and b) 59 months to 6 years. The stratification process, allocated 39 participants (60.0%) to the 0-59 months' age group and 26 participants (40.0%) to 59 months to 6 years' age group (Table 3). In addition, 14 (53.8%) of the twins were amongst the 0-59 months' age group while 12 (46.2%) were amongst the 59 months to 6 years age group.

Most of the participants were malnourished (58.5%). And so, were the twins with 61.5% prevalence. The mean Z-score for the children 0-59 months was -1.37 while the mean BMI for those of]59M- 6Years] was 15.95. The study revealed that twenty (51.3%) children out of the 39 participants aged 0-59 months had a good nutritional status while 19(48.7%) had an abnormal one. Out of the 19 malnourished children, seventeen (43.6%) had moderate acute malnutrition while two (5.1%) had severe acute malnutrition. In terms of the twin pairs, the study revealed that seven (50.0%) out of the fourteen twin pairs were malnourished. Amongst them, six (42.9%) had a moderate acute malnutrition and one (7.2%) a severe acute malnutrition (Table 3).

On the other hands, seven (26.9%) children out of the twenty-six (26) children aged 59 months to 6 years' whom the nutritional health status were classified using the BMI had a normal nutritional status and nineteen (73.1%) were abnormal. Out of these nineteen malnourished children, seventeen (65.4%) had moderate acute malnutrition and two (7.7%) had severe acute malnutrition. In terms of intensity of the anaemia amongst the twin pairs aged 59 months to 6 years', 7 (58.3%) out of the 12 twins (46.2%) had moderate malnutrition, 2 severe acute malnutrition (16.7%), while 3 were normal (25.0%) (Table 3).

Table 3: Intensity of nutritional status in twins' and overall participant's as per age group, in the western region of Côte d'Ivoire, from March 2020 to May 2021.

Severity of Nutritional status	Tested Participants		Twins		Non-twin	
	Age group		Age group		Age group	
	[0-59M]	]59M- 6Y]	[0-59M]	]59M- 6Y]	[0-59M]	]59M- 6Y]
Good/Normal	20 (51,3)	7 (26,9)	7 (50.0)	3 (25.0)	13 (52,0)	4 (28.5)
Moderate	17 (43,6)	17 (65.4)	6 (42,9)	7 (58.3)	11 (44.0)	10 (71,5)
Severe	2 (5,1)	2 (7,7)	1 (7,2)	2 (16.7)	1 (4.0)	0
Total	39 (60.0)	26 (40.0)	14 (53.8)	12 (46.2)	25 (64.1)	14 (35.9)

M: month, Y: year, Pearson chi2(χ2) = 3.3416 P: 0.342

Nutrition information

Three types of diet were observed in the study namely the exclusive breastfeeding, mixed feeding (Breastfeeding plus family meal) and exclusive family meal (Table 4).

Daily family meals were constituted predominantly in every household of cereals, root & tubers, beans & legumes, fruits, vegetables and very seldom of meat (bush meat or cow meat), poultry, dry fish and milk & other dairy products) (Data not shown). Most frequently consumed cereal and legume products included locally made maize meal and rice, cowpea and groundnuts. Fruit banana was the fruit often consumed. In terms of vegetables, spinach (*Spinacia oleracea*), water leaf, (*Talinum triangulae*), *Corchorus olitorius* and okra (*Hibiscus esculentus*) were the most consumed. Meat, milk and other dairy products as well as beverages were not frequent in their daily food intake which consisted mostly of bush meat, cow meat, powdered milk, coffee, as well as soft drinks.

The high majority of children 52 (80.0%) out of the 65 children were on family meals (weaned babies). Amongst the twin pairs, 22 (84.6%) were exclusively on family meals as well (Table 4). In terms of diet frequency, all children had an average of 3 meals a day for children aged 59 months to 6 years' while those aged 3 months to 24 months old had more depending on how often they breastfed (Data not shown).

Table 4: Type of diet used by twins and overall participant's in the western region of Côte d'Ivoire, from March 2020 to May 2021.

