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MAGNITUDE, SOCIO-ECONOMIC AND DIETARY INTAKE PREDICTORS OF MICRONUTRIENT DEFICIENCIES AMONG SCHOOL-AGED CHILDREN IN RURAL SOUTH AFRICA, A COMMUNITY-BASED CROSS-SECTIONAL STUDY

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Micronutrient deficiencies are detrimental to children's health and this study determined the prevalence and predictors of micronutrient deficiencies among children in rural Eastern Cape, South Africa. This was a cross-sectional community-based study of 237 school-aged children (6-18 years) from five purposively selected schools. Measurements included: socio-economic characteristics (parents/guardians), dietary intake (24-hour recalls evaluated using the Estimated Average Requirements (EAR) or Adequate Intakes (AI) and dietary diversity (validated nine (9) food groups Dietary Diversity Questionnaire [DDQ]) of children. Biochemical markers of micronutrient deficiencies (serum iron, zinc, vitamins A, B12 and folate) were measured using standard protocols. Data was analysed using Statistical Package for Social Sciences (SPSS) version 23. High DDS (8 median group) was reported, however, 83.3% of children had inadequate dietary iron intakes; with variations ($p < 0.001$) across the age groups (6-8 years-23.6%, 9-13 years-35.0% and 14-18 years-41.8%). No significant differences were observed in serum micronutrient deficiencies among the age groups, except for vitamin B12 ($p < 0.001$). Vitamin B12 deficiency increased with increasing age. A linear regression indicated 18.0% of the variability in serum folate (adjusted $R^2 = 0.18$; $p < 0.0001$) was explained by caregivers educational status (coefficient = 2.65; $p < 0.0001$), household income (coefficient = 1.45; $p = 0.002$) and employment status (coefficient = 1.11; $p = 0.50$). The high DDS among the children did not translate to high caregivers micronutrient intakes and status.

Keywords: Predictors, Micronutrient deficiencies, School-aged children, South Africa

INTRODUCTION

Globally, micronutrient deficiencies have devastating consequences and may cause a major threat to the health and development of populations. Iodine, zinc, iron, vitamin A and folic acid deficiencies are common among children in resource-poor countries (UNICEF, 2013; and WHO, 2015) and are of known public health importance. During childhood, micronutrients are required for the growth and development phase (Herrador *et al*., 2014).

South Africa (SA) is a country in nutrition transition, characterised by a "double burden of malnutrition" with under- and over-nutrition occurring in the same population (Shisana *et al.*, 2013). According to the South African National Health and Nutrition Examination Survey of 2012 (SANHANES-1, 2014), 10.7% of children under five years of old were anemic with 1.9% suffering from iron deficiency anemia (IDA) and 8.1% was Iron Deficient (ID). The Vitamin A Deficiency (VAD) prevalence for children under five was 43.6%, thus still a severe public health problem more than

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10 years after the implementation of a national vitamin A supplementation (2002) and food fortification (2003) programme (SANHANES-1, 2014). No information was available for school-aged children or for the other micronutrients in this report. However, the prevalence of anemia and VAD in women is 22.0% and 27.2% respectively and constituted a moderate public health problem. The prevalence of mild, moderate and severe anemia in both genders combined and older than 15 years of age was 11.6%, 5.3% and 0.6% respectively with an overall prevalence of 17.5% (SANHANES-1, 2014). The prevalence of IDA in children aged 1-9 years old was 33.3% in 2005 compared to 66.7% and 45.3% of children having an inadequate vitamin A and zinc status respectively (Labadarios *et al.*, 2007).

A number of studies have reported on micronutrient deficiencies in children in South Africa, but the epidemiological value of this study is the inclusion of multiple micronutrients, dietary intakes and dietary diversity in terms of food group intakes. This study was undertaken in the Intsika Yethu municipality (Chris Hani District Municipality, 2011) where 87.1% of the adult population is unemployed and living in poverty (Intsika Yethu Local Municipality, 2012). The aim of the present study was to determine the prevalence of iron, zinc, vitamins A, B12 and folate deficiency as well as dietary intakes, among apparently healthy school-aged girls and boys, attending five purposively selected schools in rural Cofimvaba, situated in the Eastern Cape Province of SA.

MATERIALS AND METHODS

This study followed a cross-sectional community-based study design.

Ethics

Both the Senate Research and Innovation Ethics Committee of the Vaal University of Technology (20130520-3) and the Council of Scientific and Industrial Research (CSIR) approved the study protocol and complied with the Helsinki Declaration guidelines (Council for International Organizations of Medical Sciences (CIOMS), 2002). Five schools (n = 1225 learners 6 to18 years old) out of a total of 21 schools (all beneficiaries of the National School Nutrition Programme) were purposively selected by the Department of Basic Education for inclusion in the study, thus 25% representation. Informed consent were obtained from the school management, parents/legal guardians of the children and the children. The inclusion criteria was included: all schoolchildren aged 6-18 years of age, both genders and attending one of the five purposively selected schools. A total of 523 children with signed informed consent by both the parents/legal guardians and children, were eligible to be included in the study.

