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DOI
10.18314/jnb.v3i1

Publication date
2017

Document Version
Final published version

Published in
Journal of Nutritional Biology

ISSN: 2469-4142

Gratis

Research Article

Journal of Nutritional Biology

Zinc Status in Iron Deficient Anaemic Patients in Sudan

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Received: Aug 03, 2017; Accepted: Nov 01, 2017; Published: Nov 04, 2017

Abstract

Iron deficiency anaemia is a major health problem worldwide, but may be complicated in underdeveloped nations by deficiencies of other micronutrients with consequences for adequate treatment. The World Health Organization (WHO) estimates that 2 billion people – over 30% of the world's population – are anaemic, approximately 50% of cases of anaemia are considered to be due to iron deficiency. **Aim:** Since a combined deficiency of trace elements has great consequences in the approach of this problem, zinc status was assessed in blood of iron deficient anaemic patients in Sudan representing sub-Saharan Africa and compared with Dutch anaemic patients representing Western Europe. **Method:** Zinc as well as iron were measured with instrumental neutron activation analysis (INAA) in 22 Sudanese anaemic patients and four controls as well as in 17 Dutch anaemic patients and four controls. **Result:** In the Sudanese patients very low concentrations of zinc were measured, 3 ± 1 mg/L compared to a control group of healthy individuals (5 ± 1 mg/L), while all Dutch patients had normal zinc levels (5 ± 1 mg/L). When matched for haemoglobin level, the Sudanese patients still showed lower zinc concentrations. Even in the Sudanese patients without a known history of malnutrition and in patients with the anaemia of chronic disease zinc levels were significantly lower than in the controls. A positive correlation was observed between Fe and Zn in blood of the Sudanese subjects (r=0.654), while there is no or a very week relation between Fe and Zn in blood of the Dutch group (r=0.08). The low level of Fe and Zn for the Sudanese group might be ascribed to the poor intake of these two nutrients with the main dietary food (cereals) in this area. **Conclusion:** In iron deficiency anaemia in Sudanese patients, a deficiency of zinc should be taken into account in case of supplementation.

Keywords: Iron deficiency; Anaemia; Zinc

Introduction

Anaemia affects low, middle and high income countries and causes both mortality and disability worldwide. The World Health Organization estimates that 2 billion people – over 30% of the world's population are anaemic, approximately 50% of cases of anaemia are considered to be due to iron deficiency with the highest proportion of individuals affected in Africa [1]. Anaemia affects 47.4% of children under the age of 5 years, 25.4% of school age children, 30.2% of non-pregnant women, 41.8% of pregnant women, 12.7% of men and 23.9% of the elderly [2]. Apart from inherited forms of anaemia

such as sickle cell anaemia and thalassemia, anaemia may be acquired either by disorders or deficiencies of micronutrients such as folic acid, vitamin B12 and iron. Although recent survey data are lacking, there is clear evidence that micronutrient deficiencies such as vitamin A, iodine and iron are a major public health problem in Sudan [3]. Approximately half of the cases of anaemia are due to iron (Fe) deficiency, which may be the result of an increased loss of iron from the body or an inadequate intake or uptake of this trace element. In some cases of anaemia there is a functional deficiency of iron, when iron is being stored in the body and not available for the bone marrow, the so-called anaemia of chronic disease (ACD). In food two forms of iron can be distinguished; organic and inorganic iron. The last one is present in vegetables and cereals, while organic or haem iron is present in red and organic meat. This haem-iron is more readily absorbed by the body than non-haem iron [4].

Sudan is both a developed and a low-income country, in which, according to a report of the Food and Agriculture Organization of the United Nations (FAO) more than 90% of the population suffers from poverty and food insecurity [3].

Apart from iron other trace elements, including zinc (Zn), play a role in haemoglobin synthesis. Zn is present in meat, nuts, grains, and cheese and acts as a co-factor of many enzymes in the human body [5]. The role of zinc in biology can be grouped into three general functional classes, namely catalytic, structural and regulatory functions (Table 1) [6].