Type of feeding	Tested Participants	Twins	Non-twin
Exclusive breastfeeding	6 (9.2)	0	6 (15.4)
Breastfeeding & family meal	7 (10.8)	4 (15.4)	3 (7.7)
Family meal only (Weaned baby)	52 (80.0)	22 (84.6)	30 (76.9)
Total	65	26	39

Pearson chi2(χ2): 4.9725, P: 0.083

Prevalence and distribution of anaemia according to sex and age

Overall, there were 41(63.1%) participants that were anaemic and displayed predominantly a moderate anaemia 21 (51.2%) followed by mild anaemia 19 (46.4%) and one (2.4%) severe case (Table 5 and 6). Amongst the anaemic participants, 14(34.1%) were twins (Table 5) and showed equal proportion of mild and moderate anaemia (26.9%). No severe anaemia was identified amongst the twins. The high majority of non-twin had a moderate anaemia 14(51.9%) followed by the mild one 12(44.4%) and one case of severe anaemia 1(3.7%) (Data not shown).

The characterization of the 41 anaemic participants indicated that the majority of them 24(36.9%) had a hypochromic microcytic anaemia (HMA), followed by 10(15.4%) normochromic microcytic anaemia (NMA), then 5(7.7%) normochromic

normocytic anaemia (NNA), and lastly 2(3.1%) normochromic hypochromic anaemia (NHA) (Table 5). Concerning the twin pairs, 14(34.1%) were anaemic with the majority of them 6(23.1%) having a normochromic microcytic anaemia (NMA) followed by 5(19.2%) of hypochromic microcytic anaemia (HMA). The study further noted that, participants of both genders were almost equally affected (48,8% male *vs* 51.2% female). In addition, children aged between 0-59 months were more affected than those of 59 months to 6 years' age group (66.7% *vs* 57.7% respectively). No case of Macrocytic anaemia was detected in the study (Table 5).

Table 5: Characterization and Prevalence of anaemia by sex and age, in the western region of Côte d'Ivoire, from March 2020 to May 2021.

Type of Anaemia	Anaemic Participants	Twin	Sex				Age group			
			Male	Female	χ2	P	[0 - 59 M]	]59 M - 6 Y]	χ2	P
HMA	24 (36.9)	5 (19,2)	11 (35.5)	13 (38.2)	0.0527	0.818	18 (46.2)	6 (23.1)	3.5671	0.059
NMA	10 (15.4)	6 (23.1)	4 (12.9)	6 (17.6)	0.2803	0.596	5 (12.8)	5 (19.2)	0.4924	0.483
NNA	5 (7.7)	2 (7.7)	4 (12.9)	1 (2.94)	2.2664	0.132	2 (5.1)	3 (11.5)	0.9028	0.342
HNA	2 (3.1)	1 (3.8)	1 (3.2)	1 (2.9)	0.0044	0.947	1 (2.6)	1 (3.9)	0.0860	0.769
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, M: month, Y: year, Pearson chi2(χ2), P: prevalence

The relationship between diet and anaemia.

In terms of the correlation between the type of diet and anaemia, the study firstly revealed that out of the 41 anaemic children 4 of them were on an exclusive breastfeeding (9.8%), with additional 4 (9.8%) on breastfeeding and family meal (mixed feeding) and 33 (80.4%) on exclusive family meals (weaned babies) (Table 6). With regards to the twin babies, none of them was on exclusive breastfeeding regiment. However, 4 (15.4%) were on mixed feeding while the high majority 22 (84.6%) were on family meal (Table 4). In terms of the relationship between the diet and the anaemia, the study revealed that, 19 (46.4%) participants out of the 41 anaemic children including the malnourished ones had a mild type of anaemia, 21 (51.2%) a moderate and 1(2.4%) severe anaemia (Table 6).

Table 6: Relationship between diet and anaemia, in the Western region of Côte d'Ivoire, from March 2020 to May 2021.

Type of feeding	Non anaemic	Intensity of Anaemia		
		Mild	Moderate	Severe
Exclusive breastfeeding	2 (8.3)	1 (5.3)	3 (14.3)	0
Breastfeeding & family meal	3 (12.5)	2 (10.5)	1 (4.7)	1 (100)
Family meal only (Weaned baby)	19 (79.2)	16 (84.2)	17 (81.0)	
Total	24 (36.9)	19 (46.4)	21 (51.2)	1 (2.4)

Risk factors associated to the anaemia

In terms of risk factors, the results of multivariate logistic regression analysis are summarized in Table 7. Males (odds ratio (OR) = 1.06, 95% CI: 0.37 - 3.02) and house wife (OR = 1.16, 95% CI: 0.39 – 3.47) were at a higher odds of anemia. The odds of having anemia were lower among twin pairs (OR = 0.54, 95% CI: 0.19 - 1.54) and children aged 59 months-6 years (OR = 0.69, 95% CI: 0.23 - 2.07). The associations were not statistically significant (p>0.05).