Sampling and Respondents

A sample size calculator (The Survey System, 2013) with a confidence level of 95% was used to determine a representative sample size; 218 children was required and 240 children were randomly selected from those for whom informed consent had been obtained. The extra 22 children were selected in anticipation of possible drop-out from the study as measurements were not done on one day. Data was collected in August 2013.

Data Collection

Four students from the Department of Food and Consumer Sciences at the Walter Sisulu University were recruited and trained as data enumerators. A validated socio-demographic questionnaire used in a previous study (Oldewage-Theron *et al.*, 2006), was translated into Xhosa and included questions about household size, age, level of literacy, education level and marital status of the parents/legal guardians, as well as household composition, ethnicity, income source/s and basic living conditions, such as type of house, water and sewerage facilities and the structure of the dwelling. The socio-demographic questionnaire was completed by the parents/legal guardians of the sampled children with the assistance of the trained fieldworkers.

A validated four-stage, multiple-pass interviewing procedure (Gibson, 2005) was used to complete 24-hour recall questionnaires for a week- and weekend day in two sequential weeks for the children aged 10 years and older by the fieldworkers in a one-on-one interview. Locally used household utensils and food models were used for quantifying the portion sizes of the foods consumed. The same procedures were followed for the younger children with the assistance of the parents/legal guardians.

An adapted validated Dietary Diversity Questionnaire (DDQ) (Matla, 2008) was used to collect data on Dietary Diversity (DD) indices. The DDQ was a printed list of foods grouped according to the nine food groups recommended by the United Nations Food and Agriculture Organization (FAO) (FAO, 2015). The DDQ was completed in one-on-one interviews with the children ($\geq$10 years) and for the younger children in the presence of the parents/legal guardians.

Blood was drawn from the *vena cephalica* of seated subjects after an 8-12 hour fast using a Vacutainer needle with minimal use of tourniquets by a registered nurse wearing disposable polyethylene gloves, free of talc or any other coatings. Blood was collected 5ml blood in an EDTA tube (purple lid) for full blood counts (Haemoglobin (Hb), Haematocrit (Hct), Red Blood Cell count (RBC), White blood cell count WBC). These analyses were done immediately after blood collection was completed. Other blood collection included 10ml blood in a silicone-coated tube for preparation of serum for the analysis of vitamin A, serum ferritin, serum iron, transferrin, serum folate, vitamin B12 and high-Sensitivity C-Reactive Protein (HS-CRP) as well as 5ml blood drawn into trace element-free, evacuated tubes with anti-coagulant, using silicone stoppers. The 10ml blood was covered in foil until analyses to prevent light affecting the vitamin A content. The blood was placed on ice until separation within two hours of blood collection. Serum was harvested by low-speed centrifugation at 4 °C and aliquoted into individual Eppendorf test tubes. Serum was stored at -80 °C until analyses. Analyses were performed as follows: serum iron (colorimetric, Konelab 20i analyser); ferritin (colorimetric, Konelab 20i analyser), haemoglobin (Hb) (cyanmethaemoglobin-colorimetric method, Sysmex SF300), haematocrit (Hct) (numeric integration, Sysmex SF300), red blood cell count (RBC) (direct current detection, Sysmex SF300), white blood cell count (WBC) (flow cytometry with dual angle light scattering, Sysmex SF300), transferrin (colorimetric, Konelab 20i analyser), high-sensitive C-reactive protein (HS-CRP) (colorimetric, Konelab 20i analyser), serum folate (colorimetric, Konelab 20i analyser), serum vitamin B12 (colorimetric, Konelab 20i analyser) and serum glucose (colorimetric, Konelab 20i analyser). The method used for analysing serum zinc by Atomic Absorption Spectroscopy (AAS) was described by Burtis and Ashwood (1999). Serum vitamin A was analysed by High Performance Liquid Chromatography (HPLC).

Data Analyses

The SPSS program, version 23 was used for all statistical analyses with 95% confidence level ($p < 0.05$). Normal probability plot and Kolmogorov-Smirnov tests were used for the normal distribution of variables. Most variables were not normally distributed and therefore medians as well as interquartile ranges (IQR) were calculated. The socio-demographic data were analysed for descriptive statistics (frequencies and percentages for categorical variables as well as medians for continuous variables).

The Food Finder dietary analysis software program version 3 was used for analysing the 24-hour recall questionnaires (translating the consumed food items into nutrients). Medians and IQRs were calculated for daily nutrient intakes and compared with the EAR or AI for the age groups, 4-8, 9-13 and 14-18 years (IoM, 1997; 1998; 2000a; 2000b; 2001 and 2002) respectively.