Zn deficiency has been found associated with anaemia and both Zn and Fe deficiency represent two of the most important nutritional problems in developing countries. Data on the prevalence of iron deficiency anaemia in Sudan are scarce while no data on zinc status are available [7-9]. Because of the role of zinc in iron metabolism these data are necessary for the development of adequate supplemental and nutritional recommendations, which should not only focus on iron.

In the present study we measured both Fe and Zn concentrations in Sudanese iron deficient anaemic patients and compared them with healthy controls. Furthermore the Sudanese patients were compared to Dutch iron deficient anaemic patients having a normal nutritional status and expected to have normal zinc levels.

Materials and Methods

Patients

All included anaemic patients were older than 18 years and had a haemoglobin level <11g/dL. Pregnant women, patients with cancer, renal insufficiency as well as patients taking iron or vitamin supplements were excluded. Iron deficiency anaemia (IDA) was defined as a haemoglobin (Hb) level <11 g/dL, Mean Cellular Volume (MCV) <80 MCV (10-15L), iron levels <10 µmol/L and ferritin <20 µg/L. The anaemia of chronic disease (ACD) was defined as a Hb level <11 g/dL, normal or low MCV (80-100), ferritin >20 µg/L and increased levels of CRP (at least >10 mg/l). Blood samples were collected from 26 individual adults, 12 male and 14 females with age 18-65 and mean age 44 years visiting the Al Gadarif Hospital in Eastern Sudan. Nineteen of these patients had iron deficiency, three were known with ACD and four were healthy controls, having a normal haemoglobin concentration. The Dutch part of the study included 19 individuals, eight with iron deficiency, seven with ACD and again four healthy controls. Their mean age was 52, range 22-85, and they all visited the Meander Medical Centre, Amersfoort, The Netherlands (Table 2). All subjects in the study were filling in a questionnaire of their diet, mainly asking for meat consumption and the major ingredient of their food intake.

Methods

Apart from the routine haematological tests, 5 mL blood samples of all patients were freeze dried to measure their iron and zinc concentrations. Freeze dried samples of the Sudanese patients were transferred to the Reactor Institute Delft, TU Delft, The Netherlands and, together with the material of the Dutch patients, analysed using Instrumental Neutron Activation Analysis (INAA). This is a technique for qualitative and quantitative multi-element analysis of major, minor, trace, and rare elements. INAA is based on capture of a thermal neutron by the nucleus of an element and subsequent conversion to a radioactive isotope with suitable radioactive emissions. The intensity of the radioactive emissions is proportional to the number of nuclei of the element. Neutron activation of iron results in the radionuclide ^{59}Fe (half live 44.6 d) by thermal neutron capture in ^{58}Fe. Activation of zinc results in ^{65}Zn (half live 243.7 d) by thermal neutron capture in ^{64}Zn [10].

INAA: About 200 mg of each dried sample was weighed and packed in high purity polyethylene capsules. Zinc was used as comparators in order to measure the neutron flux during irradiation [11]. Samples along with similarly

prepared blank (empty capsule) and NIST Standard Reference Material 1577c "Bovine Liver" were irradiated for 10 hours at a thermal neutron flux of ~ 4.5×10^{12} cm^{-2} s^{-1}.

Samples are allowed to decay for 2 weeks, thereby effectively eliminating all activity from the short half-life radionuclides such as ^{24}Na. The activity of the induced radioisotopes was measured during 3 hours using a well-type Ge detector, with an absolute photopeak efficiency of 16.5% for 835 keV photopeak of ^{54}Mn and 13% for the 1099 keV photopeak of ^{59}Fe. The gamma-ray spectra were analyzed using the APOLLO software [11].

Statistical analysis

Statistical analyses were performed using SPSS (IBM Statistic 23). Mann- Whitney test was used to compare the zinc concentrations between the Sudanese and Dutch anaemic patients. Pearson's regression test was used to examine the correlation between the iron and zinc concentration in both the Sudanese and Dutch group.

Ethics

All patients and controls (from Sudan and Netherlands) gave informed consent and the study was approved by the medical ethical committee of the Meander Medical Centre, Amersfoort, The Netherlands.