Table 7: Association between anaemia and gender, twins, parent profession and age category.

	Association	OR[a]	P-value	95% IC
Anaemia	Sex			
	Female	1.00		
	Male	1.06	0.911	0.37 - 3.02
	Twins			
	No	1.00		
	Yes	0.54	0.251	0.19 - 1.54
	Housewife			
	No	1.00		
	Yes	1.16	0.796	0.39 – 3.47
	Age Category			
	[0-59M]	1.00		
	]59M- 6Y]	0.69	0.503	0.23 - 2.07

OR[a]: Adjusted Odds Ratio (all models adjusted for sex, twin pair, profession and age category), CI: Confidence Interval, P: Probability

DISCUSSION

This observational community-based research study conducted in children between three (3) months to six (6) years in eight villages of the Tonkpi region in Man, Western Côte d`Ivoire finding showed a high prevalence of anaemia 63.1% confirming that Anaemia remains a major public health issue in Côte d'Ivoire. Globally, a meta-analysis study by Stevens *et al.,* in children and women from 133 countries indicated that 40% of children aged 6-59 months were anaemic in 2019 and the prevalence in 11 countries in the same age group exceeded 70%[16]. A study by Ba *et al.,* in Senegal reported[17] a prevalence of 59.9% in children of the same age group although it was conducted in a sub-urban area. A contrario, the continuous Demographic Health Survey (DHS, EDS-C) report in 2018 of Senegal indicated a prevalence of 71% of children aged 6 months to 59 months[18]. Even though our study prevalence was higher than that of Ba and collaborators, it is also less than the national Senegalese reported prevalence and that of the sub-Saharan Africa of 64.1% [95% CI: 63.9%, 64.4%][17,19]. Similarly, in Guinea-Bissau, a study by Thorne and collaborators found 80.2% prevalence of anaemia in children of the same age group[20]. In Liberia, Adugna *et al.*, reported[7] 70.8%, when Zavala *et al.,* reported[21] a prevalence of 59.6% in Chad, and Habyarimana *et al.,* reported[22] a prevalence ranging from 19% to 61.8% in some provinces in Rwanda. In terms of twin pairs, this is the very first study in Côte d'Ivoire and West-Africa. The study found 14(53.8%) of the twins to be anaemic amongst which 4(15.4%) male and 10(38.5%) female.

The intensity of anaemia in the studied population was 46.4% mild, 51.2% moderate and 2.4% severe with a slightly equal prevalence amongst participants of male and female gender respectively 48.8% and 51.8%. On the other hand, half of the anaemic twin pairs had mild and moderate anaemia (7(50.0%). No severe case was observed.

Hypochromic microcytic was the predominant type of anaemia (36.9%) observed in the overall study population followed by normochromic microcytic anaemia (15.4%). In the twin pairs the reverse order was observed *i.e.* normochromic microcytic anaemia was the predominant form (23.1%) followed by hypochromic microcytic anaemia (19,2%). Ba *et al.,* reported[17] 48.9% mild, 48.9%, moderate and 2.2% severe anaemia in Senegal with 92.1% of Microcytic while Tesema *et al.,* reported[19] 26.2% mild, 34.9% moderate and 3% hypochromia severe anaemia sub-Saharan Africa. In Liberia, Adugna *et al.,* reported[7] 3.4% severe, 38.3% moderate, and 29.1% mild anaemia while in Cabo Delgado - Mozambique, Aly *et al.,* reported[23] a prevalence of 83% anaemia of which 23% of children had a mild, 48% moderate and 12% severe although in both countries the anaemia was not categorized.

This high prevalence of anaemia observed in this study and also reported in various settings may be explained either by the high need for iron in the development of children's early-life growth[24] or a lack of access to basic healthcare services in rural

areas[7] or an increase burden of chronic malnutrition and inadequate dietary intake of nutrients in developing countries[25,26]. Lastly, it might be due to children's extended exposure to unhealthy environments with great vulnerability to diseases such as malaria, hookworms, schistosomasis, and visceral leishmaniasis[12,27-29].

