Diagnostic Patterns of Iron Deficiency and Iron Deficiency Anaemia in Children of 6 to 59 Months in Cameroon

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Abstract — A Cross sectional multicentre study was carried out to determine the prevalence of Iron deficiency (ID) and Iron deficiency anaemia (IDA) in children using Ferritin and full blood count. Full blood count was done with Mindray Bc-2800 and Ferritin test with Enzyme Linked Immuno-Sorbent Assay. Authorization was granted by Cameroonian national Ethical Committee. Data were analysed using Statistical Package of Social Sciences 21.0 significant results were considered for $P < 0.05$. ID varied from 4.2% to 9.6% for cut-offs of 30 and 50 µg/L. IDA ranged from 4.2 to 8.5% at these 2 cut-offs. Considering red cells indices, Mean Corpuscular Volume and Mean Corpuscular Haemoglobin blood count currently used should be interpreted cautiously as only Mean Corpuscular Volume and Mean Corpuscular Haemoglobin corroborate with a Ferritin level lower than 30 µg/L.

Index Terms — anaemia; Ferritin; iron deficiency; Red cells indices.

I. INTRODUCTION

Iron is an essential element involved in energy metabolism and other biochemical processes, including oxygen transport in blood, oxidative phosphorylation in cellular respiration, erythropoiesis, and DNA synthesis [1]. While studying the effect of iron supplementation in children, Lozoff *et al* [2] stated that iron deficiency (ID), a major cause of anaemia can result in intellectual reduction and motor function damage. It has also been revealed about micronutrients deficiency in developing countries, that every second, about 40% of preschool children are estimated to be anaemic [3]. In Cameroon for instance, demographic and health surveys, reported that 60 % of children aged from 6 to 59 months were anaemic [4].

Iron deficiency anaemia (IDA) is a world health problem and a burden to half of the world's population, its prevalence varies widely by geography, age and sex [5]. WHO estimates that IDA resulted in 273 000 deaths: out of which 45 % in southeast Asia, 31% in Africa, 9% in the eastern Mediterranean, 7% in the Americas, 4% in the western pacific and 3% in Europe. This means 97% of deaths occurs in low and middle income countries [6]. Economically the loss due to IDA in 10 developing countries was estimated at 4% of gross domestic income [7].

In many developing countries, iron deficiency anaemia is aggravated by worm infections, malaria and other infectious diseases such as HIV and tuberculosis. Cameroon national survey found that the prevalence of ID in children of 12-59

months of age ranged from 14% to 68% depending on the indicator and adjustment for inflammation applied [8].

The aim of this study was to determine the prevalence of ID and IDA in children through Ferritin test recommended by WHO and evaluate the effectiveness of full blood count, the commonest test available in Cameroonian health settings.

II. MATERIALS AND METHODS

A. Materials

Data were collected through a questionnaire, after parental agreement. Once the identification of the children was done, blood was drawn into EDTA tube for full blood count and dry tube for Ferritin analysis.

B. Methods

This was a cross sectional multicentre study carried out at the Bertoua regional Hospital, Douala Gynaeco-Obstetric and Paediatric Hospital and Yaounde University Hospital Centre from November 2018 to October 2019. The target population was children of 6 to 59 months attending the different study sites. Sample size was calculated using the Lorentz formula. All the children of the target age visiting the paediatric unit and having a prescription of full blood count were included. Children with Haemoglobin (Hb) level < 11 g/dL were considered anaemic and those with $Hb \geq 11$ g/dL were non-anaemic [9]. Children with neurologic affection or motor dysfunction were not included in the study. Full blood count testing was performed using Mindray Bc-2800. Anaemic children were classified into severe ($Hb < 7$ g/dL), moderate ($Hb: 7-9.99$ g/dL) and mild ($Hb: 10-10.99$ g/dL). Three Erythrocytes parameters were considered: Mean Corpuscular Volume ($MCV < 80$ fl), Mean Corpuscular Haemoglobin ($MCH < 26$ pg) and Mean Corpuscular Haemoglobin Concentration ($MCHC < 32$ g/dL). Blood in dry tubes were centrifuged at 2500G for 5 minutes to obtain serum. The serum was kept at -20°C for batch analysis of Ferritin using ERBALISA Kits by Cal Biotech Lab (USA) at the serology bench of the main laboratory of the Bertoua Regional Hospital. Normal values for this kits were given for men and women but not for children. We considered different cut-offs, of 30 and 50 µg/L to estimate the prevalence of ID and IDA. This study was approved by the Cameroon National Ethical Committee for Research in human health, the Regional delegations of public health of the East and Centre regions, the scientific committee of the Douala Gynaeco-Obstetric and Paediatric Hospital.