Results

As shown in Table 1, nine of the 19 Sudanese patients with IDA were known with a poor nutritional intake defined as a diet lacking meat and mainly consisting of bread. Their mean zinc concentration was 3.2 ± 1.0 mg/L (normal 5.4 ± 0.6 mg/L). In contrast all Dutch patients with IDA (n=8) having a normal diet, had normal zinc levels (5.3 ± 0.9 mg/L). This statistically significant difference (U=3; p= 0.001) is shown in Figure 1a. Even if patients are matched for haemoglobin concentration this difference is still existing (Figure 1b).

Zinc concentrations were also measured in Sudanese IDA patients with a normal or unknown food pattern and in ACD patients. As shown in Figure 2 zinc concentrations in these groups were comparable to those in IDA patients with malnutrition and significantly lower than in the healthy controls (U=0.00, p=0.005 for the normal nourished IDA patients vs healthy controls and U=0.00, p= 0.034 for the ACD group versus healthy controls).

Within the Dutch group of patients zinc levels were lower in the ACD patients than in IDA patients, but still in the normal range (U=10.5, p=0.042) as shown in Figure 3.

If Sudanese iron deficient patients with a normal food pattern were compared to Dutch iron deficient patients they still have significant lower zinc concentrations (U=0.00, p=0.000). This is illustrated by Figure 4.

Finally a Pearson correlation was done to examine the relations between iron and zinc for the two groups (Sudanese and Dutch). It was found that there is strong correlation between Fe and Zinc in the whole blood of Sudanese subjects (r=0.654) and p value <0.01). On the other hand there is no or a very week relation between Fe and Zn in the whole blood of the Dutch group (r=0.080; p value=0.732).

Discussion and Conclusion

Iron deficiency anaemia is a major public health problem especially in underdeveloped and developing countries. In most developed nations the main cause of this type of anaemia is blood loss either related to menses or to gastro-intestinal blood loss as in inflammation or cancer. Nutritional deficiencies, however, are not totally uncommon and can be found in anorectic patients, alcoholics, the elderly, low income families and in case of specific diets.

In developing countries a lack of sufficient micronutrients in food is the main cause of anaemia, and can be extreme in case of additional factors such as pregnancy, menses and inflammatory diseases. Anaemia based on an insufficient dietary intake is not only characterized by a deficiency of iron but also of other trace elements such as copper and zinc.

This is clearly demonstrated by the results of our study showing severe deficiencies of iron and zinc in blood of Sudanese anaemic patients. In contrast to the Dutch anaemic patients, who had a normal blood zinc level, concentrations of this element were low to very low in the blood of the majority of the Sudanese patients even those without a history of bad intake. This can be explained for the most part by a lack of red meat in their diet. On the other hand, the basic content of their food are cereals and grains, which usually contain zinc. This may indicate, as has been shown in other countries, that the soil in the Al Gadarif region is rather deficient of zinc [12]. Another possibility is that during baking or cooking zinc is transferred to a chemical form that is not easily absorbed by the human body [13].