The different dietary diversity measures were calculated for a reference period of seven days (Ruel, 1998) as follows: 1) the overall Food Variety Score (FVS) (simple count of food items), and 2) a variety score within every food group (Food Group Diversity Score [FGDS]), and 3) the median FGDS for all food groups referred to as Dietary Diversity Score (DDS) or (Hatloy *et al.*, 1998). Low food variety was defined as fewer than 30 foods consumed in the period of seven days, medium variety as 30 to 60 foods, and high variety as more than 60 foods (FVS) (Matla, 2008). Similarly, one to three food groups consumed in the period of seven days indicated low DDS, four to five groups indicated medium DDS and six to nine groups indicated high DDS (Matla, 2008).

Medians and quartiles were calculated for the haematological and biochemical variables and compared to the normal range for each variable. The percentage of respondents with abnormal values was also calculated.

Levene's test for equality of variances was performed to measure significant differences between the biochemical variables of the boys and girls. Non-parametric (Mann-Whitney) t-tests were carried to measure significant differences between the dietary intake, dietary diversity and haematological and biochemical variables of the different age groups. Spearman correlations were used to determine significant relationships between dietary intake, diversity and biochemical variables. A multiple lineal regression was used to assess the predictors of serum iron, zinc, vitamins A, B12 and folate while adjusting for confounders (age of child, caregiver's educational status, household's income and employment status).

RESULTS

Socio-Demography

The sample consisted of 49.8% (n = 118) girls and 50.2% (n = 119) boys with 23.6% of the children in the 6-8 year old, 35.0% in the 9-13 year old and 41.4% in the 14-18 year old group. Of the child caregivers/legal guardians, 87.3% were women and 12.7% were men. The mean ± SD age of the

Table 1: Analysis of 24-Hour Recall: Daily Median Intakes of the Children

	Children aged 6-8 years old (n=56)			Children aged 9-13 years old (n=83)			Children aged 14-18 years old (n=98)			
Nutrient and unit of measure	**Median (IQR)**	**Prevalence of inadequate intakes (%)**	**EAR**	**Median (IQR)**	**Prevalence of inadequate intakes (%)**	**EAR**	**Median (IQR)**	**Prevalence of inadequate intakes (%)**	**EAR**	**Significant differences before and after the intervention *P***
Energy (kJ)	6149 (4476; 7614)	**72.2**	7316 (b)/ 6896 (*g)	7172 (6083; 8599)	**50.8**	9572 (b) / 6898 (g)	7141 (5700; 9370)	**77.1**	13238 (b)/ 9940 (*g)	0.001
Total protein (g)	46.1 (35.3; 63.5)	**0.0**	19	52.6 (43.3; 65.3)	**15.3**	34	53.3 (41.3; 70.7)	**25.7**	52 (b)/ 46 (g)	0.15
Total fat (g)	41.7 (25.2; 57.8)			47.9 (31.1; 62.6)			49.9 (33.5; 67.4)			
Carbohydrates (g)	207 (160; 249)	**2.8**	100	260 (209; 294)	**0.0**	100	262 (202; 320)	**0.0**	100	0.000
Total dietary fibre (g)	10.0 (7.6; 14.3)	**100.0**	25#	12.1 (9.00; 16.25)	**98.5**	31 (b)/ 26 (*g)#	12.9 (10.3; 16.7)	**97.1**	38 (b)/ 26 (*g)#	0.008
Calcium (mg)	255 (146; 352)	**100.0**	800#	328 (212; 522)	**100.0**	1300#	324 (192; 432)	**100.0**	1300#	0.002
Iron (mg)	4.99 (4.03; 6.47)	**83.3**	4.1	6.39 (4.90; 7.97)	**35.4**	5.9 (b)/ 5.7 (*g)	6.48 (4.90; 8.36)	**57.1**	7.7 (b)/ 7.9 (*g)	0.005
Magnesium (mg)	160 (133; 193)	**2.8**	110	207 (170; 246)	**46.2**	200	206 (168; 244)	**94.3**	340 (b)/ 300 (*g)	0.001
Zinc (mg)	4.97 (3.82; 6.49)	**66.7**	4.0	5.99 (4.97; 7.43)	**55.4**	7.0	6.37 (4.67; 7.63)	**74.3**	8.5 (b)/ 7.5 (g)	0.014
Vitamin A (RE) (mcg)	224 (114; 412)	**52.8**	275	253 (133; 371)	**89.2**	445 (b)/ 420 (*g)	179 (107; 320)	**88.6**	630 (b)/ 485 (*g)	0.700
Thiamine (mg)	0.60 (0.45; 0.81)	**38.9**	0.5	0.80 (0.59; 0.97)	**38.7**	0.7	0.77 (0.64; 1.04)	**62.9**	1.0 (b)/ 0.9 (*g)	0.004
Riboflavin (mg)	1.41 (0.66; 2.49)	**16.7**	0.5	1.57 (0.99; 2.54)	**15.4**	0.8	1.54 (0.60; 3.01)	**28.6**	1.1 (b)/ 0.9 (*g)	0.20
Niacin (mg)	11.5 (8.13; 16.7)	**8.3**	6.0	12.9 (8.15; 17.0)	**33.8**	9.0	11.1 (7.82; 17.7)	**37.1**	12 (b)/ 11 (*g)	0.85
Vitamin B6 (mg)	0.77 (0.57; 1.02)	**13.9**	0.5	0.94 (0.76; 1.21)	**30.8**	0.8	0.89 (0.61; 1.21)	**54.3**	1.1 (b)/ 1.0 (*g)	0.13
Folate (mcg)	112 (77.0; 176)	**69.4**	160	145 (1052; 196)	**92.3**	250	143 (105; 213)	**97.1**	330	0.04
Vitamin B12 (mcg)	0.85 (0.58; 1.52)	**66.7**	1.0	1.36 (0.663; 2.22)	**58.5**	1.5	1.31 (0.73; 2.33)	**74.3**	2.4	0.30
Pantothenate (mg)	5.56 (4.03; 8.30)	**8.3**	3.0#	5.72 (3.73; 7.81)	**100.0**	4.0#	5.27 (3.80; 7.47)	**34.3**	5.0#	0.91
Biotin (mcg)	16.0 (12.5; 21.3)	**22.2**	12#	18.7 (14.9; 25.3)	**58.5**	20#	19.9 (14.3; 26.2)	**68.6**	25#	0.10
Vitamin C (mg)	20.0 (11.3; 32.4)	**58.3**	22	23.8 (15.0; 34.0)	**100.0**	39	18.1 (13.1; 38.3)	**91.4**	63 (b)/ 56 (*g)	0.93
Vitamin D (mcg)	1.50 (0.51; 3.35)	**100.0**	5.0#	1.43 (0.62; 3.42)	**83.1**	5.0#	2.07 (0.65; 4.99)	**71.4**	5.0#	0.07
Vitamin E (mg)	7.01 (3.83; 10.5)	**50.0**	6.0	7.63 (4.35; 12.5)	**50.8**	9.0#	9.40 (5.38; 15.7)	**45.7**	12.0#	0.003
Vitamin K (mcg)	52.3 (10.5; 114)	**41.7**	55#	70.1 (24.5; 148)	**43.1**	60#	49.7 (13.4; 154.7)	**57.1**	75#	0.67