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The collected data were computed in Excel 2013 and analysed with Statistical Package for Social Sciences SPSS (version 21.0) for Windows (SPSS Inc., Chicago, IBM, USA) using Pearson's Chi Square and Fisher exact tests, a 95% confidence interval (95% CI) with P value <0.05 for significant difference. Receiver Operating Characteristics (ROC) curves analysis was used to test the performance of Ferritin cut-offs of 30 and 50 µg/L.

III. RESULTS

383 children were recruited during this study from the three regions, 243 at the Bertoua Regional Hospital, 95 from Douala Gynaeco-Obstetric and Paediatric Hospital and 45 from Yaounde University Hospital Centre. Male were more represented than female with 56.1%. The children were mostly of the age of 6 to 23 months with a mean of 25.2 ± 0.7 months (Table I).

TABLE I: DISTRIBUTION OF CHILDREN (6 - 59 MONTHS) ACCORDING TO SOCIO-DEMOGRAPHIC DATA

Socio-demographic data N = 383	Category	Number per category n (%)
Gender	Male	215 (56.1)
	Female	168 (43.9)
Age (months)	6-23	184 (48)
	24-41	124 (32.4)
	42-59	75 (19.6)
Caregivers' education level	None	27 (7.1)
	Primary	18 (4.7)
	Secondary	236 (61.6)
	University	102 (26.6)
Child's education	None	256 (66.8)
	Pre-nursery	32 (8.4)
	Nursery	89 (23.2)
	Primary	6 (1.6)

A. ID tested by Ferritin

With 2 different cut-offs level experienced for Ferritin, iron deficiency was observed in 4.2% of children for Ferritin cut-off <30 µg/L and 9.6% for Ferritin cut-off < 50 µg/L (Fig. 1).

Fig. 1. Iron deficiency in children considering a Ferritin cut-offs < 30 µg/L and < 50 µg/L (N = 383).

B. IDA tested by Haemoglobin level and Ferritin

In total among the recruited 383 children, 44.4% (170) were non anaemic. Ferritin level did not relate to the gravity of anaemia, thus all the children with severe anaemia had a Ferritin level >100 µg/L (Table II). Additionally, all the children with Ferritin lower than 30 µg/L suffered of mild or moderate anaemia.

TABLE II: DISTRIBUTION OF ANAEMIC CHILDREN REGARDING THE TYPE AND THE SEVERITY OF ANAEMIA

Variable	Category	Severe n (%)	Moderate n (%)	Mild n (%)	Total n (%)	Fisher exact (p-value)
N = 213						
Anaemia's type	IDA	0 (0)	4 (1.9)	5 (2.3)	9 (4.2)	8.794 (0.161)
	IDA/ACD	0 (0)	26 (12.2)	21 (9.9)	47 (22.1)	
	ACD	19 (8.9)	69 (32.4)	68 (31.9)	157 (73.7)	
	Total	19 (8.9)	99 (46.5)	95 (44.6)	213 (100)	

The mean Ferritin level was 290.6 ± 12.8 µg/L for all the children, with a mean of 242.2 ± 16.2 µg/L in non anaemic lower than the mean in anaemic children (329.2 ± 18.6 µg/L). The frequency of IDA varied from 4.2% to 8.5% with respective cut-offs of 30 and 50 µg/L (Fig. 2).

