

## Iron Indices in School children in Ceres District of the Western Cape South Africa

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### ABSTRACT

Iron status of children from communities in South Africa has been a subject of debate and the lack of single biochemical indicator makes it more challenging, hence this study was designed to assess the iron status of children in a Ceres district of the Western Cape in South Africa. For this study, 265 children were randomly recruited and blood sample collected from the participating children and was used for this analysis using the following parameters; soluble transferrin receptors (sTfR), haemoglobin (Hb), mass cell volume (MCV) and ferritin (Fert). The result showed that the mean serum iron level of 13.45  $\mu\text{mol/l}$ , mean serum ferritin level of 1.85mg/l, mean serum transferrin receptor of 30.11  $\mu\text{g/l}$  were recorded in >75% of the participating children. The Haemoglobin level of less than 12.5 g/l and mass cell volume of 83.10 fl were observed in >50% of participating children. There were no gender differences for any of the iron measure. The haemoglobin level showed marginal iron deficiency while only serum iron was significantly correlated with the haemoglobin level. The serum transferrin receptors, ferritin and MCV did not indicate possible iron deficiency. The results of other factors such as socioeconomic status and demographic data did not show any significant interference either. The implication of the finding is been discussed.

**KEY WORDS:** Blood, iron, children, Hb, STR, (MCV), (Fert).

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### INTRODUCTION

Iron deficiency is the most common nutritional deficiency in Africa, Asia and most developing world; affecting as much as 66–80% of the world's population (UNICEF, 1998 and Nwafia *et al.*, 2006) and is the leading nutritional cause of anemia (Allen and Casterline-Sabel, 2001). It is easily noticed in anemia and those particularly at risk include: children, pregnant women, women with heavy menstruation and people with mal-absorption problems.

Although over the years iron status of children has improved according to data published by (National Health and Nutrition Examination Survey 111 or NHANES 111) and (NHANES 11) (Betsy *et al.*, 2006), despite these improvement iron deficiency is still common in most countries.

Infants and young children are especially vulnerable to iron deficiency because of their rapid growth and increased physiologic demands for iron (Nwafia *et al.*, 2006) in order to fulfill major body functions. Also diets high in cereals and low in meat and fish products may cause iron deficiency due to poor dietary iron bioavailability. Studies also show that ascorbic acid increases iron absorption Margarita (Diaz *et al.*, 2003). Of particular significance is the fact that iron deficiency in children can adversely affect cognitive and psychomotor development during vulnerable periods such as the toddler years (Oti-Boateng, *et al.*, 1998). Iron deficiency during childhood has multiple consequences like neurochemistry disorder; alteration of dopamine receptors (Beard, 2003) and decreased monoamine oxidase activities (Prpic-Majie *et al.*, 2002).

Dietary intake requirement, inflammation and most biochemical indicators are age-related and constitute a major factor influencing iron concentration in children (David, *et al.*, 2005). Iron deficiencies in children are generally estimated from studies using healthy adults, but they differ in many ways from that of adults. In children, the most likely cause is an inadequate amount of iron in the diet, coupled with the extra requirement for iron because of growth, also children present particular problem as they are highly susceptible to diseases

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and require more nutrients than adults (David, *et al.*, 2005). Other features are non-hematological but are clinically important and are clearly defined in children than in adults. Presently, there is no single biochemical indicator available to reliably access iron inadequacy in children (RDA, 1989).

## AIM OF THE STUDY

This study focused on determination of iron status using the following parameters; serum ferritin, serum transferrin receptor, MCV, and Haemoglobin values as possible indices for assessment of iron status in children in the Ceres district of the Western Cape.

## METHODOLOGY

### Study design

This study aimed to access the iron status of children using the blood serum technique. This technique is widely used in accessing the iron status although no single biomarker is adequate.

### Subjects

This paper presents the biochemical indicators of iron in school children in Ceres district. A total number of 265 grade one learners aged between 7-9 years in six primary schools in Ceres district of the Western Cape, South Africa, were involved in this survey. These schools serve communities that duly represent the diverse ethnic groupings as well as socio-economic groupings within the bigger South African community. Informed consent was received.

### Exclusion and inclusive criteria

Children with CRP concentration greater than 10mg/l were excluded while those with CRP concentration less than 10mg/l were included. The CRP concentration was used to include participants as a sign of apparent healthy state and to exclude participants as a sign of inflammation and ill health. The 265 pupils met the criteria of  $\geq$  abnormal iron measure.

### Data collection

Sample and data collection took place during school hours at each school over a period of one week in 2003 and 2004 respectively. Samples were prepared for analyses within one week of sample collections according to the following methodology:

### Blood analysis

Information as supplied by Pathcare laboratory. 5mL of whole venous blood was collected in a zinc free heparinised tube (LSRO, 1985). This process was carried out by a trained community staff nurse. Through the process care was taken to avoid any health risk situation that will endanger the subject or the nurse through blood contamination, infections or psychological situation. All blood samples collected were adequately marked and labelled. The blood samples were then stored in coolers under ice bags at 0 to -4 °C, for usually <24hrs (Connie *et al.*, 1990; Tietz, 1994).