Note: b = boy, *g = girl; EAR – Estimated Average Requirement; # = AI – Adequate Intake; mcg = Microgram; mg = Milligram; IQRs = Inter Quartile Ranges; n = number of children ad p was significant at $p<0.05$.

child caregivers/legal guardians was 53.8 ± 16.1 years and ranged from 21-103 years. The majority of the households stayed in clay houses (51.5%) with either two (31.1%), three to four (34.9%) or Greater than four (33.0%) rooms. The average household size accommodated five people. The majority of the child caregivers were single (62.7%) and a large percentage had received primary (46.8%) and secondary (30.3%) education, but only 7.7% of the caregivers/legal guardians and 11.4% their spouses were employed with 35.0% who received a state pension. The majority of the households (80.7%) had a household income of less than ZAR2000 per month. This amounts to ZAR12.58 per person per day, which is about US$1.25 per person per day, the cut-off point for people living in poverty, according to the World Bank (The World bank, 2016).

Dietary Intake and Diversity (Table 1&2)

The nutrient intake analysis of the diets indicated deficient median intakes for a large number of nutrients for the six to eight year group of children when compared to the EAR. These were total energy, dietary fiber, calcium, iodine, as well as vitamins B12, C, D, K, folate and pantothenate. On the other hand, despite sufficient median intakes for dietary iron, 83.3% of the children had deficient iron intakes (<100.0% of EAR). The same was observed for magnesium, zinc, chromium, selenium, vitamins B1, B2, B3, B6 and E.

The same trend was observed for the 9-13 year old children who showed inadequate median intakes for even more of the nutrients (13/26) than the six to eight year old children. Adequate median intakes were observed for total protein, carbohydrates, dietary fibre, dietary iron, magnesium, chromium, vitamins B1, B2, B3, B6, K and pantothenate in the 9-13 year old children, but the prevalence of inadequate intakes for these nutrients ranged from 0.0% for carbohydrates and 98.5% for dietary fibre in the macronutrients respectively. For the micronutrients, the prevalence of inadequate intakes ranged from 15.4% for vitamin B2 to 100% for calcium, pantothenate and vitamin C respectively. The 14-18 age group showed the most nutrients (18/26) with inadequate median intakes when compared to the EAR. These included total energy, total dietary fibre, calcium, dietary iron, magnesium, zinc, chromium, selenium, iodine, vitamins A, B1, B6, B12, C, D, E, K, folate and biotin. Despite sufficient median intakes for the remaining nutrients, the prevalence of inadequate intakes ranged from 0% for carbohydrates to 42.9% for chromium.

