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Original Article

Biochemistry Section

Anaemia in Adult Nigerians in Ebonyi State, South Eastern Nigeria is not Related to Plasma Mineral Element Levels

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ABSTRACT

Introduction: Anaemia remains one of the major public health challenges with global impacts, especially in developing countries. Causes of anaemia are multiple and variable among which are social, dietary, physiological and environmental factors with evidences suggestive of contributory roles of metal interactions.

Aim: To determine the relationship of anaemia with plasma levels of lead, iron and zinc in adult Nigerians.

Materials and Methods: Total 428 subjects (111 males, 184 non-pregnant female and 133 pregnant female), aged ≥18 years (mean=38.4±13.7 years) were enrolled in the study. Sociodemographic data were collected using structured questionnaire while blood samples were collected for the determination of haemoglobin and mineral elements using standard methods and techniques. Anaemia was defined as haemoglobin <12.0g/dl (non-pregnant women), <11.0g/dl (pregnant women) and <13.0g/dl (men), respectively. Ninety three anaemic subjects were compared with 335 non-anaemic subjects.

Results: Although the plasma levels of all the elements were lower in anaemic in comparison to non-anaemic subjects, only lead was significant (0.004±0.002 vs 0.005±0.012 µg/dl; p = 0.027). While none of the elements showed any relationship with haemoglobin, plasma iron was positively correlated with zinc (r = 0.837; p = 0.001).

Conclusion: The absence or weak significant relationship between anaemia and any of the elements suggests that relative concentrations of the elements may be important determinant of anaemia in this population. While further studies are desired to substantiate these findings, food diversification and reduction in toxic metals exposure are recommended to improve the nutritional status of residents and reduce anaemia prevalence with its attendant health consequences.

Keywords: Food diversification, Heavy metal, Micronutrient interaction

INTRODUCTION

Anaemia, a leading cause of morbidity and mortality among children, pregnant and lactating women [1], still remains one of the major public health challenges with global impacts, especially in developing countries [2]. Causes of anaemia are multiple and variable among which are social, dietary, physiological and environmental factors [3,4] with evidences suggestive of contributory roles of metal interactions [5-7]. Although, iron deficiency in experimental animals has been shown to increase lead absorption from intestinal tract, human study has not been able to demonstrate the impact of iron deficiency on increased lead absorption [8]. Again, it has been shown that lead increases zinc excretion [5] and that zinc deficiency enhances lead absorption [7]. Furthermore, a close inverse relationship between blood lead and activity of zinc-containing heme-enzymes, particularly delta-aminolevulinic acid dehydratase suggests that lead replaces zinc in these enzymes [6].

Many metals have been found in high concentration in the soil, food and water in Ebonyi state [9-11] including cadmium, lead, zinc, copper, iron etc. Heavy metal uptake by crops growing on contaminated soil has been recognised as a potential health hazard to human, because of transmission in food chain [12-14].

With recent report of anaemia, prevalence of 21.7% among adult Nigerians in Ebonyi state, which has no definite relationship with BMI and sociodemographic characteristics [15], there is need to explore other possible causes of anaemia. The present study therefore, aimed at determining the relationship of anaemia with plasma levels of lead, iron and zinc in adult Nigerians in Ebonyi state.

MATERIALS AND METHODS

Study area

Ebonyi state is located on longitude 8°E and latitude 6°N with moderate relief of between 125m and 245m above sea level. Ebonyi state has 3 Senatorial Districts (Ebonyi South, Central and North), 13 Local Government Areas (LGAs) and 215 political wards [16].

Methodology

This prospective cross-sectional study was conducted between the period of April 2013 and June 2014, in the 13 LGAs of Ebonyi state. It was a part of a larger study intended to correlate some toxic metal contents of food and water in the state with their levels in blood of residents and associated biochemical consequences. The subject selection and sample collection has been previously described in details [15].

Briefly, blood sample for haemoglobin and mineral element determination was collected in EDTA and trace element-free heparinised bottles. Blood sampling was done in line with the International Zinc Nutrition Consultative Group (IZiCG) technical document [17]. Haemoglobin concentration was determined by cyanmethaemoglobin method as previously described [18]. Plasma for the estimation of iron, lead and zinc was obtained by centrifugation of the heparinised samples at 2000g for five minutes. Samples were stored frozen at -8°C until they were analysed. Plasma iron, lead and zinc were determined by atomic absorption spectrophotometer (Bulk Scientific, AVG model 201) using certified metal standards (obtained from Sigma-Aldrich Co LLC, USA) for atomic spectrometry as control. To control for lead contamination, all glassware were washed and soaked in two successive dilute nitric acid bathes (0.8 mg/L). These were then rinsed in ultra-pure double distilled de-ionized water. Samples (of all reagents, glassware and sample collection devices) were randomly selected for Pb assay and no contamination was found. The tubes were washed with 10% nitric acid (HNO3). Atomic absorption spectrophotometer was used as described by Jacobson et al., for low Pb concentration [19].