Fig. 2. Iron deficiency anaemia in children considering a Ferritin cut-offs < 30 µg/L and < 50 µg/L (N = 213).

The ROC curves testing the performance of Ferritin cut-offs at 30 and 50 µg/L showed that the area under curve (AUC) of 30 µg/L (0.985) was greater than the one of 30 and 50 µg/L (0.936) (Fig.3).

Fig. 3. Receiver Operating Characteristics (ROC) curves for 2 cut-offs of Ferritin in IDA.

C. Red cells indices and ID

Using full blood count to determine iron deficiency showed consistent results while considering low MCV, low MCH and a Ferritin cut off <30 µg/L (4.2%). Oppositely low MCHC revealed iron deficiency only in one quarter (1%) of the children tested by Ferritin.

Looking into anaemia and red cells indices, we found that MCHC showed a significant difference between anaemic and non anaemic children with a P = 0.002 (Table III).

TABLE III: ASSOCIATION BETWEEN ANAEMIA AND RED CELL INCICES

Variable N = 383	Category	Anaemia (213)	No anaemia (170)	Chi square (p-value)
		n (%)	n (%)	
MCV	Microcytic	173 (45.1)	145 (37.9)	2.671 (0.263)
	Normal to Macrocytic	40 (10.5)	25 (6.5)	
OR (CI) 0.711 (0.409-1.235)				
MCH	Hypochromic	164 (42.8)	121 (31.6)	1.681 (0.195)
	Normal	49 (12.8)	49 (12.8)	
OR (CI) 1.355 (0.855-2.148)				
MCHC				9.824 (0.002)
	Hypochromic	69 (18.0)	31 (8.1)	
	Normal	144 (37.6)	139 (36.1)	
	OR (CI) 2.149 (1.325-3.485)			

There was no significant difference between microcytic and macrocytic status regarding Ferritin level with a P value of 0.564. This was the same for hypochromic or normal status found by MCH and MCHC levels with P value of 0.169 and 0.598 respectively (Table IV).

Looking at IDA (Table V), our results showed that the best red cells index was MCV followed by MCH with no significant difference found (P = 0.349 and P = 0.314 respectively).

TABLE IV: ASSOCIATION BETWEEN RED CELLS INDICES AND FERRITIN

Variable	Category	Low (16) <30 µg/L n (%)	Normal (98) 30-100 µg/L n (%)	High (279) >100 µg/L n (%)	Fisher Exact (p-value)
N = 383					
MCV	Microcytic	16 (4.2)	75 (19.6)	227 (59.3)	3.924 (0.133)
	Normal to Macrocytic	0 (0)	13 (3.3)	52 (13.6)	
MCH	Hypochromic	14(3.7)	70 (18.3)	201 (52.5)	3.195 (0.221)
	Normal	2(0.5)	18(4.7)	78 (20.4)	
MCHC	Hypochromic	5 (1.3)	25 (6.8)	70 (18.3)	0.611 (0.737)
	Normal	11 (3.9)	63 (16.4)	209 (54.6)	

TABLE V: RED CELLS INDICES AND IRON DEFICIENCY ANAEMIA

Variable	Category	Ferritin (9) <30µg/L Hb<11g/dL n (%)	Ferritin (204) ≥30 µg/L Hb<11g/dL n (%)	Fisher Exact (p- value)
N = 213				
MCV	Microcytic	9 (4.2)	164 (77)	0.878 (0.349)
	Normal to Macrocytic	0 (0)	40 (18.8)	
MCH	Hypochromic	8 (3.8)	156 (73.2)	1.012 (0.314)
	Normal	1 (0.4)	48 (22.6)	
MCHC	Hypochromic	4 (1.9)	65 (30.5)	1.602 (0.206)
	Normal	5 (2.3)	139 (55.3)	

IV. DISCUSSION

Male sex and the younger age mostly represented in our study, were consistent with the study of Moschovis et al while studying risk factors among young children in sub-Saharan Africa [10], this may be explained by the accelerated growth velocity during the first year of life, since growth velocity is higher in boys when compared to girls during this period [11].