In the laboratory the whole sample was then centrifuged to separate the serum. The serum was then stored at -15°C for all analyses. Prior to each analysis the serum was then thawed once only (Tietz *et al.*, 1994). Iron reagent was used to measure the iron concentration by a timed-endpoint method. In the reaction, iron is released from transferrin by acetic acid and is reduced to the ferrous state by hydroxylamine and thioglycolate. The ferrous ion is immediately complexed with the FerroZine Iron Reagent. The SYNCHRON LX<sup>®</sup> i725 (Beckman Coulter, 1998 – 2006) system automatically proportions the appropriate sample and reagent volumes into a cuvette. The ratio used is one part sample to 8 parts reagent. The system monitors the change in absorbance at 560 nm. The change in absorbance is directly proportional to the concentration of iron in the

sample and is used by the SYNCHRON LX (Beckman Coulter, 1998 – 2006; Looker, *et al.*, 1997) systems to calculate and express the iron concentration.

Serum ferritin measurement was based on radio-immunometric assay (Pathcare laboratory Ltd Cape Town). Combined measurement of ferritin, soluble transferrin receptor, and C-reactive protein was performed by a sandwich enzyme-linked immunosorbent assay technique. The external standard for ferritin consisted of the IBS standard diluted to 19.2 µg/l ferritin. The coefficient of variation for within assay-variation was <4.5% for all ELISA assays, with a between-batch coefficient of variation of 6.2–7.4%.

All children had data for Hb (hemoglobin), MCV (mean cell volume). Value for transferrin saturation, ferritin, TfR, and body iron were available for all infants. A total of 236 children had complete data for the measures used to determine iron status in NHANES II or III (i.e., Hb, MCV, transferrin saturation, and ferritin) (Tietz, 1994; Looker, *et al.*, 1997). Missing data were due to technical problems, such as trouble obtaining sufficient blood, samples with CRPC conc. >10mg/L and ethical exceptions.

For CRP, within-batch variation was <3% and between batches, it was <5%.

### **Precautions: Blood specimen storage and stability**

Tubes of blood were kept closed at all times in a vertical, stopper-up position. Serum was physically separated from contact with cells as soon as possible. A maximum limit of two hours from the time of collection is needed for the assay to expire if not used (NCCLS, 1990). Separated serum should not remain at 15-30°C longer than 8hrs. Serum assays should be completed within 8hrs and the separated samples should be stored at 2-8°C. If the assays are not completed within 24hrs, serum samples should be re-centrifuged and separated from precipitates before testing. Frozen samples should be thawed only once. Analysis deterioration may occur in samples that are repeatedly refrozen and thawed (NCCLS, 1990).

### **Ethical Considerations**

The Senate Research committee of the University of the Western Cape provided ethical approval for this study (SHD of 2004/6). The participation of learners was voluntary following informed consent by parents or guardians. The participants were free to terminate participation at their convenience. Confidentiality of the data collected and subsequent findings were assured by using only code numbers for each participant.

### **Statistical Analysis**

Statistical analyses were performed using SAS Version 8.12 (SAS, 1999). The theory of Oliver Jean Dun (1971) was used in comparing of test of quality of dependent correlation coefficient (Pearson Correlation Coefficients) between blood indexes, food, vegetables and water samples. The health of learners plays a major role in determining the response of learners to elements utilization, therefore, paired student t-tests were used to relate variability to physical factors as well as objective observational conditions.

## **RESULTS**

The male: female ratio in this study is 1:1. The mean age was 7.73 ±0.60yrs. The average weight and height of the children are 21.93 ±4.8kg and 118.69 ±7.2cm, respectively. The median household income contributors were 2 persons and that of income was R250–R999 per month (Table 1). The mean value of the serum iron (umol/l) of the participating children was within the range of the book reference but 20%, 75% and 5% of the children presented with serum iron value below, within and above the standard reference range. This showed no significant drop in serum iron  $P>0.05$ . See table 2.

Similarly, the mean soluble transferrin receptor (mg/l) and mean serum ferritin of the participating children were within the range of the standard reference. However, 24%, 75% and 1% of the children presented with values below, within and above the standard reference range in each case. This value did not show any significant drop in soluble transferrin value  $P>0.05$ . See table 2.

The mean haemoglobin (g/dl) was within the range of the standard reference but 5%, 95%, and 0%, and none of the children presented with values below, within and above the standard reference range. There was no significant drop in haemoglobin  $P>0.05$ . See table 2. The mean mass cell volume (ft) of the participating children was also within the range of the book reference, however, 15% and 85% of the children presented

with values below and within the book reference range. There was no significant drop in MCV  $P>0.05$ . See table 2.