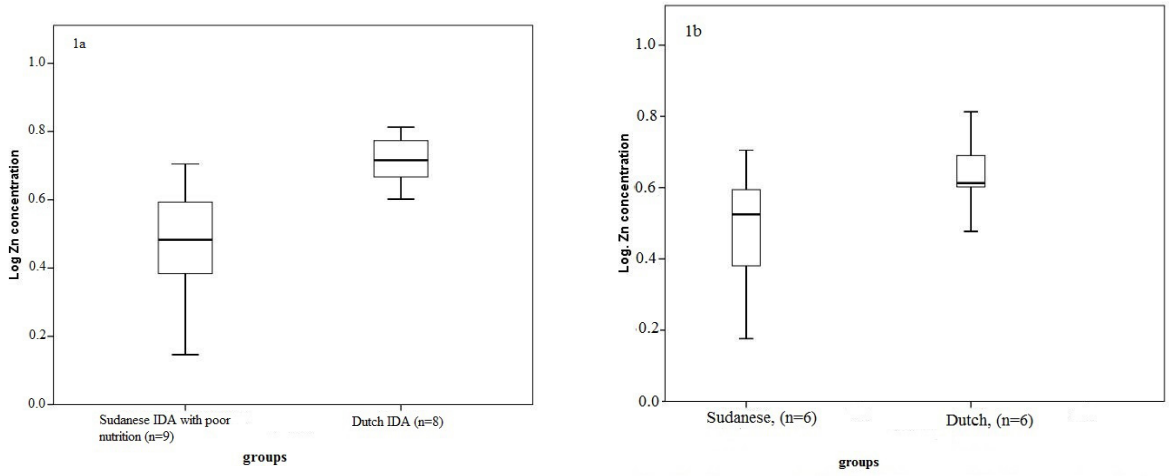

Figure 1a: Zinc concentrations in the Sudanese IDA group with malnutrition and the Dutch IDA group.
Figure 1b: Zinc concentrations in Sudanese and Dutch anaemic patients matched for haemoglobin level.

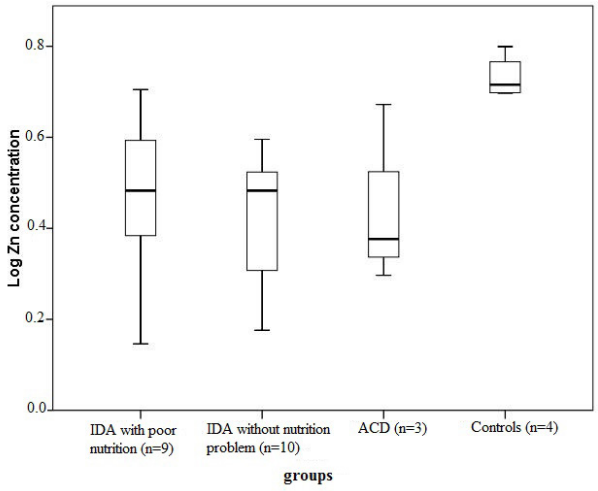

Figure 2: A comparison of Zinc concentrations in blood of Sudanese IDA patients with and without known malnutrition, ACD patients and healthy controls.

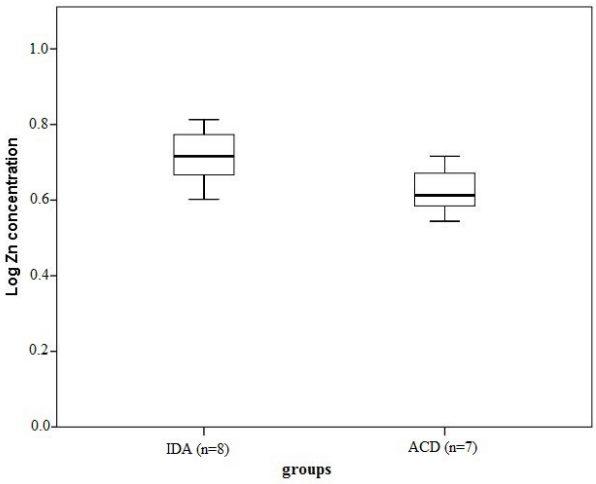

Figure 3: A comparison of Zinc concentrations in Dutch IDA and ACD patients.

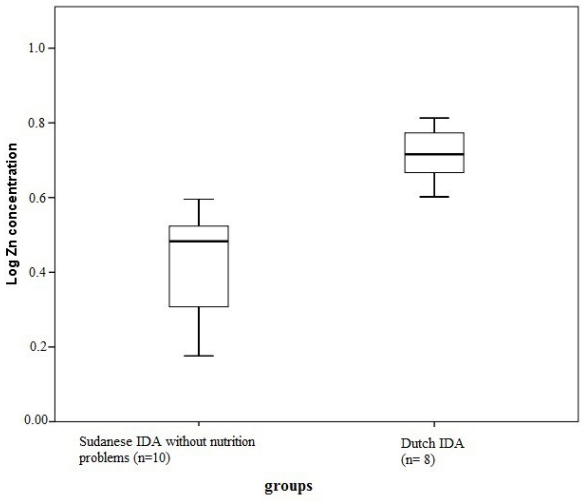

Figure 4: Zinc concentrations in blood of Sudanese IDA patients without a nutrition problem and Dutch IDA patients.

