

## **Factors affecting Ferritin level in children of 6 to 59 months in the Eastern region of Cameroon.**

Agokeng D. Sylvie<sup>1,3</sup>, Tayou T. Claude<sup>2</sup>, Assob N. Clement<sup>1</sup>, NJunda Anna L.<sup>1</sup>

- 1) University of Buea, faculty of health sciences, Medical Laboratory Sciences
- 2) University Hospital Centre Yaounde
- 3) Regional Hospital Bertoua, Laboratory Service

### **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.

#### **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 recently in 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.

**Keyword: anaemia, inflammation, Ferritin, Children**

### **1-Introduction**

Iron deficiency with or without concurrent anaemia affects  $\approx 30\%$  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 inflammatory 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 METHOD**

### **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 <11g/ dL were classified as cases and those with  $Hb \geq 11g/dL$  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 (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 ( $Hb < 7g/dL$ ), moderate ( $Hb: 7-9.99g/dL$ ) and mild ( $Hb: 10-10.99g/dL$ ). Leucocytosis was considered as white blood cells(WBC)  $> 10\ 000$  cells/ $\mu L$ . Blood in dry tubes were centrifuged at 2500tr/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 6mg/dl. The remaining serum was kept at  $- 20^{\circ}c$  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  $\mu g/L$  was considered as proposed by Turgeon *et al*[12]. Additionally children in different categories integrated the cut-off of 30  $\mu g/L$  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.

## **2-5- 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'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 > 6mg/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/ $\mu$ L. 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 346.5µg/l ranging from 13 to 1,126µg/L. 3.2% had Ferritin under 30µg/L and 9.5% under 50µg/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 <30µg/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 seem 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 result 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 enhances 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 ( $<30\mu\text{g/L}$ );  $P=.07$ (Table 5).

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].

**Table 1: Socio-demographic data, anaemia and inflammation (CRP) in recruited children.**

| Data | Anaemia( Cases) |          |      | Controls<br>No | CRP      |          | Total<br>n | Total<br>% |      |
|------|-----------------|----------|------|----------------|----------|----------|------------|------------|------|
|      | Severe          | Moderate | Mild |                | Positive | Negative |            |            |      |
| Sex  | Male            | 3        | 19   | 16             | 33       | 24(36.5) | 47         | 71         | 56.3 |

|                       |             |                 |                 |                 |                |                 |                 |            |            |
|-----------------------|-------------|-----------------|-----------------|-----------------|----------------|-----------------|-----------------|------------|------------|
|                       | Female      | 2               | 11              | 12              | 30             | 23(38)          | 32              | 55         | 43.7       |
| <b>Total</b>          |             | <b>5 (7.9*)</b> | <b>30(47.6)</b> | <b>28(44.5)</b> | <b>63(100)</b> | <b>47(37.3)</b> | <b>69(52.3)</b> | <b>126</b> | <b>100</b> |
| Age class<br>(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       |
| <b>Total</b>          |             | <b>5 (7.9*)</b> | <b>30(47.6)</b> | <b>28(44.5)</b> | <b>63(100)</b> | <b>47(37.3)</b> | <b>69(52.3)</b> | <b>126</b> | <b>100</b> |
| Parent's<br>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        |
| <b>Total</b>          |             | <b>5 (7.9*)</b> | <b>30(47.6)</b> | <b>28(44.5)</b> | <b>63(100)</b> | <b>47(37.3)</b> | <b>69(52.3)</b> | <b>126</b> | <b>100</b> |
| Child's<br>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        |
| <b>Total</b>          |             | <b>5 (7.9*)</b> | <b>30(47.6)</b> | <b>28(44.5)</b> | <b>63(100)</b> | <b>47(37.3)</b> | <b>69(52.3)</b> | <b>126</b> | <b>100</b> |

