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Association between iron status and white blood cell counts in African schoolchildren of the North-West Province, South Africa

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Abstract Iron deficiency with or without anemia is associated with increased susceptibility to infection owing to impaired immune function; this study aimed to examine the associations between markers of iron status and white blood cell counts in African schoolchildren. This cross-sectional study is part of the larger BeForMi study done in the North-West province of South Africa. A total of 556 African schoolchildren (aged 7–10 years) were recruited from the three schools participating in the BeForMi multiple micronutrient intervention study. Demographic information of the children was obtained from their parents/caregivers/guardians in the language of choice using validated questionnaires. Anthropometric indices (weight and height), iron status parameters, hematological parameters (hemoglobin (Hb), red blood cell count (RBC), total and differential white blood cell counts) were measured using standard procedures. No significant gender differences were observed in most of the iron markers and hematological parameters except in C-reactive protein (CRP) ($p = 0.004$) and eosinophils ($p = 0.042$) which were higher in boys while RBC ($p = 0.018$) and Hb ($p = 0.023$) levels were higher in girls. No relationships were observed between the different iron markers and differential white blood cell counts. A positive correlation was observed between serum ferritin (SF) and CRP in girls only ($r = 0.336$, $p < 0.01$), and a positive correlation between SF and mean cell volume (MCV) in boys only ($r = 0.197$, $p < 0.01$). In both genders, no correlations were observed between the different iron markers and the differential white blood cell counts. The study revealed no associations between iron status and differential

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white blood cell counts in children that participated in the BeForMi study calling for more studies to be done in the area of the significance of iron supplementation in healthy children.

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1. Introduction

Iron deficiency anemia is the most common nutritional deficiency in the world and brings with it negative consequences on growth and health [1,2]. In developing countries, the prevalence of anemia is widespread in children, and three to four times higher than in developed countries [3,4], making it a significant public health problem worldwide. The World Health Organization (WHO) estimated the national prevalence of anemia in South Africa (Hb <11 g/dl) to be 24.1% (WHO, 2008). Also, there have been three national nutritional surveys in the last 20 years and comparing the results of the three surveys shows that iron deficiency with or without anemia is prevalent in the country ranging from 5% to 14.3% [5–7]. The studies have reported the prevalence of anemia to be between 21% and 27.9% in children [5–7]. With regard to iron intake, it was reported that 25%–37% of children between the ages of 1 and 9 years had less than the recommended daily allowance, whereas 36–57% had iron intakes of less than two-thirds of the recommended daily allowance (RDA) [6]. The prevalence of poor iron status in children appeared to have increased when compared with previous national data [5], which point to the fact that the iron status of children in South Africa was deteriorating.

It is well recognized that the consequences of iron deficiency with or without anemia include increased susceptibility to infection owing to impaired immune function, poor growth development, reduced work capacity and mental performance, retardation of psychomotor development and reduced learning capacity [8]. Experimental evidence in the last decade showed that iron regulates the function of T-lymphocytes, and in most studies, a deficiency results in impaired cell-mediated immunity [9–12]. Iron deficiency may also delay the normal development of the cell-mediated immunity [9]. The various mechanisms that could explain the effects of iron deficiency on the immune system have been highlighted and reviewed by several authors [9,11].

Several studies have examined the associations between iron status and immune response in different populations [13–19], most of which were conducted on infants, adults and women. It has

been shown that measures of iron status such as ferritin, which is an acute-phase protein, are elevated in the presence of infection or inflammation [20]. Despite indications of a high prevalence of iron deficiency, the effect of iron deficiency on immune response in schoolchildren has not been well elucidated, especially when using white blood cell counts as immune markers. However, because of the high prevalence of iron deficiency among South African children, it is probable that cell-mediated immune response may be impaired in many of these children, which will be reflected as abnormal differential white blood cell counts. This study, therefore, examined the associations between iron status and immune response as measured by differential white blood cell counts in apparently healthy African schoolchildren.