Anaemia was defined in accordance with WHO [20] criteria as haemoglobin concentration <12.0g/dL (non-pregnant women), <11.0g/dL (pregnant women) and <13.0g/dL (men), respectively. Data for 93 anaemic subjects and 335 non-anaemic subjects were compared for a relationship.

Ethical Consideration

The study was approved by the Ethics and Research Committee of Federal Teaching Hospital, Abakaliki, Ebonyi state. The research was performed following the international guidelines for human experimentation in clinical research [21]. During the study, subjects who were found to have serious medical condition were referred to the nearest hospital or to the Federal Teaching Hospital, Abakaliki for further assessment and management.

STATISTICAL ANALYSIS

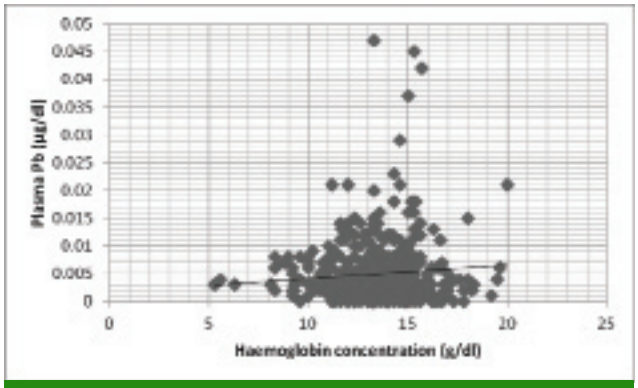
Data was analysed using Statistical Package for Social Sciences (SPSS®) for Windows ® and results were expressed as mean±standard deviation. Comparison was done using One-way Analysis of Variance (One-way ANOVA) with level of significance set at $p < 0.05$. Correlation analysis was used to determine the relationship among the elements and anaemia.

RESULTS

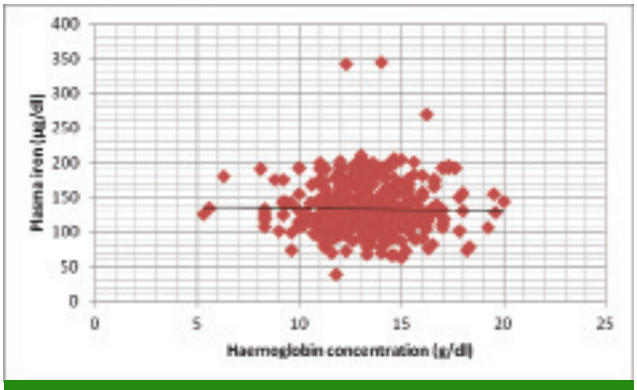
Haemoglobin concentration was significantly lower in anaemic in comparison to non-anaemic subjects [Table/Fig-1]. Also, although the plasma levels of all the elements were lower in anaemic subjects in comparison to their non-anaemic counterparts, only lead was found to be statistically significant.

Parameters	Non-anaemic (n=335)	Anaemic (n-93)	p-values
HBC (g/dl)	14.1±1.6	10.6±1.3	0.001*
Lead (µg/dl)	0.005 ±0.006	0.004±0.004	0.027*
Iron (µg/dl)	134.9 ±36.5	129.6±30.8	0.160
Zinc (µg/dl)	95.2±18.7	92.7±15.2	0.176

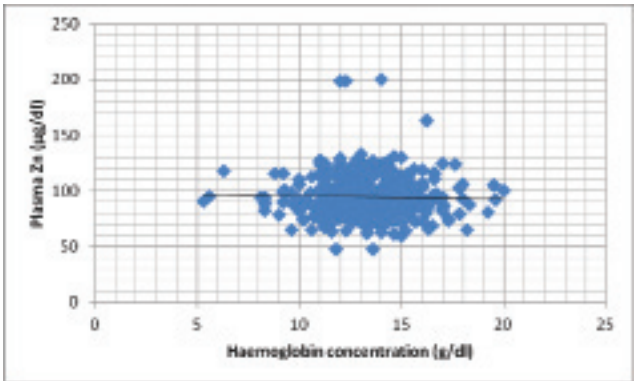
[Table/Fig-1]: Comparison of plasma haemoglobin concentration, lead, iron and zinc between anaemic and non-anaemic adults.
HBC: Haemoglobin concentration * $p < 0.05$