A. ID tested by Ferritin

While using cut-off level of 30 and 50 µg/L experienced for Ferritin level, iron deficiency that varied from 4.2% to 9.6% questioned the real level of Ferritin to be used in children to assess iron shortage. This has been previously raised by Turgeon and collaborators in Canadian children who was proposed to raise the level to 50 µg/L [12] and Phiri's team proposing a value of 273 µg/L in area with high pressure of infection and inflammation [13].

B. IDA tested by Haemoglobin level and Ferritin

In this study Ferritin did not relate to the gravity of anaemia, as observed, children with severe anaemia instead had high level of Ferritin. This may highlight the fact that anaemia of chronic disease had an impact on Ferritin known as an acute phase protein [14, 15]. Iron deficiency often considered the primary cause of (severe) anaemia, as a result, the terms anaemia, iron deficiency and iron deficiency anaemia were often interchanged. This is incorrect as

anaemia can occur with sufficient iron stores and iron deficiency in the initial phase does not necessarily lead to anaemia [16]. The prevalence of ID and IDA in our study population were lower as compared to previous study in Cameroon [8] and elsewhere [17].

The mean Ferritin level was $290.6 \pm 250 \mu\text{g/L}$ for all the children, the mean in non anaemic ($242.2 \pm 211.9 \mu\text{g/L}$) was lower than the mean in anaemic children ($329.2 \pm 241 \mu\text{g/L}$). This was different from what was obtained by Kisangani et al who concluded that anaemia predisposed to iron deficiency [18]. This difference may be due to variation in infection rates according to the area of study or related to the national implementation of iron supplementation in pregnant women as done in Cameroon. The aim of this supplementation is to have in normal circumstances iron replete mothers that will deliver iron replete children [19].

In all the children suffering of IDA, none had a request for Ferritin test, showing that physician is not following WHO recommendations on the algorithm of IDA. The reason may be the cost and availability of the test. It was observed that in 2 of the 3 health settings where the research was done, Ferritin test was available but not forcefully prescribed for the diagnosis of IDA. This can be managed if there are means to sensitize prescribers to ask for Ferritin test in case of suspicious IDA so that when used frequently, the cost will also be reduced.

C. Red cells indices, ID and IDA

Using full blood count to determine iron deficiency showed that low MCV and low MCH were good predictors of ID compared to low MCHC. These results are in accordance with Turkish study revealing that MCV and MCH are parallel parameters [20] but not consistent with those of Koren *et al.* who observed that MCV and MCHC were a good predictor of iron deficiency in African refugees in Syria [21]. This suggest that Microcytic and hypochromic erythrocytes are more reflected by these 2 parameters in addition to the increase of red cell distribution width (RDW) more and more recommended by researchers in the diagnosis of ID and IDA [22], [23]. We did not consider RDW in our study due to units variation used by the different sites.

V. CONCLUSION

Ferritin recommended by WHO for ID and IDA diagnosis was not used in our health settings. Full blood count currently used should be interpreted cautiously as only MCV and MCH corroborate with a Ferritin level lower than $30 \mu\text{g/L}$ not MCHC.

The use of Ferritin for the diagnosis of iron deficiency and iron deficiency anaemia should be promoted in health settings where it is available and oriented for other purposes. Alternatively, full blood count should be considered for monitoring iron deficiency anaemia when Ferritin cannot be included in the diagnostic process but inflammation impact should be integrated.

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