2. Subjects and methods

2.1. Study design

The study was a cross-sectional study done in the North-West province of South Africa, not known to be a malaria endemic area. The selection of the study sites was the result of a consultation process with the most important stakeholders taking all priorities into account. Study sites were comprised of three primary schools in Jouberton in the greater KOSH (Klerksdorp, Orkney, Stilfontein, Hartebeesfontein) municipal area. The specific schools were identified in collaboration with the KOSH area Department of Education. Efforts were made to identify the most needy schools as these stood to benefit the most from the micronutrients to be provided. Three primary schools in Jouberton in the Matlosana municipal area of the North-West province of South Africa were included in the study. This study made use of the baseline data from the BeForMi multiple micronutrient beverage intervention study. Of the 1106 learners enrolled in the schools, 547 were excluded based on their age and 10 were not present during the days of measurements. The study population consisted of 556 schoolchildren between the ages of 7 and 10 years. This study was conducted according to the guidelines laid down in the Declaration of Helsinki. Written informed consent was obtained from the

parents of each child, and oral assent was obtained from each child. Permission to undertake the study was obtained from the Ethics Committees of the North-West University (**Research Ethics number: NWU-00065-09-A1**). The study was registered with the North-West Provincial Department of Health before the beginning of the study.

2.2. Demographic and anthropometric measurements

Demographic information was collected using a validated structured questionnaire, and the children's anthropometric measurements were done by trained anthropometrists, with the subjects barefoot and in their underwear. Boys' and girls' anthropometric measurements were performed separately in a private room. Weight was measured on a portable electronic scale (Precision Health Scale, A&D Company, Saitama, Japan) and height was measured with a stadiometer (IP 1465, Invicta, London, UK), with the subjects standing upright with their heads in the Frankfort plane. Z-Scores were calculated by using WHO Anthro Plus software (version 1.0.4).

2.3. Collection of blood samples and laboratory analysis

Venous blood samples (10 ml) were collected and delivered in two containers as follows: (i) 4 ml blood was collected in EDTA-containing tube for hemoglobin (Hb) and full blood count; (ii) 6 ml blood was collected in coagulant free tubes and centrifuged for the estimation of serum ferritin (SF), serum transferrin receptor (sTfR) and C-reactive protein (CRP). Hemoglobin (Hb) and full blood count (WBC, RBC, MCV, neutrophils, lymphocytes, monocytes, eosinophils and basophils) were measured at North West University Nutrition laboratory using an AcT 5diff Cap Pierce Hematology Analyzer (Beckam Coulter, Miami, Florida, USA), using 3-level controls provided by the manufacturer, within 2 h of blood sampling. Zinc Protoporphyrin (ZnPP) analysis was measured on washed red blood cells (from the EDTA tubes) using a hematofluorometer (Aviv Biomedical, Lakewood, NJ, USA) and 3-level control material provided by the manufacturer. Enzyme-linked immunosorbent assays were used to measure sTfR and SF (Ramco Laboratories, Inc, Stafford, Texas). CRP was measured by immunoturbidimetric test (Human Biochemical and Diagnostical Laboratories, South Africa). The coefficients of variance (CV) for all assays were <10%.

2.4. Statistical analyses

Data were analyzed using the Statistical Package for Social Sciences (SPSS) version 18 package. The data were log transformed and after log transformation most were still not normally distributed, hence non-parametric tests were used for the analysis. The results are presented as medians and interquartile ranges (IQR). Spearman's correlations were used to assess the relationships between indices of iron status (SF, TfR, transferrin to serum ferritin [TfR/SF] ratio), Hb and ZnPP) and other hematological parameters (RBC, WBC, Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils). Additionally, for both genders, SF was used to divide subjects into two groups to ascertain if iron depletion affects differential WBC count—Group 1: SF <12 µg/L; Group 2: SF between 12–150 µg/L; and differential WBC counts were compared. These cut-off points were used as they are the clinical cut-off points recommended for standard dietetics practice [21] and have been successfully used by Gopane et al. [22]. For both genders, Mann–Whitney *U* test was used to assess differences in WBC counts between the two SF groups ($p < 0.05$ was considered statistically significant).