Significant different intakes were observed between the different age groups for total energy (p = 0.001), carbohydrates (p < 0.0001), total dietary fibre (p = 0.008), calcium (p = 0.002), dietary iron (p = 0.005), magnesium (p = 0.001), zinc (p = 0.014), thiamine (p = 0.004), folate (p=0.036) and vitamin E (p = 0.003) intakes. As expected, the intakes were progressively higher as the age increased for all of these nutrients, except for calcium, magnesium, vitamin B1, and folate where the 9-13 year old group had significantly higher intakes than the 6-8 year old and 14-18 year old groups respectively. It thus seems as if the 9-13 year old group had significantly better intakes than the 6-8 and 14-18 year old groups.

The total number of food items consumed by the 237 children was 54 for the period of seven days however, the number of individual foods mostly consumed by an individual ranged between 7 and 29, indicating a low FVS. This was confirmed by the low median FVS of 23.5, 20.0 and 17.0 for the 6-8 year old, 9-13 year old and 14-18 year old groups respectively (Table 2). None of the age groups consumed a low DDS (1-3 groups). The majority of the children consumed a high DDS with 96.4% in the 6-8 year old group compared to 92.8% and 94.7% in the 9-13 and 14-18 year old groups respectively. The DDS did not differ significantly between the age groups and all consumed a median of 8 food groups, indicating a high DDS.

Table 2: Food Variety Within the Food Groups

Food groups	Children aged 6-8 years old (n=56) Median (IQR)	Children aged 9-13 years old (n=83) Median (IQR)	Children aged 14-18 years old (n=98) Median (IQR)	Range
Group 1: Flesh Foods	4.00 (3.00; 5.00)	3.00 (2.00; 4.00)	3.00 (2.00; 4.00)	0-10
Group 2: Eggs	1.00 (1.00; 1.00)	1.00 (1.00; 1.00)	1.00 (1.00; 1.00)	1-1
Group 3: Dairy	2.00 (1.00; 3.00)	2.00 (1.00; 3.00)	1.00 (1.00; 2.00)	0-6
Group 4: Cereals	7.00 (5.00; 8.00)	7.00 (6.00; 8.00)	7.00 (5.00; 8.00)	1-15
Group 5: Legumes	1.00 (1.00; 2.00)	1.00 (1.00; 2.00)	1.00 (1.00; 2.00)	0-4
Group 6: Vitamin A	2.00 (1.00; 3.00)	2.00 (1.00; 3.00)	2.00 (1.00; 3.00)	0-5
Group 7: Fruits and Juice	3.00 (2.00; 4.00)	2.00 (1.00; 4.00)	2.00 (1.00; 2.00)	0-9
Group 8: Vegetables	3.00 (2.00; 4.00)	3.00 (2.00; 4.00)	2.00 (2.00; 4.00)	0-9
Group 9: Oils and Fats	2.00 (1.00; 2.00)	1.00 (1.00; 2.00)	2.00 (1.00; 2.00)	0-3
Food variety score (FVS)	23.5 (19.0; 29.8)	20.0 (17.0; 28.0)	17.0 (21.0; 27.5)	8-49
Dietary diversity score (DDS)	8.00 (7.25; 9.00)	8.00 (7.00; 9.00)	8.00 (7.00; 9.00)	4-9

Note: IQRs = Inter Quartile Ranges.

Hematological and Biochemical Results

The results in Table 3 showed normal median serum levels for all micronutrient status parameters, except for serum folate and zinc levels that were low in all the age groups. The prevalence of zinc deficiency was the highest in the 6-8 year old group (97.7%), followed by the 9-13 (95.8%) and 14-18 (93.3%) year old groups respectively. Although the median vitamin A was normal, 62.2%, 60.5% and 63.9% of the children in the 6-8, 8-13 and 14-18 year old groups respectively had levels reflecting Vitamin A Deficiency (VAD). Metabolic differences were observed despite no significant statistical differences being observed in serum micronutrient levels, except for vitamin B12, between the age groups. Median serum vitamin B12 levels were much higher than the reference value, however, 5.36%, 6.02% and 9.18% of the children in the 6-8, 9-13 and 14-18 year old age groups had low serum vitamin B12 levels, thus deficiency. Furthermore, the median serum vitamin B12 levels significantly ($p < 0.0001$) decreased with age.