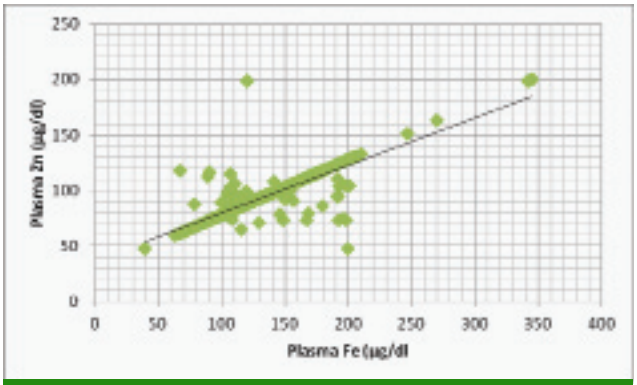
[Table/Fig-2]: Scatter plots of plasma lead against haemoglobin concentration.



[Table/Fig-3]: Scatter plots of plasma iron against haemoglobin concentration.



[Table/Fig-4]: Scatter plots of plasma zinc against haemoglobin concentration.



[Table/Fig-5]: Scatter plots of plasma Zn against Fe.

Although, none of the elements showed any significant correlation with haemoglobin concentration [Table/Fig-1-4], plasma iron was significantly positively correlated with zinc ($r = 0.837$; $p = 0.001$) [Table/Fig-5].

DISCUSSION

This study has revealed that anaemic adult Nigerians in Ebonyi state have lower plasma levels of iron, lead and zinc, but only lead was statistically significant with plasma iron positively correlated with zinc. Also, none of the elements (iron, lead and zinc) showed any relationship with haemoglobin concentration.

The lower plasma iron and zinc in anaemic subjects in comparison to the non-anaemic controls, though not statistically significant is in corroboration with the findings of Hegazy et al., [22]. Similar findings have been reported in anaemic pregnant Nigerian women recruited at gestational age of ≥25 weeks [23]. Micronutrient deficiencies have been associated with several factors, including low dietary intake [24], low bioavailabity as in the case of copper, iron and zinc due to inhibition [25] poor utilization due to environmental factors such as poor hygiene that lead to increased infections and infestations, adverse nutrient-nutrient interactions and genetic causes. The reason for the significantly lower plasma lead found in anaemic in comparison to non-anaemic subjects is obscure, but however, suggests that the anaemia in this population may be due to other factors other than lead toxicity. Anaemia in this population may not also be attributed to iron deficiency as comparable levels of iron were found in anaemic and non-anaemic subjects. However, Alabdullah et al., had earlier reported that the inverse association between blood lead and serum iron in studies carried out on children does not occur in adults [26]. Anaemia has been shown to be affected by many factors including ethnicity, gender, age, dietary habits, physical and mental health, environment, gynaecological/obstetric history, cancers, and anti-cancerous drugs and genetic makeup [4]. However, anaemia in this population has previously been found to lack association with sociodemographic data [15].

Although the reason for the lack of association of anaemia with plasma lead, iron and zinc in the present study is not obvious, metal-metal interactions may be a factor. A number of mechanisms have been proposed through which micronutrient interactions can affect the absorption and bioavailability of other micronutrients [27]. For instance, competition for transport proteins or uptake mechanisms may either facilitate or inhibit absorption [28]. Therefore, intestinal competition of zinc with copper, iron and lead may accentuate the nutritional deficiencies of copper and iron and toxicity of lead. Abdel-Mageed et al., had earlier reported that the intestinal competition of zinc with copper, iron, lead, calcium and cadmium may accentuate nutritional deficiencies or toxicities from these environmental metals [29].

Iron and zinc are important trace elements in human nutrition. Iron is a component of haemoglobin and deficiency of iron has been associated with microcytic hypochromic anaemia. Similarly, zinc is an antioxidant trace metal and an important component of over 300 enzymes, particularly the glutathione peroxidase, which protects the cellular integrity of the red blood cell. Among children, high blood levels of copper, cadmium and lead have been found to decrease iron absorption and affect haematological parameters [30]. It is however not clear if the same mechanism applies for the adults.