3. Results

The baseline clinical and anthropometric characteristics of the 556 children who participated in the study are presented in Table 1. The median age for both males and females was 8 years, and 53% of the participants were boys. The results revealed no significant differences in most parameters between boys and girls with the exception of CRP ($p = 0.004$) and eosinophils ($p = 0.042$) which were higher in boys, whereas Hb ($p = 0.023$) and RBC ($p = 0.018$) were higher in girls. The measured iron markers' median values were within the normal reference values. White blood cell (WBC) and red blood cell (RBC) medians were within the normal reference values [21] in both genders (Table 1). Also, the differential WBCs were within the normal reference values with the exception of monocytes, which were slightly lower than normal values in both boys and girls (Table 1).

Table 2 shows the classification of the children according to their iron status. The overall prevalence of anemia (Hb <11.5 g/dl) was 6.8%, iron deficiency (Hb \geq 11.5 g/dl and SF <12 µg/L) was present in 13.9% and iron deficiency anemia (Hb <11.5 g/dl and SF <12 µg/L) was present in 5.6% of the children.

Correlations between the measured iron markers, CRP and hematological parameters in the total

Table 1 Clinical and anthropometric characteristics of the children according to gender.

Variables	Boys (N = 283)	Girls (N = 273)	Normal reference range (20)	P value
	Median (IQR)	Median (IQR)		
Age (years)	8.05 (1.20)	8.27(1.35)		NS
BAZ	-0.57(1.32)	-0.52(1.53)		NS
HAZ	-1.04 (1.40)	-0.75 (1.18)		NS
WAZ	-1.05 (1.50)	-0.83 (1.49)		NS
Serum ferritin (µg/l)	24.74(21.72)	26.90(28.29)	12–150	NS
TfR (µg/l)	5.77(1.69)	5.69(1.65)	2.9–8.3	NS
TfR/Serum ferritin ratio	0.24(0.24)	0.21(0.28)	>0.975	NS
CRP (mg/l)	3.70(3.10)	3.30(2.70)	3–10	0.004
ZnPP (µmol/mol heme)	56.00(21.38)	56.00(24.50)	<40	NS
RBC (1,000,000/µl)	4.58(0.47)	4.74(0.47)	3.5–5.0	0.018
WBC (1000/ µl)	6.80(2.70)	6.90(2.70)	4.0–11.0	NS
Hb (g/dl)	12.60(1.20)	12.80(1.15)	11.5–13.0	0.023
MCV (fL)	83.00(5.00)	83.00(6.00)	76–96	NS
Neutrophils (1000/µl)	2.78(2.20)	2.80(2.02)	2.0–7.5	NS
Lymphocytes (1000/µl)	3.02(1.10)	2.87(1.01)	1.5–4.0	NS
Monocytes (1000/µl)	0.45(0.22)	0.43(0.19)	0.5–1.5	NS
Eosinophils (1000/µl)	0.34(0.36)	0.29(0.30)	0.04–0.4	0.042
Basophils (1000/µl)	0.03(0.02)	0.04(0.02)	0.0–0.1	NS

N = number, IQR = Interquartile range, BAZ = BMI-for-age z-score, TfR = Transferrin receptor, HAZ = Height-for-age z-score, WAZ = Weight-for-age z-score, CRP = C-reactive protein, ZnPP = Zinc protoporphyrin, RBC = red blood cell, WBC = white blood cell, MCV = mean cell volume, Hb = Hemoglobin, NS: Not significant.

Table 2 Classification of the children according to iron status.

