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Factors Affecting Ferritin Level in Children of 6 to 59 Months in the Eastern Region of Cameroon

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Authors' contributions

This work was carried out in collaboration among all authors. Author SDA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author CTT managed the analyses of the study, managed the literature searches and reviewed the first draft of the manuscript. Authors JCNA and ALN supervised the study and reviewed the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Ferritin reflects total iron storage and is also the first laboratory index to decline with iron deficiency. It may be less accurate in children with infectious or inflammatory conditions as an acute phase reactant. Considering the fact that Cameroonian children live in malaria endemic and high risk hookworm infection area, our objective was to study factors affecting Ferritin level including socio-demographic data, child nutrition, anaemia and inflammatory status.
Study Design: A case control study was carried out with anaemic children as cases and non-anaemic as controls.
Place and Duration: Paediatric and laboratory units of the Bertoua regional Hospital, from November 2018 to January 2019.
Methodology: A case control study was carried out in children of 6 to 59 months attending the Bertoua regional hospital. Data were collected and blood distributed in EDTA and dry tubes for full blood count, C - reactive protein (CRP) and Ferritin analysis. Obtained data were analysed with SPSS 21.0 using Pearson's Chi Square test.

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Results: 126 children were included, 63 anaemic (Haemoglobin<11 g/dL) as cases and 63 non anaemic as controls. The Mean age of children was 27.3+/- 15.4 months, the mean haemoglobin was 10.4+/- 1.6 g/dL. Ferritin as state by WHO for the diagnosis of iron deficiency anaemia, was below 30 µg/L in 3.2% independently of anaemic status. Inflammation tested by CRP occurred in 37.3% of children. When the ferritin cut-off value was shifted to 50 µg/L, Ferritin was low in 9.5% thus approaching the stated frequency of iron deficiency obtained recentlyin Cameroon. Mean Ferritin level was 346.5 µg/L.

Conclusion: The relatively high level of Ferritin showed that iron storage seems to remain intact in most children despite anaemic or inflammatory status. The level of Ferritin in children is highly dependent on haem iron consumption and food diversification also has a role to play.

Keywords: Anaemia; inflammation; Ferritin; children.

1. INTRODUCTION

Iron deficiency with or without concurrent anaemia affects ≈30% of the global population, making it the most widespread nutrient deficiency [1]. The early stage of iron deficiency can be recognized by abnormalities in serum Ferritin (SF), zinc protoporphyrin (ZP), and serum transferrin receptor (sTfR), whereas the more advanced stage of iron deficiency, iron deficiency anaemia (IDA), occurs when anaemia develops. The detrimental public health effects of IDA include retarded infant development, increased morbidity and mortality at childbirth, and reduced work performance [2-4]. Initially, as specific tests were not available, the prevalence of anaemia was used to estimate the prevalence of iron deficiency and IDA [5]. However, in many developing countries, anaemia can also result from infections such as malaria, chronic inflammatory disorders, or other nutritional deficiencies like folate or vitamins B12 and A [6-8]. It is well known that infection and inflammation influence haemoglobin and iron-status indexes such as ZP and SF [9].

Iron status is determined by a combination of factors which influence iron losses and iron uptake, althoughresearch to date has not been able to describe those factors in detail [10].

Iron stores in the body exist primarily in the form of Ferritin. In the body, small amounts of Ferritin are secreted into the plasma. The concentration of this plasma (or serum) Ferritin is positively correlated with the size of the total body iron stores in the absence of inflammation. A low serum Ferritin value reflects depleted iron stores, but not necessarily the severity of the depletion as it progresses [1].

While low SF is a sensitive and specific indicator of low total body iron stores, elevated SF is sensitive but very nonspecific for iron overload [10].

It may be less accurate in children with infectious or inflamatory conditions because ferritin is also an acute phase reactant. Considering the fact that Cameroonian children live in a context of endemic malaria and frequent hookworm infection, our objective is to point out factors affecting Ferritin level in young children. Specifically sociodemographic factors, nutrition factors and clinical conditions like anaemia or inflammation.