Regarding iron status, most of the variables showed significant differences between the age groups except for serum ferritin ($p = 0.69$) and serum iron ($p = 0.13$). All the heamatological median serum levels were within the normal range for all the age groups except for Hct in the 6-8 year old group and Mean Corpuscular Volume (MCV) in the 9-13 year old group that showed borderline levels. Poor iron status is associated with low Hb, low serum iron, low transferrin and low ferritin levels. Based on serum iron, the prevalence of children with poor iron status was 28.6%, 22.9% and 25.5% compared to 10.7% (n=6), 3.61% (n=3) and 14.3% (n-14) with low iron stores (based on serum ferritin) in the 6-8, 9-13 and 14-18 year old groups respectively. A low serum ferritin level is the first indication of a poor iron status, thus iron status is compromised in 9.7% of these group of children. Based on decreased serum iron, transferrin and ferritin levels, none of the children was classified as iron depleted. Furthermore, no iron deficiency (based on all the iron parameters + decreased MCV, but normal Hb) or iron deficient anaemia (all low heamatological parameters including Hb and hct) was prevalent in this group of children.

The prevalence of microcytosis (based on MCV) was, however, prevalent in 44.6% of the children aged 9-13 years old, followed by 35.7% in the 14-18 year old and 30.4% of the

Table 3: Prevalence of Micronutrient Deficiency Status and HS-CRP Status

		6-8 years old (n=56)		9-13 years old (n=83)		14-18 years old (n=98)		
Haematological parameters	**Cut-off levels**	**Median (IQR)**	**Prevalence of abnormal blood values (%)**	**Median (IQR)**	**Prevalence of abnormal blood values (%)**	**Median (IQR)**	**Prevalence of abnormal blood values (%)**	**Significant differences between the age groups *p***
Haemoglobin (g/dL)	<11.5 g/dL (6-11 year) <12 g/dL (≥12 years) (25)	12.9 (12.3; 13.6)	**1.79**	13.3 (12.9; 14.0)	**6.02**	14.2 (13.4; 15.0)	**3.06**	<0.001
Haematocrit (%)	<36 (25)	35.8 (34.3; 37.4)	**46.4**	37.1 (35.1; 39.0)	**25.3**	38.9 (36.6; 40.9)	**17.3**	<0.001
Transferrin (g/L)	<2 g/L (26)	2.86 (2.54; 3.08)	**0.00**	2.78 (2.50; 3.14)	**1.20**	3.10 (2.66; 3.56)	**1.02**	0.001
Serum ferritin (µmol/L)	< 12 µmol/L (girls) < 15 µmol/L (boys) (25)	28.9 (17.5; 51.4)	**10.7**	31.2 (20.0; 41.2)	**3.61**	27.7 (15.4; 43.3)	**14.3**	0.69
Serum iron (µmol/L)	<10 µmol/L (25)	13.1 (8.68; 16.24)	**28.6**	14.9 (8.37; 18.37)	**22.9**	14.2 (9.56; 19.5)	**25.5**	0.13
Mean corpuscular volume (fL)	<82 (6-7.9 years) <84 (8-11.9 years) <85 (boys ≥12 years)	83.5 (81.2; 85.6)	**30.4**	84.5 (82.2; 86.8)	**44.6**	86.7 (84.2; 89.4)	**35.7**	<0.001

Table 3 (Cont.)

	<86 (girls≥12 years) (3)							
WBC	< 4X10^3/uL (27)	6.56 (5.27; 7.66)	**5.36**	6.75 (5.04; 8.53)	**7.23**	6.27 (4.96; 4.71)	**4.08**	0.59
RBC	< 3.9X10^6/uL (27)	4.32 (4.09; 4.57)	**8.93**	4.41 (4.12; 4.60)	**6.02**	4.47 (4.24; 4.71)	**5.10**	0.02
Serum folate (nmol/L)	< 21 ng/ml (28)	13.3 (10.5; 16.9)	**91.1**	12.3 (8.55; 15.4)	**80.7**	10.8 (7.50; 13.4)	**85.7**	0.78
Serum vitamin B12 (pmol/L)	< 200 pg/ml (28)	470 (364; 623)	**5.36**	428 (329; 589)	**6.02**	358 (275; 480)	**9.18**	<0.001
Serum vitamin A	1.05-3.32 umol/l (27)	1.28 (0.67; 1.85)	**62.2**	1.36 (0.65; 1.98)	**60.5**	1.74 (0.67; 0.89)	**63.9**	0.56
Serum zinc	10.7-18.4µmol/l (27)	2.8 (2.00; 22.6)	**97.7**	3.6 (1.80; 23.2)	**95.8**	6.7 (2.40; 26.3)	**93.3**	0.69
Serum calcium	2.15-5.57 g/dL (27)	2.40 (2.34; 2.48)	**3.4**	2.43 (2.35; 2.47)	**3.6**	2.41 (2.32; 2.48)	**3.1**	0.95
Serum magnesium	0.66-1.07 mmol/L (27)	0.84 (0.75; 0.88)	**9.3**	0.82 (0.72; 0.88)	**10.1**	0.83 (0.78; 0.89)	**7.9**	0.42
HS-CRP	> 3 mg/dL (29)	1.68 (1.00; 3.01)	**23.2**	1.13 (0.71; 2.61)	**18.1**	1.37 (0.78; 2.67)	**17.3**	0.57

Note: IQRs = Inter Quartile Ranges; RBC = Red Blood Cell Count; HS-CRP = High-Sensitivity, C - reactive protein; WBC = White Blood Cell Count; µ = Micro gram; dL = Deciliter; fL = Femtolitre; g = Gram; L = Liter: mcg = Microgram; mL = Milliliter; mg = Milligram; mol = Mole; nmol = Nanomols; pg = Picogram; and pmol = Picomols.