Diagnosis/condition	Indicators [7]	Total N (%)	Females (n = 273)	Males (m = 283)
Anemia	Hb <11.5 g/dl	38 (6.8)	22 (8.0)	16 (5.6)
Iron deficiency	Hb ≥11.5 g/dl & Serum ferritin <12 µg/L	77 (13.9)	36 (13.2)	41 (14.5)
Iron deficiency	Hb <11.5 g/dl & Serum ferritin <12 µg/L	31(5.6)	17 (6.2)	15 (5.3)
Anemia				
Iron sufficient	Hb ≥11.5 g/dl & Serum ferritin ≥12 µg/L	410 (73.7)	198(72.5)	210 (74.2)

N = Number.

group are displayed in Table 3. SF correlated positively with weight-for-age z-score (WAZ) ($r = 0.124$, $p < 0.01$), Hb ($r = 0.191$, $p < 0.01$), CRP ($r = 0.167$, $p < 0.01$), and MCV ($r = 0.159$, $p < 0.01$). No correlations were observed between any iron markers and differential WBC counts. Table 4 shows the correlations between iron markers, CRP and hematological parameters after splitting by gender. A positive correlation was observed between SF and CRP in girls only ($r = 0.336$, $p < 0.01$), and a positive correlation between SF and MCV in boys only ($r = 0.197$, $p < 0.01$). In both genders, no correlations were observed between the different iron markers and differential WBC counts. In both genders adjusting for CRP levels did not change the associations observed.

In the total group when comparing children with low SF (SF <12 µg/L) and those with high SF (SF

≥12 µg/L), the only difference observed was with eosinophils which were higher in the low SF group (0.38; [IQR 0.44]) than in the high SF group (0.30; [IQR 0.31]) ($p = 0.026$). Splitting the children by gender showed no differences in boys, but did in girls. There were differences in lymphocytes ($p = 0.033$) with the low SF group recording higher values (3.26; [IQR 1.38]) than the high SF group (2.88; [IQR 0.95]). The same was observed with eosinophils ($p = 0.011$) with the low SF group recording higher values (0.40; [IQR 0.54]) than the high SF group (0.27; [IQR 0.28]).

4. Discussion

In this study, the associations between iron status and WBC counts of healthy South African school-children were examined. The study generally

Table 3 Correlations (r_s) between markers of iron status, CRP; and hematological and anthropometric parameters of children ($N = 556$).

Variable	Ferritin	TfR	TfR/Ferritin	HB	ZnPP	CRP
Age	0.258**	-0.086*	-0.249**	0.194*	0.032	0.050
BAZ	0.051	0.042	-0.054	0.054	0.067	0.141
WAZ	0.124**	0.078	-0.031	0.214**	0.051	0.083
HAZ	0.074	0.085	-0.074	0.231**	0.009	0.052
Ferritin	—	-0.285**	-0.967**	0.191**	0.007	0.167**
TfR	-0.285**	—	0.490*	-0.017	0.029	0.001
TfR/ratio plu	-0.967**	0.490**	—	-0.177*	0.010	-0.149**
CRP	0.167**	0.001	-0.149**	-0.005	-0.073	—
ZnPP	0.007	0.029	0.010	-0.026	—	-0.073
RBC	0.023	0.149**	-0.006	0.640**	-0.015	-0.046
WBC	-0.049	-0.014	0.032	-0.044	-0.049	-0.038
Hb	0.191**	-0.017	-0.177**	—	-0.026	-0.005
MCV	0.159**	-0.188**	-0.165**	0.274*	0.000	0.008
Neutrophils	-0.071	-0.030	0.054	-0.032	-0.054	-0.062
Lymphocytes	-0.045	0.013	0.039	0.034	-0.051	-0.008
Monocytes	-0.021	-0.015	-0.007	-0.036	-0.061	0.007
Eosinophils	-0.024	0.007	0.015	0.004	-0.038	0.017
Basophils	-0.056	0.005	0.033	0.018	-0.011	-0.045

r_s = Spearman correlation, BAZ = BMI-for-age z-score, WAZ = Weight-for-age z-score, HAZ = Height-for-age z-score, TfR = Transferrin receptor, CRP = C-reactive protein, ZnPP = Zinc protoporphyrin, RBC = red blood cell, WBC = white blood cell, MCV = Mean cell volume, Hb = Hemoglobin.