Although, studies that have analysed the interactions between iron and zinc have shown conflicting results, the present study revealed a positive association between plasma zinc and iron. It has been suggested that iron and zinc are absorbed by different mechanisms in the presence of yet to be identified organic substance, with interaction larger when both are provided as supplement [28]. Using kinetic analysis of zinc and iron uptake individually and in combination under normal and altered cellular mineral concentrations in human intestine Caco-2 cell line, Iyenger et al., revealed that cellular Zn status profoundly influence Fe uptake and its interactions with Zn during uptake [31]. If that is true, it means that the interaction between Zn and Fe depends on the relative cellular concentrations of the two elements. For instance, previously, cellular Zn repletion has been found to double iron uptake and

eliminates inhibition, with Zn depletion decreasing Fe uptake [31]. Therefore, the positive correlation between Zn and Fe in the present study may be attributed in part to the relative cellular level of the two elements among the population. It has also been reported that dietary proportions of Zn, Cu, and Fe appear to influence their metabolism at intestinal and cellular transport levels over a given period of time [32]. Moreover, according to earlier hypothesis of Levander and Cheng and Mills, over abundance of one trace element can interfere with the level and metabolic utilisation of another element present in normal or marginal concentration [33,34]. Although the dietary intakes of Fe and Zn were not assessed, it may be speculated that copper and zinc are present at different proportions in the diet of the subjects, and it seems the level of one may have affected the absorption/utilisation of the other. It has been reported that Fe deficiency does not only increase the efficiency of Fe absorption, it seems to also affect the absorption of chemically related elements and toxic elements [35]. Nevertheless, it is yet to be ascertained by which mechanism dietary Fe enhanced the absorption or metabolism of Zn and vice-versa.

CONCLUSION

Relative concentrations of essential and toxic metals among adult residents of Ebonyi state are important contributors of anaemia. While further studies are desired to substantiate this finding, food diversification and reduction in exposure to toxic metals are recommended to improve the nutritional status of residents and reduce anaemia prevalence with its attendant health consequences.

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REFERENCES

[1] Gambling L, Andersen HS, McArdle HJ. Iron and copper, and their interactions during development. *Biochemical Society Transactions*. 2008;36:1258-61.

[2] Bhattacherjee S, Banerjee R, Roy JK, Mandal S, Biswas R, Chakraborty M. Under nutrition and anaemia in rural adults- a cross-sectional study in rural North Bengal. *Indian J Prev Soc Med*. 2010;41(1&2):33-36.

[3] World Health Organization. Iron Deficiency Anaemia. Assessment, prevention and control. A guide for program managers. Geneva, Switzerland; WHO; 2001.

[4] Sinha NK, Chattopadhyay JC, Das PK, Maiti S, Maiti K. Prevalence of anaemia and its possible attributing factors in psychologically healthy women of reproductive ages in Midnapore (Jangalmahal area), India. *Indian Journal of Community Health*. 2013;25(3):226-32.

[5] Victery W, Thomas D, Shoeps P, Vander A. Lead increases urinary zinc excretion in rats. *Biol Trace Elem Res*. 1982;4:211.

[6] Goyer RA. Toxic and essential metal interactions. *Annu Rev Nutr.* 1997;17:37-50.

[7] Goyer RA. Nutrition and metal toxicity. *Am J Clin Nutr.* 1995;61(Suppl.):646-50S.

[8] Dietrich KN. Lead toxicity, mineral intake and child development. *Clin Appl Nutr*. 1991;1:27-38.

[9] Onwuchekwa IS, Etok CA, Ogbonna CE, Ezekwe CI. Effect of mine-water on agricultural soil quality in Ishiagu, Ebonyi state. *Nigerian J Microbiol.* 2009;23(1):1823-29.

[10] Ogbodo EN. Heavy metal levels in paddy soils and rice (Oryxa sativa (L)) exposed to agrochemicals at Ikwo South East Nigeria. *International Journal of Agric and Innovative Research*. 2013;2(3).

[11] Edeogu CO, Ekuma CE, Okaka ANC, Ezeonu FC, Uneke CJ, Elom SO. Public health significance of metal concentrations in soil, water and staple foods in Abakaliki, South eastern Nigeria. *Trend in Applied Sciences Research*. 2007,2(5):439-44.

[12] Zhuang P, Zou B, Li NY, Li ZA. Heavy metal contamination in soils and food crops around Dabaoshan mine in Guangdong, China: implication for human health. *Environ Geochem Health*. 2009;31:707–15.

[13] Addo MA, Darko EO, Gordon C, Nyarko BJB. Heavy metal contaminations in soil and cassava harvested near a cement processing facility in the Volta Region, Ghana: implications of health risk for the population living in the vicinity. e-*Journal of Science and Technology*. 2013;8(3):71-83.

[14] Ekpo FE, Ukpong EJ, Udoumoh IDJ. Bioaccumulation of heavy metals on soil and arable crops grown in abandoned peacock paint industry in Ikot Ekan, Etinam Local Government Area, Akwa Ibom State, Nigeria. *Universal Journal of Environmental Research and Technology*. 2014;4(1):39-45.