In comparing each biochemical indicator against serum iron using SAS (Pearson correlation coefficient), there was a significant ( $P<0.01$ ) correlation between serum iron and hemoglobin, as shown in Graph 1 with few out-layers. Removing all out-layers (Graph 2) a strong correlation could be seen, indicating marginal serum iron decrease in children investigated and the lack of consistence of serum iron decrease was also observed.

Only one child in this study had a value above this cut-off. MCV showed a corresponding level of low iron deficiency.

## DISCUSSION

The results have clearly showed a non prevalent of iron deficiency situation, with two or more biochemical indicators (indices) not showing abnormal levels of iron in serum as noticed in whole serum iron. This finding is in agreement with the theory that at least two biochemical indicators must be abnormal to ascertain iron deficiency (United Nations. 2000). Also that iron deficiency is the most common nutritional deficiency in the world, affecting as much as 66–80% of the world's population (United Nations. 2000) and is the leading nutritional cause of anemia in the developing world (Allen and Casterline-Sabel. 2001). Anemia been the third stage of iron deficiency may occur only when the total iron hemoglobin level is reduced below normal which is  $\leq 13$  for children (United Nations. 2000; WHO, 1968). It is contrary to most findings that showed high level of iron deficiency among disadvantage children, although other factors might have been responsible but the appropriate cut-offs for iron deficiency in infant and children will remain controversial.

Table 1: Socio-economic characteristics of participating children and family

		Age of participants (years)	Weight (kg)	Height (cm)	Family members contributing to household income	Family average wage
Phase One		Average in yrs	(Kg)	(Cm)	Income *	
1	November 2003	7.60	20.46	118.71	4.82	2.19
2	September 2004	7.84	22.48	118.62	4.12	1.84
1 & 2	2003/04	7.73 SD 0.60	21.93 SD 4.82	118.69 SD 7.23	4.73 SD 1.28	2.73 SD 1.24

Table 2: Blood and serum biochemical makers for iron

Parameters	Mean±Sd	**Standard reference range for category of participants	Number of participants with values within, above and below the standard reference range			SEM
			Below (%)	Within (%)	Above (%)	
Serum iron (umol/l) (n=236)	13.45±5.19	9.5 – 21.3	20	75	5	0.34
sTfR (mg/l) (n=235)	1.83±0.67	0.8 – 2.3	24	75	1	0.04
Ferritin (ug/l) (n=235)	30.11±15.52	20 – 100	24	75	1	1.01
Hb (g/dl) (n=232)	12.53±1.10	11.5 – 15.5	5	95	0	0.07
MCV (fl) (n=232)	83.10±4.42	77 – 95	15	85	0	0.29

sTfR = Soluble transferrin receptor, Hb = Hemoglobin, MCV = Mass cell volume, \*\*Ref Std (12)

Individual iron index levels showed a non-perfect distribution in comparison with the laboratory references, except in MCV and Hb where a strong relationship can be observed and statistical significance showed a strong correlation between samples mean and standard means, as observed in serum iron and hemoglobin, sTfR, and MCV.

One of the factors that is regularly overlooked when assessing iron status of children is the concentration of several nutrients such as zinc, vitamin A and iodine. Dietary intake requirement, inflammation and most biochemical indicators are age-related (Oti-Boateng, 1998).