** Correlation coefficient is significant at the 0.01 level (2-tailed).

* Correlation coefficient is significant at the 0.05 level (2-tailed).

showed no relationships between iron status and WBC counts in this population. There were significant gender differences in the population with regard to the distribution of CRP, RBC and Hb. With regard to iron deficiency, though not significant, boys tended to have higher prevalence rates which are contrary to previous findings where girls have been shown to be more anemic [23].

This study showed no association between the measured iron markers and some immune variables (differentiated WBC). A number of studies have found that several immune parameters are affected during iron deficiency, especially cell-mediated immunity and bactericidal activity of neutrophil granulocytes [17,24]. In this study this was not the case probably because the sample was drawn from a subpopulation of children with low prevalence of iron deficiency (Table 2). According to Chandra [24], the ability of blood neutrophils and peritoneal macrophages to kill ingested bacteria and fungi is reduced in iron-deficient individuals and is associated with alterations in intracellular metabolic activity. Conversely, in a study among homebound, older women in Pennsylvania, no differences were seen between the iron-sufficient and iron-deficient groups with respect to total lymphocytes or their subpopulations, granulocytes, or Monocytes, when they were expressed as absolute counts or percentages [19]. Krause et al., [25] have also shown that

several immune parameters, including circulating T-lymphocyte number, natural killer cell cytotoxicity, phagocytosis, and bactericidal function, did not differ in generally healthy and well-nourished women. Lesourd [26] reported that these changes in the immune system are more important in populations that are not well nourished and in populations who exhibit decreased iron status mainly because immune responses are related to health status. This could explain why there were no associations observed between the iron markers and immune parameters in this study population.

This study demonstrated a positive association between SF and CRP in girls. Numerous studies of iron status conducted in developing countries have used biomarkers of inflammation to determine the influence of this inflammation on markers of iron-status [27–29]. SF is an acute phase reactant which is elevated during inflammation [30]. These associations could be a result of the weight differences observed to be higher in girls. Other studies in the literature have confirmed the association between weight and CRP and pointed toward BMI as a major contributor to the observed variation in CRP levels in different populations [31–34]. But, surprisingly, there were no differences in BAZ between boys and girls in this study. BMI now recognized as an inflammatory marker [35,36] may have contributed indirectly to the observed

Table 4 Correlations (r_s) between markers of iron status, CRP and hematological parameters of children by gender ($N = 556$).

Variable	Ferritin		CRP		ZnPP		TfR		Hb		TfR/Ferritin	
	Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls
Age	0.227**	0.295**	-0.017	0.099	0.015	0.055	-0.142*	-0.043	0.168**	0.229**	-0.226**	-0.287**
BAZ	0.024	0.094	0.154**	0.079	-0.076	0.238**	0.037	0.146	0.057	0.014	-0.002	-0.038
WAZ	0.045	0.134	0.102	0.078	-0.074	0.197	0.029	0.139	0.152*	0.084	-0.047	-0.108
HAZ	0.032	0.135	0.057	0.093	-0.018	0.042	0.048	0.049	0.127	0.127	-0.039	-0.103
Ferritin	—	—	0.011	0.336**	0.093	-0.083	-0.266**	-0.292**	0.214**	0.159*	-0.955**	-0.977**
TfR	-0.266**	-0.292**	-0.008	-0.024	0.075	-0.011	—	—	0.039	-0.039	0.498**	0.469**
TfR/Ferritin ratio	-0.955**	-0.977**	-0.008	-0.319**	-0.062	0.084	0.498**	0.469**	-0.170*	-0.161*	—	—
CRP	0.011	0.336**	—	—	0.042	-0.183**	-0.008	-0.024	-0.039	0.071	-0.008	-0.319**
ZnPP	0.093	-0.083	0.042	-0.183**	—	—	0.075	-0.011	0.055	-0.112	-0.062	0.084
RBC	0.024	0.009	-0.050	-0.006	0.069	-0.098	0.195**	0.130*	0.630**	0.648**	0.011	0.005
WBC	0.003	-0.101	-0.094	0.034	-0.054	-0.045	0.009	-0.023	-0.046	-0.046	-0.005	0.077
Hb	0.214**	0.159*	-0.039	0.071	0.055	-0.112	0.039	-0.039	—	—	-0.170*	-0.161*
MCV	0.197**	0.129	0.002	0.026	-0.023	0.012	-0.178**	-0.187**	0.324**	0.213**	-0.185*	-0.146*
Neutrophils	-0.128	-0.011	-0.112	0.003	-0.240**	0.145*	-0.045	-0.006	-0.024	-0.039	0.114	-0.006
Lymphocytes	-0.009	-0.072	-0.105	0.097	-0.061	-0.036	-0.014	0.017	0.079	-0.007	-0.016	0.081
Monocytes	-0.072	0.032	-0.019	0.013	-0.147*	0.038	-0.054	0.014	-0.054	-0.010	0.024	-0.039
Eosinophils	0.074	-0.107	0.010	-0.005	-0.076	0.004	-0.008	-0.010	0.000	0.030	-0.096	0.100
Basophils	-0.075	-0.036	-0.066	0.002	-0.098	0.087	0.019	0.005	0.006	0.028	0.054	0.016