2. MATERIALS AND METHODS

2.1 Study Design

This was a case-control study carried out at the Bertoua regional Hospital from November 2018 to January 2019.

2.2 Subjects and Sampling

The target population was made up of children of 6 to 59 months attending the Bertoua regional hospital. The sample size was calculated using the case-control formula for continuous exposure [11]. 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 classified as cases and those with Hb≥11 g/dL were controls. Children with neurologic impairment were not included in this study.

2.3 Measurements and Laboratory Analysis

Data were collected through a questionnaire, after parental agreement. Questions about family, nutrition habits, environmental factors, child and parent education, child's feeding (type of meals, haem iron and non-haem iron consumption) and child's clinical history were

administered to the parent/guardian directly or by phone if they were not available at the time of blood collection.

A sample of 2 ml of blood distributed in EDTA and dry tubes were collected from each child for laboratory analysis. Full blood count testing was performed using Mindray Bc-1800. In the cases group, anaemia was subdivided into severe (Hb<7 g/dL), moderate (Hb: 7-9.99 g/dL) and mild (Hb: 10-10.99 g/dL). Leucocytosis was considered as white blood cells (WBC) >10 000 cells/µL. Blood in dry tubes were centrifuged at 2500 tr/min for 5 minutes to obtain serum. Fresh serum was used to detect CRP by latex agglutination using Fortress Diagnostic Limited (UK) Kits following the procedure with a cut-off value of 6 mg/dl. The remaining serum was kept at – 20ºC for subsequent Ferritin analysis using ERBALISA Kits by Cal Biotech Lab (USA) accordingly. Normal values for this kits, were given for men and women but not for children. A cut-off value 50 µg/L was considered as proposed by Turgeon et al. [12]. Additionally children in different categories integrated the cut-off of 30 µg/L proposed by Phiri et al. [13]. Batch analysis of Ferritin was done at the serology bench of the main laboratory of the Bertoua Regional Hospital.

This study was conducted in accordance with the standards set forth in the Declaration of Helsinki [14], and all procedures involving human subjects were approved by the National Ethical Committee for Research in human health of Cameroon and the Regional delegation of public health of the East region. Each parent or caregiver signed an informed assent form. Children who presented with anaemia and low Ferritin level were particularly referred to the paediatrician for care.

2.4 Statistical Analysis

The collected data were computed in Excel 2010 and analysed with Statistical Package for Social Sciences SPSS (version 21.0) for Windows (SPSS Inc., Chicago, IBM, USA) using Pearson's Chi Squared test, a 95% confidence interval (95% CI) with p value <0.05 were considered for significant difference.

3. RESULTS AND DISCUSSION

3.1 Sociodemographic Data

For this study, 126 children were recruited, 63 in the case group and 63 in the control group. The mean age was 27.3+/- 15.4 months and the age class mostly observed was 25-36 months. Males (71;56.3%) were more represented than females. These results are consistent with that of Semedo et al. [15] with 56.3% of male in their study population and contrary to those of Ahmad et al. [16] with less male than female affected by anaemia.

3.2 Anaemia, Inflammation and Leucocytosis

The mean haemoglobin was 10.4+/- 1.7 g/dL, in the control group the mean haemoglobin was 11.6 g/dL, in the case group the mean was 10.1 g/dL. Severe anaemia was revealed in 7.9% and moderate in 47.6% in the case group. This distribution of anaemia related to sex showed that males as well as females were exposed to anaemia occurrence (Table 1).

Sickle cell children in this study despite the anaemia had a normal Ferritin level, going on the same line with the findings of Odunlade et al. in Nigeria [17] concluding that despite the anaemic status of sickle cell patients, their Ferritin level is usually normal or high.

Inflammation measured by CRP> 6 mg/dL was present in 37.3%. For the case group inflammation was observed in 23 (36.5%) and 24(38%) was observed in the control group, meaning that inflammation is a reality in the East region for anaemic and non-anaemic children (Table 1). Mean WBC level was 9940 cells/µL. Leucocytosis described was observed in 56 (44.4%) of children but mostly in non-anaemic children with 31 (49.2%) (Table 2). This can be explained by the endemic effect of infections like Malaria and hookworm.