Table 1: Biological functions of zinc [6].

Zinc Functions	Comments
Catalytic factor	Enzyme cofactors in six main enzyme classes Oxidoreductase; alcohol dehydrogenase, sorbitol dehydrogenase Transferase; the major function of zinc is not catalytic in this class (only 34% are catalytic) Hydrolase; carboxypeptidases, alkaline phosphatases, angiotensin-converting enzyme Lyase; carbonic anhydrase, δ-aminolevulinic acid dehydratase Isomerase; phosphomannose isomerase Ligase; the major function of zinc is not catalytic in this class (only 39% are)
Structural component	*Zinc fingers C2H2-like finger; classical zinc finger motif, transcription factor TFIIIA, 20–30 amino acid sequence Zinc ribbon; many transcription factors, ribosomal proteins, RanBP Treble clefs; RING finger domain, Arf-GAP domain, LIM domain, FYVE domain, PHD domain, MYND domain, nuclear receptor DNA-binding domain, GATA, PKC Zinc necklaces; TAZ domain in transcriptional adaptor protein CBP/p300 Interprotein binding mediator (e.g., zinc hock motif) Crystallization of peptides such as insulin
Signaling mediator (Zinc signaling)	Extracellular zinc signaling Neuromodulating functions in the central nervous system Activating zinc receptor (ZnR/GPR39) Reducing insulin secretion and suppressing hepatic insulin clearance Intracellular zinc signaling Second messenger functions Inhibition of enzyme activity (caspases, protein tyrosine phosphatases,phosphodiesterases) Modulation of signaling pathways (PKC, ERK, JAK/STAT, BMP/TGF-β, NF-$_{k}$B,cAMP-CREB, PI3K/Akt, B-cell receptor) Zinc wave; zinc release in cytosol from perinuclear region Zinc spark; zinc ejection from oocyte, which is necessary for the egg-to-embryo Transition

* A zinc finger is a small protein structural motif that is characterized by the coordination of one or more zinc ions in order to stabilize the fold.

Phytate is the storage form of phosphorous in plants. Whole grain cereals contain high levels of minerals (Fe, Zn, Mg) but also of phytate, which due to formation of insoluble complexes makes the minerals unavailable for absorption. Phytic acid (inositol hexa- and penta-phosphate) is the principal dietary factor known to limit zinc bio-availability by strongly binding zinc in the gastrointestinal tract [14].

We realize that our study has some limitations. The size of the population is small, especially of the Dutch patients. There are more differences between the Sudanese and the Dutch patients than nutritional ones. In Sudanese patients there is a higher incidence of infections such as malaria, which can also cause anaemia, so many patients have a combination of underlying factors responsible for the anaemia. These factors may also be involved in their zinc deficiency.

Zinc plays an essential role in a number of enzymatic processes in the synthesis of haemoglobin and a deficiency may therefore contribute to the extent of anaemia. This is relevant in case of supplementation because the focus should not only be on iron but also on zinc. Since zinc and iron show some chemical similarity and they do share transport pathways such as the divalent metal transporter 1 (DMT-1) in the intestinal cell, they may therefore be competitive. It was found that zinc administration with an iron aqueous solution lead to acute inhibition of iron bioavailability when both elements are given together in fasting condition [15,16]. It has also been reported that, inhibition of iron absorption

Table 2: Diagnosis, gender, number of individuals, mean Hb and mean age of Sudanese and Dutch groups participating in this study.