6-8 year old groups. Microcytosis (based on MCV >95 fL) was prevalent in none of the 6-8 year old group, and only one and two children in the 9-13 (1.2%) and 14-18 (2.4%) year old groups respectively. The sample further showed a prevalence of 85.2% and 7.6% for folate and vitamin B12 deficiency in the children. Infection was prevalent in these children with the 6-8 year old group (23.2%) being most affected, followed by the 9-13 year olds (18.1%).

Associations and Factors Predicting Micronutrient Status

The child's age was predictive of 18.0% of serum folate (adjusted R^2 = -0.18; p = 0.013) when gender of the child and the marital status of his/her caregiver was constant in a regression model. Thus, low folate status was negatively correlated with age (p < 0.0001) and age could independently predict about 27% of the variability in low serum folate status (r = 0.27; p-value < 0.0001).

Serum vitamin A strongly correlated with age (p < 0.001) however no predictive association was observed in a linear regression model using serum ferritin, serum iron, serum zinc, gender and age of the child. A linear regression indicated that serum zinc (adjusted R^2 = 0.03, p-value = 0.043) was marginally predicted (3.0%) by serum ferritin and age in such a away that for every 1 µmol/l increase of serum zinc, serum ferritin will reduce by 0.17 µmol/L (coeffienient = -0.17, p-value = 0.010) holding serum iron, folate, age and vitamin A status constant. Also for every 1 µmol/l increase of serum zinc, age will reduce by 0.13 years (coefficieint = -0.13, p-value = 0.05) holding serum iron, folate, ferritin and vitamin A status constant. Thus, the older the child got the better their ferritin levels but not their serum zinc levels.

A linear regression indicated that 18.0% of the virability observed in serum folate (adjusted R^2 = 0.18; p < 0.0001) was explained by caregivers educational status (coeffiecnt -2.65; p < 0.0001), household income (coefficient 1.45; p = 0.002) and employment status of caregiver (coefficient 1.11; p = 0.50). However, no predictive associaton was observed in serum iron, serum ferritin, serum vitamin A or serum zinc among the children.

In an adjusted model ($R^2 = 0.11$; $p < 0.001$), serum vitamin B12 was observed to be predicted by a child's age (coefficient = -0.28; $p < 0.0001$) and caregivers' educational status (coefficient = 0.18; $p < 0.018$). Serum folate significantly correlated negatively with only RBC (coefficient = - 0.16; $p = 0.015$) and Hct (coefficient = -0.22; $p = 0.001$), but not MCV.

DISCUSSION

Co-existence of multiple micronutrient deficiencies, as observed in this study, is more common in developing countries and more specifically high prevalence of folate, vitamin A, zinc, iron and iodine deficiencies (Bailey *et al*., 2015).

The dietary diversity results showed conflicting results in that the majority of the children had a low FVS and a high DDS. This indicates that although most food groups were consumed by the children, only a few foods from each group were included. Consuming one or two foods from each of the nine groups does not, therefore, constitute a varied intake. Low dietary diversity indicates monotonous diets with poor quality, resulting in poor consumption of energy and nutrient dense foods (Herrador *et al.*, 2014). A study in Ethiopia suggested that school-aged children with low DDS consumed diets rich in cereals, roots and tubers, with low animal source foods that are also rich in micronutrients, particularly iron and zinc (Speedy, 2003; and Herrador *et al.*, 2015), thus increasing risk of iron and zinc deficiencies (Ayele and Peacock, 2003). In this study, the prevalence of iron deficiency was 10.7% among 6-8 year, 3.6% among 9-13 year and 14.3% among 14-18 year old children. Dietary intake results supported this finding as more than half of all the children did not have sufficient dietary intake of iron- or vitamin C rich foods, except in the 9-13year old group. In addition, significant low dietary intakes were observed for thiamin, vitamin E, magnesium and calcium, and deficient intakes of all these vitamins and minerals hinder children's growth and development (FAO and WHO, 2005).