3.3 Ferritin and Various Factors

Ferritin level in children was relatively high with a mean of 346.5 µg/l ranging from 13 to 1,126 µg/L. 3.2% had Ferritin under 30 µg/L and 9.5% under 50 µg/L.

Ferritin level with regards to different factors was tested. Looking at the association of Ferritin with socio-demographic data, no significant difference was found for age, parent education level, profession and child's education. Sex distribution of Ferritin revealed that all the 4 children with Ferritin <30 µg/L were male, *P=.09*, with a relative higher mean (364.4 µg/L) than female (332.2 µg/L).

Table 1. Socio-demographic data, anaemia and inflammation (CRP) inrecruited children

Data		Anaemia(Cases)			Controls	CRP		Total	
		Severe	Moderate	Mild	No	Positive	Negative	n	%
Sex	Male	3	19	16	33	24(36.5)	47	71	56.3
	Female	2	11	12	30	23(38)	32	55	43.7
Total		5 (7.9*)	30(47.6)	28(44.5)	63(100)	47(37.3)	69(52.3)	126	100
Age class (Months)	0-12	0	8	8	16	14	18	32	25.4
	13-24	0	9	8	9	9	17	26	20.6
	25-36	2	4	9	22	15	22	37	29.4
	37-48	2	5	2	6	4	11	15	11.9
	49-60	1	4	1	10	5	11	16	12.7
Total		5 (7.9*)	30(47.6)	28(44.5)	63(100)	47(37.3)	69(52.3)	126	100
Parent's education	Primary	0	1	1	4	1	5	6	4.8
	Secondary	4	20	19	34	28	49	77	61.1
	Tertiary	1	6	6	21	13	21	34	27.0
	Arabic	0	0	0	1	1	0	1	0.8
	None	0	3	2	3	4	4	8	6.3
Total		5 (7.9*)	30(47.6)	28(44.5)	63(100)	47(37.3)	69(52.3)	126	100
Child's education	Pre-nursery	0	2	3	5	5	5	10	7.9
	nursery	2	5	4	19	9	21	30	23.8
	None	3	22	21	38	32	52	84	66.7
	Primary	0	1	0	1	1	1	2	1.6
Total		5 (7.9*)	30(47.6)	28(44.5)	63(100)	47(37.3)	69(52.3)	126	100

**Represent the percentage of the category*

Table 2. Leucocytosis in recruited children

Group	Leucocytosis						P value (Chi square)
	Yes		No		Total		
	n	%	n	%	n	%	
Controls	31	49.2	32	50.8	63	100	.37 (1.157)
Cases	25	39.7	38	60.3	63	100	
Total	56	44.4	80	55.6	126	100	

For socio-economic data, parent and child's drinking water, number of meals per day, child's appetite, Child's feeding, family habits were surveyed but none of them seems to influence directly Ferritin level.

Concerning clinical data, transfusion history, iron supplementation, vaccination, allergy, mosquito net use, hookworm medication, malaria treatment, fever experience during the last three months , they all seems to have no impact on Ferritin level. However, sickle cell should be considered while analysing Ferritin level, because in this study though not statistically significant, the case observed here had a Ferritin level of 57.9 µg/L after red blood cell transfusion (*P =.07*).

Low Ferritin level (<50 µg/ L) was observed in 4 (6.3 %) children among cases and 8(12.7%) in the controls showing more cases of iron deficiency in children without anaemia, *P =.36*(Table 3). This results revealed the presence of Iron deficiency in 9.5% of the study population and iron deficiency anaemia in 6.3% of the cases. All the children with severe anaemia instead had high Ferritin level (>50 µg/L) but with inflammation (3/5) and leucocytosis (2/5) different ratios were obtained.

While analysing nutritional status, most of the children 88 (69.8%) were eating the same food as the whole family, although statistically we had a*P=.05*, this result means that food diversification enhance iron intake and absorption though having an impact on iron store.

Furthermore we found a strong correlation between the type of iron ingested and ferritin level. For instance haem iron appears to influence Ferritin level more than non-haem iron with a *P<.001* (Table 4), showing that with growing age children should consume food rich in Haem iron.