	Sudanese group					Dutch group				
Diagnostic	Total Number of individuals	With *malnutrition	Without malnutrition	Mean Hb/ group	Mean age/ group	Total Number of individuals	With malnutrition	Without malnutrition	Mean Hb/ group	Mean age/ group
IDA	19, (M=8, F=11)	9	10	5.6	40	8	-	8	9.7	47
ACD	3, (M-2, F=1)	-	3	4.0	51	7	-	7	9.6	75
Healthy	4, (M=2,F=2)	-	4	12.9	34	4	-	4	13.1	38

*Malnutrition refers to deficiencies, excesses or imbalances in a person's intake of energy and/or nutrients
Hb: haemoglobin, M: male, F: female, IDA: Iron deficiency anaemia, ACD: anaemia of chronic disease

by zinc depends on the ratio of these two elements in the solution [17,18]. Within the normal range of dietary intakes (ratio 1:2 to 2:1), no significant interaction was observed. Adverse effects of Zn supplementation on Fe status have been demonstrated in rat pups. Pups supplemented with high Zn supplementation showed lower haemoglobin concentration [19]. A lack of iron and zinc increases the risk of infections, as iron-deficiency anaemia dampens immunity and zinc plays a role in immune function [20]. It is reported that, even mild to moderate zinc deficiency can impair killer-cell activity that helps the immune system fight off infections and illnesses [21-25].

As a result of lack of a reliable indicator for zinc status and the absence of a specific syndrome or biomarker linked to zinc deficiency, it took so long before zinc was recognized as an important mineral for public health in humans. The best marker to assess zinc deficiency is a matter of debate. Although indirect indicators such as stunting or iron deficiency prevalence have been used, these are imprecise and may lead to erroneous estimates of zinc deficiency. Direct indicators of zinc status such as plasma and urinary zinc concentrations are probably the best indicators currently available, but more research is needed to gain better insight in the sensitivity and specificity of these [26].

Zinc deficiency is usually due to insufficient dietary intake, but can be associated with malabsorption, chronic liver disease, kidney disease, diabetes and malignancies. Groups at risk include the elderly, children in developing countries and those with renal dysfunction. The intestine is the site of zinc absorption and the major route of zinc excretion. Dietary inadequacy or conditions that decrease zinc absorption or increase its losses from the gastrointestinal tract, urine, or skin may quickly cause zinc deficiency due to the limited availability of rapidly exchangeable zinc pools in the body [14]. Diarrheal disease is the second leading cause of death in children under 5 years. It is both a sign and a cause of zinc deficiency and can be treated by zinc supplementation in young children. A recent review on infectious diarrhea supports this [27].

From the FAO's food balance data on zinc intake and bioavailability, it has been calculated that about 20% of the world's population could be at risk of zinc deficiency. The geographical regions most affected are believed to be, in descending order of severity, south Asia, Africa and the western Pacific. All population age groups are at risk of zinc deficiency, but infants and young children are probably the most vulnerable. The main food in these areas including the area under study (Al Gadarif, Sudan) is cereals and legumes, on which the phytic acid can account for up to 80% of seed total P [28].

The goal of biofortification is to develop plants that have an increased content of bioavailable nutrients in their edible parts. Biofortification strategies demand

that essential micronutrients leave the root and are targeted to the edible parts of the plant, such as cereal grains. At the same time, it is essential to ensure that toxic metals remain in the root. The major bottlenecks in plant biofortification appear to be the root–shoot barrier and – in cereals – the process of grain filling. Bottlenecks for zinc biofortification vary between cereals. In some species, continuous zinc uptake during grain filling and continuity in the loading of endosperm from the xylem might be key processes. Continued zinc uptake requires genetically improved uptake capacity combined with properly managed soil availability [29].

Zinc supplementation trials conducted over the last few decades in children from developing countries have clearly demonstrated the positive benefits of improved zinc status, including improved growth rates and reductions in the incidence of various infectious diseases.

In conclusion many Sudanese patients with iron deficiency anaemia also have a zinc deficiency reflecting a lack of both micronutrients in their intake. In case of supplementation a product also containing Zn should be considered.

Acknowledgement

The authors would like to acknowledge the Netherlands Fellowship Programme (NFP) for funding the PhD study of one of the authors (TY) under which this research has been done. They also thank the Meander Medical Centre, Amersfoort and Al Gadarif Hospital in Sudan for recruiting patients and controls and supervising the sample collection. Finally they thank Dr. P Pasker-de Jong of the Meander Academy for her contribution to the statistical analysis.

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