r_s = Spearman correlation, BAZ = BMI-for-age z-score, WAZ = Weight-for-age z-score, HAZ = Height-for-age z-score, TfR = Transferrin receptor, CRP = C-reactive protein, ZnPP = Zinc protoporphyrin, RBC = red blood cell, WBC = white blood cell, MCV = mean cell volume, Hb = Hemoglobin.

** Correlation coefficient is significant at the 0.01 level (2-tailed).

* Correlation coefficient is significant at the 0.05 level (2-tailed).

positive association between SF levels and CRP in girls. The weight differences between boys and girls could serve as an additional explanation for the positive association between SF and CRP. However, it was odd to find that hemoglobin levels were higher in girls than in boys while it is known that at this age there are no significant gender differences.

It is also known that MCV is a marker of iron deficiency erythropoiesis; as such its relationship with TfR is in agreement with previous findings which have shown that red cell parameters decreased gradually with increasing negative iron balance as iron deficiency occurred [23]. However, it is hard to explain the relationship between MCV and SF only in boys. But it is possible that since it is also an iron marker, the relationship is related to that of its receptor. It is also worthwhile to note that boys had slightly higher percentages of those who were iron deficient though this was not statistically significant. Recent evidence seems to be pointing to the direction that improving iron status in children may be a potential risk in stimulating the development of infection [37,38]. Although iron supplementation improves cognition and growth in deficient children [39–42], this may not be so when improving immune function in a healthy population as the increased available iron may be harmful through the proliferation of bacteria by the iron concentration of the culture medium [43] and iron supplements can produce oxidative stress and cause damage to cells mediated through free radicals [44]. Because iron is not easily eliminated from the body, attention has to be paid to circumstances in which excess iron may be absorbed or used inappropriately. In Africa, infections are a major factor when considering supplementation because of the potential risk that improving iron status may have on bacterial growth, especially in healthy children. Oppenheimer et al., [45] have previously reported increased infection rates after iron intervention.

5. Conclusion

It is concluded that there are no associations between the measured iron markers and white blood cell counts in healthy children that participated in the BeForMi study. It is thus possible that in healthy children, the association is shielded or that the population has a different genotype, an area that needs to be explored further. Furthermore, this highlights the need for studies around the issue of supplementing iron sufficient children to explore whether this is beneficial or detrimental to them with respect to their immune function (especially

the reported increases in infection rates) [45] and weighing against the fact that iron supplementation improves cognitive development.

Conflict of interest

There are no supplementary material submitted on line neither is there any potential conflict of interest. The article contains no libelous or unlawful statement none infringe upon anyone else's copyright.

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