Original Research Article

Factors Affecting ferritin level in children of 6 to 59 Months in the Eastern region of Cameroon

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 inflamatory conditions as an acute phase reactant. Considering the fact that Cameroonian children live in such context, 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 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 using SPSS 21.0.

Results:

126 children were included, 63 anaemic (Haemoglobin<11g/dL) as cases and 63 non anaemic as controls. The Mean age of children was 27.3 months+/- 15.4, the mean haemoglobin was 10.4+/- 1.6g/dL. Ferritin as preconized by WHO for the diagnosis of iron deficiency anaemia, was below 30µg/l in 3.2% independently of anaemic status. Inflammation tested by CRP occurred in 37.3% 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 in 2013 in Cameroon. Mean ferritin level was 346.5µg/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.

Keyword: 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, although research 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 level <11g/ dL were classified as cases and those with haemoglobin>11g/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 and child's clinical history were administered to the parent/caregiver 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. Children were either classified as cases (Hb<11g/dl) or controls (Hb ≥ 11g/dl). Blood in dry tubes were centrifuged at 2500tr/min for 5 minutes to obtained serum. Fresh serum was used to detect CRP by latex agglutination using Fortress diagnostic limited Kits (UK) following the procedure with a cut-off value of 6mg/dl. The remaining serum was kept at – 20°c 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.

2-4-Ethical considerations

Ethical approval was obtained from the East Regional Delegation of Public Health and the National Ethical Committee.

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.

2-5- 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 Chi 2 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 months+/- 15.4, the age class mostly observed was 25-36 months. Male were more represented than female 71(56.3%). Occurrence of anaemia in children in this study was independent of sex but male being more represented than female, this 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 control group the mean haemoglobin was11.6 g/dL, in case group the mean was 10.1 g/dL. Severe anaemia was revealed in 7.9% and moderate in 47.6 %. This distribution of anaemia related to sex showed that male children as well as female children were exposed to anaemia occurrence (Table 1).

Sickle cell child 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 are usually normal or high.

Inflammation measured by CRP> 6mg/dL was present in 37.3%. For cases inflammation was observed in 23(36.5%) and 24(38%) was observed in the control group. Meaning that inflammation is a reality in our context in anaemic and non-anaemic children (Table 1). Mean WBC level was 9940cells/µL. Leucocytosis was observed in 56 (44.4%) of children but mostly in non-anaemic children with 31 (49.2%). These 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 346.5µg/l ranging from 13 to 1,126 µg/L. 3.2% had Ferritin under 30µg/L and 9.5% under 50 µg/L.

Ferritin level with regards to different factors were tested. Looking 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).

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 2). 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 (>50µg/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 3). 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 Saoudi Arabia in children of 6 to 18 months and regarding nutrition in children as a whole [18,19].

Fruits time of 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.

Inflammation status results 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 4).

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 reveal 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 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- Tables

Table 1: Socio-demographic data, anaemia and inflammation (CRP) in children of 6 to 59 months

Data		**Anaemia(case)**			**Control**	**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: Ferritin level in control and case group

Group	**Ferritin level**						**P value**
	<50		**>50**		**Total**		
	n	**%**	**n**	**%**	**n**	**%**	
Control	8	12.7	55	87.3	63	100	0.363
Case	4	6.3	59	93.7	63	100	
Total	12	9.5	114	91.5	126	100	

Table 3 : Haem iron consumption and ferritin level in children of 6 to 59 months

Haem iron	**Ferritin level**				**Total**	**P value**
	<30	**>100**	**30-50**	**50-100**		
Yes	2	98	8	14	122	
No	2	1	0	1	4	<0.001
Total	**4**	**99**	**8**	**15**	**126**	

Table 4: Distribution of children by Inflammation tested by CRP and Ferritin level

CRP	FERRITIN LEVEL								Total		P value
	<30	%	>100	%	30-50	%	50-100	%	n	%	
Positive	0	0	42	83.4	3	6.4	2	4.2	47	100	
Negative	4	5.1	57	72.1	5	6.3	13	16.5	79	100	0.07
Total	4	100	99	100	8	100	15	100	126	100	

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 still come in.

COMPETING INTERESTS

Authors declared there is no competing interests in this study.

CONSENT

A parental agreement was needed before children could be included in the study and a consent

ETHICAL APPROVAL

This study was approved by the National ethical committee for health under 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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