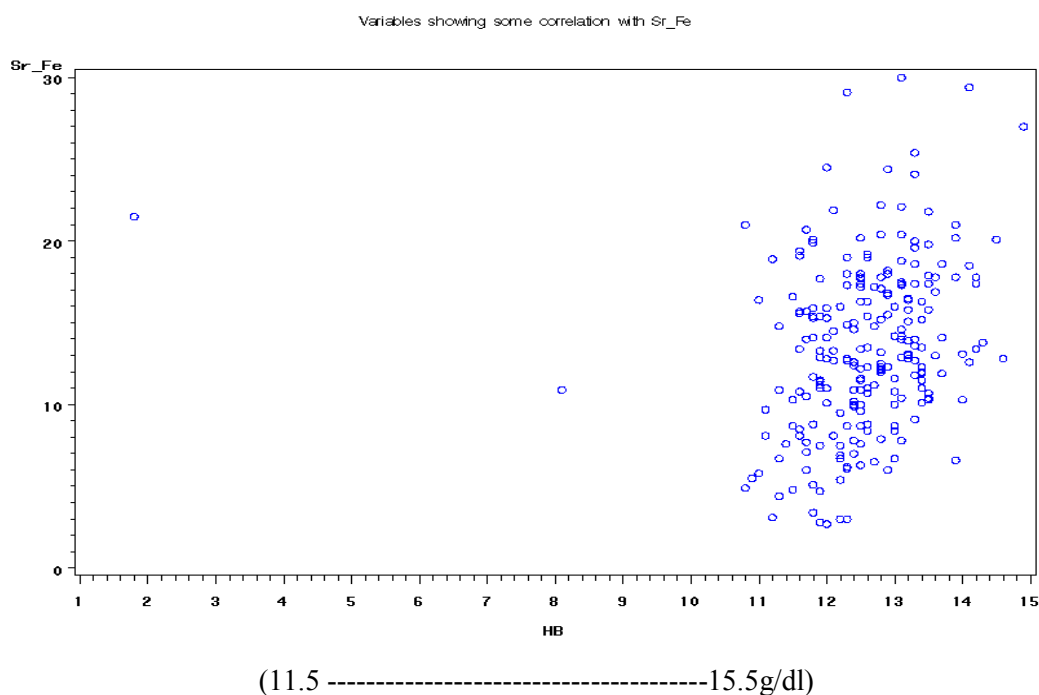
Although there were higher levels of sTfR and very low Ferritin and MCV observed in one of the samples, the results did not show exact relationship between both, but need further investigation especially when there are very high levels of sTfR which might result in lowering of both Ferritin and MCV. However, the proportion meeting criteria for iron deficiency was considered low ( $\leq 5\%$ ) and regular iron absorption and storage is not fully developed in infants therefore making it difficult to interpret measures connected to iron storage such as ferritin and sTfR and standards used for children results will tend to lack consistency.

Other results showed no differences between male and female children on any individual iron measure or the proportion meeting criteria for iron deficiency, this result is a contrast to gender difference observed in some other studies (Domellöf, *et al.*, 2002; Thorsdottir, *et al.*, 2003; Hay, *et al.*, 2004). Although the explanation for the differing finding is not readily available, iron food fortification might have played an important part.

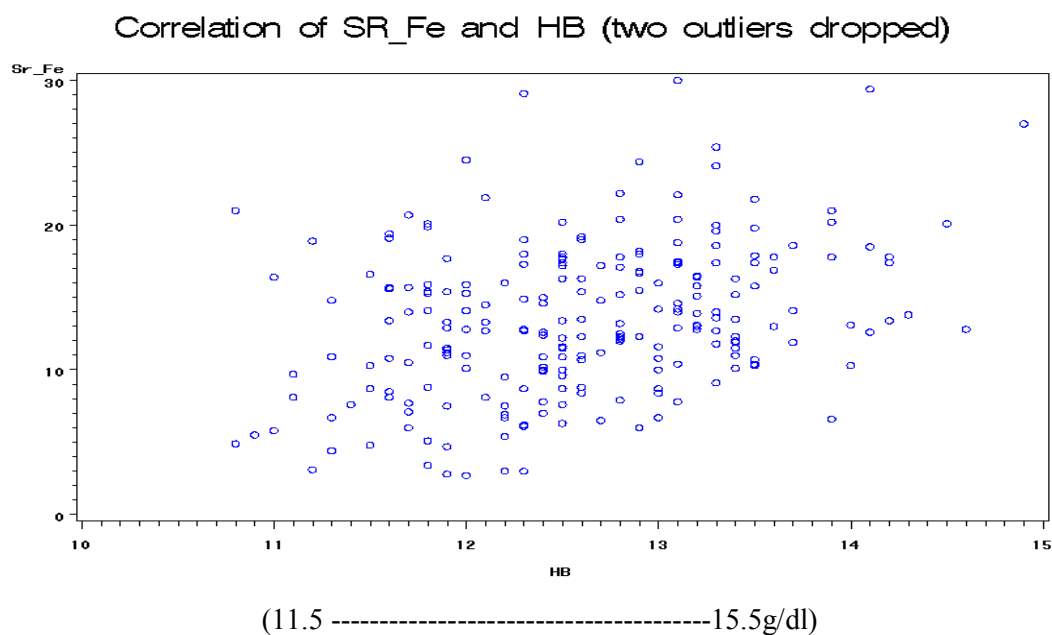
## CONCLUSIONS

Caution should be taken in drawing conclusion on the nutritional status (iron) of children, as many factors can easily influence the low biochemical iron indicators. Children's immune systems are still developing and there are higher frequencies of sickness than in adults. Sub-clinical inflammation in apparently-healthy children, ethnic life style, and state of other nutrients can easily lead to misinterpretation of iron status and overestimation of those with deficiency.

Graph: 1



Graph: 2



These results had shown marginal iron deficiency in the children population and might have been influenced by non active infection (inflammation and worms). Hence, the need for more investigation on their involvements prior to treatment is very important as most direct intervention has not been effective. Iron deficiency can easily be corrected with supplementation and food rich in iron before it gets to a clinical deficiency state.

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