In terms of diet, the majority of participants in the study were weaned babies (80.0% followed by mixed fed children (10.8%), and then the exclusively breastfed children (9.2%). Similarly, 22(84.6%) of the twin pairs were weaned babies as well while 4(15.4%) were mixed feed. Fabunmi et *al.,* reported[25] 12.8% exclusively breastfeeding in their study in Nigeria. As per Tesema *et al.,* study, 31% (range 7-70%) of infants aged 0-5 months in sub-Saharan African countries were predominantly breastfed[19]. In Senegal, Ba and collaborators reported[17] in their study that 42.1% of children were on family meals, and 75.7% were breastfeeding inclusive of complementary feeding (mixed feeding).

This study showed an overall anaemic prevalence of 63.1% for the participants aged 0-59 months (younger) and 36.6% for children aged between 59 months through 6 years (older). The male and the female gender were almost equally affected (48.8% *vs.* 51.2%) respectively. In addition, 46.2% of younger children and 23.1% of older ones were predominantly hypochromic microcytic anaemic. They were followed by the normochromic microcytic anaemic children at a respective rate of 12.8% and 19.2%. This was also observed by Aly *et al.,* in Cabo Delgado- Mozambique, and Zavala *et al.,* in Chad where younger children were more anaemic than the older ones with no sex difference[21,23]. The fact younger children were the most affected (anaemic) may be explained by the fact that these kids were born from anaemic pregnant mothers (poor maternal iron reserve during pregnancy). Therefore, they did not receive sufficient amounts of micronutrients including iron, zinc, folate and vitamin B_{12} from their respective maternal breast milk [30] since both mother and child share a strong haemoglobin concentration relationship [31]. Indeed, iron is a major factor of anaemia in the first 46 months; the interval from conception of the foetus to 2 years of life is a critical window when the needs of nutrition must be secured. If not, the nutritional deficiencies could lead to early life child morbidity or mortality including irreversible cognitive dysfunctions, low-expected school performance, and growth affection and its sequel[32-35]. The high majority of the hypochromic microcytic is due to the abnormal haemoglobin synthesis in the erythroblasts, due to unavailability of iron for haemoglobinosynthesis in these children (iron deficiency anaemia)[36,37].

In the younger children, we noted the highest prevalence of anaemia being the hypochromic normocytic anaemia (46.2%), followed by normochromic microcytic anaemia (12.8%). A similar pattern was also observed in the older children with hypochromic normocytic and normochromic microcytic anaemia displaying 23.1% and 19.2% prevalence respectively. Furthermore, the weaned children expressed the highest prevalence of mild (84.2%) and moderate (81.0%) anaemia. These may be explained first by the fact that the complementary foods, intended for the progressive adaptation of these children (≥ 6 months) to the family food was neither correctly introduced nor these children received any supplementary/formulated foods (ready-to-eat or milled form[38-41]. Secondly, the fact that maternal education is a precise and strong indicator of children's nutritional outcomes as it assists in the improvement of the child healthcare system and good nutritional feeding practices so that the promotion of exclusive breastfeeding and the introduction of appropriate complementary feeding are correctly done[38,39,42,43]. In our study, the majority of parents were of low-socio economic incomes, mostly housewives (46.2%), followed by farmers (38.5%) and retailers (9.2%). All living in a rural area where a proper feeding of a normal household is a daily challenge. Similar pattern was observed in the twin pairs' parents with 46.1% housewives, followed by 23.1% farmers and 15.4% retailers. No parent received a proper education

with a steady job that could provide some substantial incomes to support their respective family. A study by Beni *et al.,* in five West African Countries revealed that children of mothers with no education were more susceptible to be malnourished (OR=1.243; p < 0.0001; 95% CI: 0.996-1.345) compared to children of mothers with only primary or tertiary education[44]. Indeed, an educated mother could influence the well-being and survival of their offspring by introducing healthy behaviour in the household leading to a more equitable distribution of the resources, or challenge the traditional beliefs and attitudes and, or introduce developmental initiatives using modern health care[45,46]. Furthermore, the size of the household and the Fathers' education are critical determinants that positively correlated with nutritional status of a child health since in most settings and particularly in Africa, fathers are usually the bread winners and decision makers[47-49].