[15] Ugwuja EI, Ogbonnaya LU, Obuna JA, Awelegbe F, Uro-Chukwu H. Anaemia in relation to body mass index (BMI) and sociodemographic characteristics in adult Nigerians in Ebonyi State. *Journal of Clinical and Diagnostic Research*. 2015;9(1):LC04-07.

[16] Ebonyi state Independent Electoral Commission (EBSIEC). Delineation of political Wards in Ebonyi State. EBSIEC, Abakaliki, 2011.

[17] Brown KH, Rivera JA, Bhutta Z, Gibson RS, King JC, Lönnerdal B, et al. International Zinc Nutrition Consultative Group (IZiNCG) technical document #1. Assessment of the risk of zinc deficiency in populations and options for its control. *Food Nutr Bull*. 2004;25:S94-S203.

[18] Dacie JV, Lewis SM. Practical hematology 10th edition Edinburgh: Churchill Livingstone, 1995; pp. 25-57.

[19] Jacobson BE, Lokltch O, Qulgley GC. Improve sample preparation for accurate determination of low concentration of Pb in whole blood by graphite furnace analysis. *Clin Chem*. 1991;37:515–19.

[20] WHO. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Vitamin and Mineral Nutrition Information System. Geneva, World Health Organization, 2011(WHO/NMH/NHD/MNM/11.1) (http://www.who.int/vmnis/indicators/haemoglobin.pdf, [Accessed 15/01/2015].

[21] World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects. World Medical Association, 2000. Available at http://www.wma.net/e/policy/b3.htm. [Accessed June 15, 2013].

[22] Hegazy AA, Zaher MM, Abdel-hafez MA, Morsy AA, Saleh RA. Relation between anaemia and blood levels of lead, copper, zinc and iron among children. *BMC Research Notes*. 2010;3:133.

[23] Ugwuja EI, Akubugwo EI, Ibiam UA, Obidoa O, Ugwu NC. Plasma copper and zinc among pregnant women in Abakaliki, Southeastern Nigeria. *The Internet Journal of Nutrition and Wellness*. 2010;10(1).

[24] Keen CL, Clegg MS, Hanna LA, Lanoue L, Rogers JM, Daston GP, et al. The plausibility of micronutrient deficiencies being a significant contributing factor to the occurrence of pregnancy complications. *J Nutr*. 2003;133:(5 Suppl 2):1597S-605S.
[25] Sanstead HH. Requirements and toxicity of essential trace elements, illustrated by zinc and copper. *Am J Clin Nutr.* 1995;61:6215-45.
[26] Alabdullah H, Bareford D, Braithwaite R, Chipman K. Blood lead levels in iron-deficient and non iron-deficient adults. *Clin Lab Haem*. 2005;27:105-09.
[27] Arredondo M, Martinez R, Nunez MT, Ruz M, Olivares M. Inhibition of iron and copper uptake by iron, copper and zinc. *Biol Res*. 2006;39:95-102.
[28] Sandström B. Micronutrient interactions: effects on absorption and bioavailability. *Br J Nutr.* 2001;85(Suppl 2):181-85.
[29] Abdel-Mageed AB, Oehme FW. A review of the biochemical roles, toxicity and interactions of zinc, copper and iron: I. Zinc. *Vet Hum Toxicol.* 1990;32(1):34-39.
[30] Turgut S, Hacioglu S, Emmungil G, Turgut G, Keskin A. Relations between iron deficiency anaemia and serum levels of copper, zinc, cadmium and lead. *Polish J Environ Stud*. 2009;18(2):273-77.
[31] Iyengar V, Pullakhandam R, Nair M. Iron-zin interaction during uptake in human intestinal Caco-2 cell line: kinetic analyses and possible mechanism. *Indian J Biochem Biophy*. 2009;46:299-306.
[32] Adel-Mageed AB, Oehme FW. The effect of various dietary zinc concentrations on the biological interactions of zinc, copper and iron in rats. *Biol trace Elem Res*. 1991;29(3):239-56.
[33] Levander OA, Cheng L. Micronutrients interaction: vitamins, minerals and hazardous elements. *Ann NY Acad Sci.* 1980;355:1-35.
[34] Mills CF. Interactions between elements in tissues: Studies in animal models. *Fed Proc*. 1981;40:2138-43.
[35] Wright RO, Tsaih SW, Schwartz J, Wright RJ, Hu H. Association between iron deficiency and blood lead level in a longitudinal analysis of children followed in an urban primary care clinic. *Journal of Pediatrics*. 2003;142:9–14.

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