\*Represent the percentage of the category

**Table 2: Leucocytosis in recruited children**

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

**Table 3: Ferritin level in controls and cases groups**

| Group           | Ferritinlevel |      |     |      | Total |     | P value |
|-----------------|---------------|------|-----|------|-------|-----|---------|
|                 | <50           |      | >50 |      | n     | %   |         |
|                 | n             | %    | n   | %    |       |     |         |
| <b>Controls</b> | 8             | 12.7 | 55  | 87.3 | 63    | 100 | 0.36    |
| <b>Cases</b>    | 4             | 6.3  | 59  | 93.7 | 63    | 100 |         |
| <b>Total</b>    | 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<br>(Chi square) |
|--------------|----------------|---------------|-----------|-----------|------------------|-------------------------|
|              | <30            | 30-50         | 50-100    | >100      |                  |                         |
| <b>Yes</b>   | 2 (50)         | 8(100)        | 14(93.3)  | 98(98.99) | 122(96.8)        | <.001                   |
| <b>No</b>    | 2(50)          | 0             | 1(6.7)    | 1 (1.01)  | 4(3.2)           |                         |
| <b>Total</b> | <b>4(100)</b>  | <b>8(100)</b> | <b>15</b> | <b>99</b> | <b>126 (100)</b> |                         |

**Table 5: CRP effect on Ferritin level in children of 6 to 59 months**

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

**ACKNOWLEDGMENTS:** To all the parents and guardians who kindly accepted the participation of their children in this study.

## COMPETING INTERESTS

Authors declared there is no competing interests in this study.

## AUTHORS' CONTRIBUTIONS



Agokeng D. Sylvie:designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript.

Tayou T. Claude:managed the analyses of the study, managed the literature searches and reviewed the first draft of the manuscript.

Assob N. Clement: supervised the study and reviewed the first draft of the manuscript.

NJunda Anna L.:supervised the study and reviewed the first draft of the manuscript.

All the authors agreed for the publication of this manuscript.

## **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 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.

## **REFERENCES**

- 1- Franziska Staubli Asobayire, Pierre Adou, Lena Davidsson, James D Cook, and Richard F Hurrell. Prevalence of iron deficiency with and without concurrent anemia in population groups with high prevalences of malaria and other infections: a study in Côte d'Ivoire. *Am J Clin Nutr* 2001;74:6 776-782.
- 2- Cornet M, Le Hesran JY, Fievet N, Cot M, Personne P, Gounoue R. et al. Prevalence of and risk factors for anemia in young children in southern Cameroon. *Am J Trop Med Hyg.* 1998;58:5606-611.
- 3- Nojilana B, Norman R, Dhansay MA, Labadarios D, van Stuijvenberg ME, Bradshaw D. Estimating the burden of disease attributable to iron deficiency anaemia in South Africa in 2000. *S Afr Med J.* 2007;97:741-46.
- 4- Cook JD. Defining optimal body iron. *Proc Nutr Soc* 1999;58:489-95.
- 5- Yip R, Dallman PR. The roles of inflammation and iron deficiency as causes of anemia. *Am J Clin Nutr* 1988;48:1295-300.
- 6- Suharno D, West CE, Muhilal, Karyadi D, Hautvast JG. Supplementation with vitamin A and iron for nutritional anaemia in pregnant women in West Java, Indonesia. *Lancet* 1993;342:1325-328.
- 7- Savage D, Gangaidzo I, Lindenbaum J, et al. Vitamin B-12 deficiency is the primary cause of megaloblastic anaemia in Zimbabwe. *Br J Haematol* 1994;86:844-50.