Child's nutrition was an important characteristic and our results showed that with diversified food, iron stores are reinforced and preserved and furthermore emphasis should be made on consumption of iron from animal sources as they seem to be more valuable in improving the iron status in children and covering iron need more conveniently. The same findings were obtained from a recent study carried out in Saudi Arabia in children of 6 to 18 months and regarding nutrition in children as a whole [18,19].

Time of fruits consumption did not directly affect the level of Ferritin, but this does not mean that the role of fruits in iron deficiency is minor as it has been proved that ascorbate is needed for iron absorption [20].

Most of the children were breast fed at least for 6 months 51(40.5%) without a specific impact on their Ferritin level.

The results show that inflammation status appears to have an influence on Ferritin level, but here only children with negative CRP 5.1% with or without anaemia had a low Ferritin level (<30 µg/L);*P=.07*(Table 5).

Table 3. Ferritin level in controls and cases groups

Group	Ferritin level						P value
	<50		>50		Total		
	n	%	n	%	n	%	
Controls	8	12.7	55	87.3	63	100	0.36
Cases	4	6.3	59	93.7	63	100	
Total	12	9.5	114	91.5	126	100	

Table 4. Haem iron consumption and Ferritin level in children of 6 to 59 months

Haem iron	Ferritin level				Total (%)	P value(Chi square)
	<30	30-50	50-100	>100		
Yes	2 (50)	8(100)	14(93.3)	98(98.99)	122(96.8)	<.001
No	2(50)	0	1(6.7)	1 (1.01)	4(3.2)	
Total	**4(100)**	**8(100)**	**15**	**99**	**126 (100)**	

Table 5. CRP effect on Ferritin levelin children of 6 to 59 months

CRP	Ferritin level								Total		P value
	<30	%	30-50	%	50-100	%	>100	%	n	%	(Chi square)
Positive	0	0	3	6.4	2	4.2	42	83.4	47	100	.07
Negative	4	5.1	5	6.3	13	16.5	57	72.1	79	100	(7.17)
Total	4	100	8	100	15	100	99	100	126	100	

It has been widely proved that Ferritin is affected by inflammation and thus will remain normal or high in case of inflammation. In this study all the children with low Ferritin level had a negative CRP, this reveals the fact that true iron deficiency is easy to diagnose using Ferritin when there is no inflammation, but the invisible part of the iceberg being that functional and/or absolute iron deficiency could be misdiagnosed if solely based on Ferritin level [21]. This latter fact may concern a larger population in a context of endemic infection like malaria and hookworm as it is the case in Cameroon as a whole and in the Eastern region in particular.

Of recent, questions about the relationship between inflammation and Ferritin are still ongoing. Serum Ferritin presents a paradox, as the iron storage protein Ferritin is not synthesised in serum and yet is to be found there. Serum Ferritin is also a well-known inflammatory marker, but it is unclear whether serum Ferritin reflects or causes inflammation, or whether it is involved in an inflammatory cycle [22].

Growing attention is now being paid to the iron status of patients with inflammatory conditions, which predispose them to iron deficiency [23, 24].

As a matter of fact differentiating iron deficiency from normal iron status in inflammatory context is of great complexity association of other tests is currently examined and studied worldwide [25-29].

4. CONCLUSION

At the end of this study, we noticed that the level of Ferritin in children of 6 to 59 months in the Eastern region of Cameroon was relatively high. Food diversification and consumption of haem iron had an impact on Ferritin level by contributing to its increase in the studied population. Additionally, the use of Ferritin in diagnosing iron deficiency in children is interesting but the question of differentiating functional and absolute iron deficiency in inflammatory conditions stillremains.

CONSENT

A parental agreement was needed before children could be included in the study and a clear consent given by the parent/guardian before selecting the children as participant in accordance with inclusion and exclusion criteria.

ETHICAL APPROVAL

This study was approved by the National ethical committee for heal thunder an ongoing research on diagnostic biomarkers of iron metabolism, namely soluble transferrin receptor.

All the data collected from the research were codified, kept confidential and analysed anonymously.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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