In this study, the reported low dietary diversity and nutrient intakes were consistent with the biochemical results observed. The consumption of zinc-rich foods was poor, confirming our finding of more than 90% prevalence of zinc deficiency; much higher than the national level of 45.3% (Labadarios *et al*., 2007; and Labadarios *et al*, 2008) Zinc deficiency relates to many other micronutrients in the body. An association between zinc and folate was observed as has been reported in some studies (Tamura, 2002) and a deficiency of both these nutrients observed among children with Attention Deficit Disorder (ADD) (Bryan *et al*., 2004; Sinn, 2008; and Millichap and Yee, 2012). The ADD condition is characterized by hyperactivity, poor attention and reduced academic performance. Thus, the observed prevalence of zinc and folate deficiencies and the well documented effect of iron deficiency anemia on cognitive development, stress the importance of interventions with a potential to address many micronutrient deficiencies in schoolchildren as the effects go beyond the health and development of children (WHO, 2015). Furthermore, age and low serum ferritin acted as predictors of low serum zinc levels in this study. A study in Thailand among children aged 6-12 years indicated a high proportion of school-going children with low serum zinc concentrations and the prevalence varied in terms of age and gender. In our study, boys and older children had a higher prevalence of zinc deficiency. In addition, serum zinc and hemoglobin levels were positively associated with low serum retinol (<1.05 μmol/l) which was consistent with a study in Thailand (Thurlow *et al*., 2006).

A large percentage of children across all age groups had poor iron status. In Mexico the prevalence of anemia among children aged 5 to 11 years was 9.7% and risk was higher for the younger children in comparison with older children. Low serum retinol (<1.05 μmol/l) is also a predictor of low hemoglobin level (Thurlow *et al*., 2006). More than 60% of all the children had VAD, which was higher than the national South African prevalence (Shisana *et al*., 2013). The prevalence of microcytosis, usually associated with poor dietary intakes, absorption problems, blood loss and/or an underlying chronic infection or inflammatory process (van Vranken *et al.*, 2010), further indicate IDA in this group of children. Microcytosis is often caused by inadequate vitamin B12 and folate intake (Gibson, 2005). The high folate deficiency prevalence may explain the high prevalence of microcytosis (Mischoulon *et al*., 2000).

Linear regression of this study data showed that caregivers' literacy rate, household income and caregivers' employment status had an association with serum folate level, but not with any of the other micronutrients. In most societies, employment influence income levels (Ferguson *et al*., 2011), however, income in this study had a stronger predicting power of serum folate compared to employment status, consistent with several other studies (Darmon *et al.*, 2008; and Nilsson *et al.*, 2011). Recently, folate deficiency among children has also become a public health

concern (Das *et al.*, 2013) since folate is essential for many biosynthesis reactions in the human body, especially in growing children (Labadarios *et al*., 2008). Vitamin B12 deficiency was also observed in a small sample (7.8%) in this study, and showed a significantly higher prevalence with age.

No significant statistical differences were observed in micronutrients prevalence across the various ages except for vitamin B12. Age was a predictor of most of the micronutrient deficiencies in this sample, which makes a call for age-appropriate nutrition education for school feeding programmes and households relevant (Alderman and Bundy, 2011). Micronutrient deficiencies and infections are interrelated since many micronutrients play antioxidant and immunodulating functions. Inadequate intake of micronutrients impair immune systems, thus making the body less resistant to infections (Bhaskaram, 2002; and Allen, 2014). The HS-CRP indicates concomitant infection as it indicates acute response proteins (Burtis and Ashwood, 2008). Among the sample, high HS-CRP decreased as children grew older, but still showed that almost one in five children had an infection.

CONCLUSION

After implementing the national school nutrition programme in 2003 and fortification of maize and wheat flour in 2004 in SA, after 10 years the country is still experiencing micronutrient deficiencies among its population, especially in children (Shisana *et al.*, 2013) and similarly among children in this study where multiple micronutrient deficiencies were observed (zinc, iron, vitamins A, B12 and folate). This study proved that caregiver income, employment and education levels are predictors for folate intakes, but failed to prove this relationship for any of the other micronutrient deficiencies. Furthermore, the high DDS did not translate to high micronutrient intake with very high inadequate dietary intake of vitamin B12 and folate. This may be due to the overall low FVS. More research is needed to investigate the predictors of multiple micronutrient deficiencies in these children in order to address this public health concern.

The importance of primary prevention is very important in early childhood. A suitable intervention study should be planned for both these child caregivers and children. Healthy lifestyles, including physical exercise, should be promoted through nutrition education and awareness programmes, focusing on balanced diets based on the food-based dietary guidelines for SA (Levine *et al.*, 2012).

Education of caregivers is relevant since it was observed to predict serum folate and vitamin B12 levels in an inverse and positive nature respectively in this study. Though higher education especially among mothers has a positive influence on nutrition and health (Levine *et al*., 2012), our findings emphasize that education it is not synonymous to nutrition education. Thus, a concerted nutrition education of child caregivers, school management and school children may improve and maintain optimum nutritional status of children in this area.

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