- 8- Amanda J. Patterson, Wendy J. Brown and David C.K. Roberts. Dietary and lifestyle factors influencing iron stores in Australian women: an examination of the role of bio-available dietary iron. *Aust J Nutr Diet* 2001; 58: 2107-113.
- 9- **Frederick KE Grant, Reynaldo Martorell, Rafael Flores-Ayala, Conrad R Cole, Laird J Ruth, Usha Ramakrishnan et al.** Comparison of indicators of iron deficiency in Kenyan children. *Am J Clin Nutr* 2012; 95: 51231–1237.
- 10- Domellöf M, Dewey KG, Lönnerdal B, Cohen RJ, Hernell O. The diagnostic criteria for iron deficiency in infants should be reevaluated. *J Nutr* 2002; 132: 3680-3686.
- 11- Jay H. Lubin, Mitchell H. Gail, Abby G. Ershow. Sample size and power for case-control studies when exposures are continuous. *Stat Med* 1988; 7: 3363-376.
- 12- Huguette Turgeon O'Brien, Rosanne Blanchet, Doris Gagné, Julie Lauzière, and Carole Vézina. Using soluble Transferrin Receptor and taking inflammation into account when defining serum ferritin cut-offs improved the diagnosis of iron deficiency in a group of Canadian preschool Inuit children from Nunavik. *Anemia* 2016; 10 pages, ID 6430214.
- 13- KS. Phiri, J CJ Calis, A Siyasiya, I Bates, B Brabin, M Boele van Hensbroek. New cut-off values for ferritin and soluble transferrin receptor for the assessment of iron deficiency in children in a high infection pressure area. *Bmj journals* 2018; 62: 12.
- 14- American medical association, world medical declaration of Helsinki ethical principles for medical research involving human subjects. *JAMA* 2013; 310: 20.
- 15- Rosa M.L. Semedo, Marta M.A.S. Santos, Mirian R. Baião, Ronir R. Luiz, and Gloria V. da Veiga. Prevalence of Anaemia and Associated Factors among Children below Five Years of Age in Cape Verde, West Africa. *J Health Popul Nutr.* 2014 Dec; 32(4): 646–657.
- 16- Mirza Sultan Ahmad, Hadia Farooq, Sumaira Noor Maham, Zonaira Qayyum, Abdul Waheed, and Waqar Nasir. Frequency of Anemia and Iron Deficiency among Children Starting First Year of School Life and Their Association with Weight and Height. *Anemia.* 2018; 5 pages, ID 8906258.
- 17- Olufunke Odunlade, Olugbenga Adeodu, Joshua Owa, Efere Obuotor. Iron deficiency, still a rarity in children with sickle cell anemia in Ile-Ife, Nigeria. *Hematol Transfus Cell Ther* 2019; 41: 3216-221.
- 18- Zainab Alghamdi. Iron Status of Infants and Toddlers Age 6 to 18 Months and Association with Type of Milk Consumed from DNSIYC Secondary Analysis. *J Nutr Food Sci* 2017; 7: 596. doi: 10.4172/2155-9600.1000596.
- 19- S. M. Namaste, F. Rohner, and J. Huang, “Adjusting ferritin concentrations for inflammation: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project. *Am J Clin Nutr* 2017; 106: 1359S–371S.
- 20- Soliman G, Azmi M, El Said S. Prevalence of anemia in Egypt (Al-Gharbia Governorate). *Egypt J Hosp Med* 2007; 28: 395-305.
- 21- D. W. Thomas, R. F. Hinchliffe, C. Briggs, I. C. Macdougall, T. Littlewood, and I. Cavill. Guideline for the laboratory diagnosis of functional iron deficiency. *Br J Haematol* 2013; 161: 5639–648.
- 22- Kell DB, Pretorius E. Serum ferritin is an important inflammatory disease marker, as it is mainly a leakage product from damaged cells. *Metallomics* 2014; 6: 4748-753.
- 23- M. D. Cappellini, J. Comin-Colet, A. de Francisco et al. Iron deficiency across chronic inflammatory conditions: International expert opinion on definition, diagnosis, and management. *Am J Hematol* 2017; 92: 101068-1078.

- 24- L. De Franceschi, A. Iolascon, A. Taher, and M. D. Cappellini. Clinical management of iron deficiency anemia in adults: Systemic review on advances in diagnosis and treatment. *Eur J Int Med* 2017; 42: 16-23.
- 25- C. Camaschella. New insights into iron deficiency and iron deficiency anaemia. *Blood Reviews* 2017;31: 4 225–233.
- 26- N. M. Archer and C. Brugnara. Diagnosis of iron-deficient states. *Crit Rev Clin Lab Sci* 2015; 52:5 256-272.
- 27- Sherwin DeSouza, Anita Shet, Prasanna Kumar Kapavarapu and Arun S. Shet. Evaluating biomarkers of iron deficiency anemia in anemia of inflammation. *Blood* 2013; 122:21,948.
- 28- Giridhar Kanuri, Deepti Chichula, Ritica Sawhney, Kevin Kuriakose, Sherwin De'Souza, Faye Pais, Karthika Arumugam, Arun S. Shet. Optimizing diagnostic biomarkers of iron deficiency anemia in community-dwelling Indian women and preschool children. *Haematologica* 2018 ;103:121991-1996.
- 29- Drakesmith H. Next-Generation Biomarkers for Iron Status. Nestle Nutrition Institute workshop series 2016;84: 59-69.