In terms of nutritional status, the younger children were more affected compared to the older ones (60% *versus* 40% respectively). The association between the type of diet and anaemia showed that the high majority of anaemic participants were the weaned children (84.2% mild and 81.0% moderate with no severe case) inclusive of the 84.6% of the weaned twin pairs. The children on mixed diet displayed the only severe case of anaemia with 10.5% mild and 4.7% moderate anaemia. On the other hand, the exclusive breastfeeding diet showed 5.3% mild and 14.3% moderate with no severe case. These observations may be explained firstly by the fact, all participants resided in rural settings where their staple foods were made mainly of unrefined grains and derived products (example cereals, breads, legumes, and vegetables products including rice, maize, cowpea, groundnut, spinach, *Hibiscus esculentus* (okra) and flour) (data not shown). These foods are potentially rich in substances that inhibit the intestinal absorption of non-haem iron (phytates, which cause poor bioavailability of iron)[25,50]. Their animal food intake was very seldom and linked to few households (data not shown). In addition, the insufficient micronutrient dietary intake (vitamin A, iodine and iron deficiencies) and the low-quality of absorbed food or the grain-based complementary foods are poor sources of absorbable iron leading to unhealthy child growth and development[51]. Secondly, the sociodemographic factors such as maternal education, household wealth status, number of household members, sex of the child, type of birth, and maternal age[19] as well as early introduction (at age 3 - 6 months) of non-fortified complementary plant-based foods is a risk factor for anaemia[42, 43].

Lastly, the association between anemia and gender, twin pairs, parent profession and age category showed no significant correlation (p>0.05) even though the adjusted OR (OR^a:) of having anemia were lower among twins (OR^a = 0.54, 95% CI: 0.19 - 1.54) and children aged 59 months - 6 years (OR^a = 0.69, 95% CI: 0.23 - 2.07). Nonetheless, studies conducted in Ethiopia and Brazil showed different outcomes with an association between the age category and the anaemia[52-53]. An additional study in Ethiopia displayed an association between the anaemia and the age category, the sex, and no formal education[54].

Our study showed some limitations; firstly, the size of the study population including the set of twin pairs. Secondly, we did not assess the presence of other parasites that may have co-infected the same participant causing the observed anaemia. Thirdly, we also did not 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 neither the aetiology of malnutrition, the anaemia nor the educational status and incomes of the parents in our study group.

CONCLUSION

From the presented data, the prevalence of anaemia among twin pairs children aged less than seven (7) years is still high (34.1%) with the moderate and mild normochromic microcytic anaemia (NMA) sharing equally predominance (26.9%) in the Western region of Côte d`Ivoire. 84.6% of twin children are on family meals and,

15.4% on mixed feeding. Furthermore, 50.0% of twin children aged between 0-59 months were malnourished with 42.9% moderate acute malnutrition and 7.2% severe acute malnutrition compared to 75.0% of the age group 59 months to 6 years amongst which 58.3% moderate malnutrition and 16.7% severe acute malnutrition. We therefore, recommend similar studies countrywide to identify the problem through continued and effective efforts in order to find suitable solutions to improve the general health of the high-risk population in each community.

Conflict of Interest:

The authors have declared that no competing interests exist.

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Supplemental Table: Questionnaire on socio-demographic characteristics and nutritional parameters at household level

A- General Information

Type of visit: ----------

- Date of visit [DD/MM/YYYY]: -- --/-- --/-- -- -- --
- Participant Screening No.: ---------- Enrolment No. ----------
- Site or Centre (Village): ----------
- Address: ----------
- Telephone:

B- Socio-demographic information

- Participant's surname & first names: ----------
- Date of birth [DD/MM/YYYY]: ------/------/--------- or (Age) -------- years months
- Occupation (Mother/Father/Legal Representative): ----------
- Gender: ----------
- Ethnic Group: ----------

C- Constants

- Body temperature (°C): ----------
- Weight (Kg): ----------
- Height (cm): ----------

D- Feeding details

- Number of meals/day: ----------
- Type of meal: ----------
- Nutritional status: Malnourished (moderate □, severe □) or, Normal (Good) □