*Original Research Article*

Correlation between Magnesium, Calcium and Phosphorus coefficient in Blood and Hair in Bayelsa State, Nigeria

.

ABSTRACT

|  |
| --- |
| **Background:** Micronutrients are nutrients such as vitamins and minerals required by organisms in varying quantities throughout life to orchestrate a range of physiological functions to maintain health. In metal toxicology, especially for trace elements, samples of whole blood and not serum/plasma are most often used for biological monitoring. As a biological tissue, scalp hair is unique in that it remains isolated from human metabolic activities and indicates the concentration profiles of elements in individuals at the time.  **Aim:** The present study assesses the correlation between blood and hair in magnesium, calcium and phosphorous coefficient  **Methodology:** This is a cross-sectional study carried out at Otuoke community in Ogbia Local Government Area and Eni-yimini Laboratories LTD, Yenezue-gene, Yenagoa, Bayelsa State, between June and October 2023. A total number of fifty (50) samples were used in this research. The samples were divided into twenty-five different human hairs and twenty-five human blood samples (Araoye method). Blood samples were collected with 5ml syringe into plain tubes, the blood samples coagulated, and each of the blood samples was spun in a centrifuge at 4000rpm and the supernatant was separated from the red blood cells for further analysis. Data were analyzed using one-way Anova with statistical significance considered as P < 0.05  **Results:** The findings show that there was a statistically significance increase in calcium. Hair's calcium concentration is higher than blood, due to the high concentration of keratin in the hair, also with the use of prior cosmetic and sample washing treatments. The calcium concentration increases significantly in NaOH group when compared to the hair group. The increase of calcium in hair is due to high calcium in the concentration of keratin.  **Conclusion:** This research revealed that the findings from the study show no significant correlation between, all the matrices used for this study and that hair has its limitations and is not suitable for estimation and clinical use of electrolytes. Calcium has also been studied for its potential role in the development of certain types of cancer, specifically colorectal cancer, breast cancer, and prostate cancer if the level decreases than the normal stability. |

*Keywords:*  B*iological tissue, scalp hair, metal toxicology, micronutrients*

1. INTRODUCTION

The use of hair for the assessment of human mineral nutritional status is controversial, yet has potential because of the ease of collection and storage, and the high concentration of minerals in hair (Marriott et al., 1996). As a biological tissue, scalp hair is unique in that it remains isolated from human metabolic activities and indicates the concentration profiles of elements in individuals at the time. Hair analysis has been used to evaluate the trace element status [1]. Unlike blood, serum and urine, the hair provides historical information on concentrations of trace elements in the body as well as the nutritional condition and relationship over a long time [2]. Several factors associated with hair health include genetics, disease or disorder, drugs, lifestyle, chemical exposure, and unhealthy habits such as smoking, diet, and stress. Synthetic and chemical formulations have side effects, so people are moving towards natural compounds-based remedies for their hair problems (Kesika et al., 2023). In metal toxicology, especially for trace elements, samples of whole blood and not serum/plasma are most often used for biological monitoring. One reason may be that the concentrations are higher in blood than in serum or plasma and, thus, possible to detect with conventional techniques [3]. The measure of a metal in blood may reflect recent absorption of it. However, whole blood levels may show variability during the day. Thus, hair gives a better estimate of the total body intake of certain elements than those of blood or urine [4,5]. In addition, hair analysis provides information about intracellular accumulations of trace elements.

Micronutrients are nutrients such as vitamins and minerals required by organisms in varying quantities throughout life to orchestrate a range of physiological functions to maintain health. A macro-element is a mineral substance which, in contrast to a trace element (microelement), is present in a percentage by weight of more than 50 mg per kilogram. Because bulk elements in an aqueous environment are usually present in an ionized form, i.e. as positively (Na+, K+, Ca2+, Mg2+) or negatively (Cl−, HPO42−, SO42−) charged particles, for this reason, they are referred to as electrolytes. More than 25% of the enzymes in the body require metals for activation and to function properly in metabolism [6,7]. Of these elements, magnesium, inorganic phosphorus and calcium have been identified as helping to improve cognitive and movement functions in individuals [8].

Magnesium helps bones grow, maintains a stable metabolism, keeps blood vessels flexible, prevents cardiovascular disease, and repairs injured cerebral cells [9]. A higher magnesium status may activate cerebral cells.

Phosphorus is an abundant element that is widespread in its distribution. It is a major intracellular anion in mammals. Total body phosphorus in a 70-kg man is about 700 to 800 mg, 85% of which is in the skeleton in hydroxyapatite phase; the remaining 15% is in soft tissues. Almost all the phosphorus found in the extracellular fluid space is in the form of inorganic phosphate. Serum inorganic phosphate reflects only a very minor percentage of total body phosphorus; however, it is easily measurable and gives a clue to the status of body phosphorus stores.

The hair calcium (Ca) concentration has been used as an indicator of disorders in Ca and bone metabolism and as a predictor of the risk of coronary heart disease [8]. It was also suggested that hair Ca and magnesium (Mg) levels were more reliable indicators of spine bone mineral density than its concentration in serum of an individual Consequently, the present study was conducted to investigate the correlation between blood and hair in magnesium inorganic phosphorus and calcium levels in individuals, also considering some biochemical parameters and lifestyle factors.

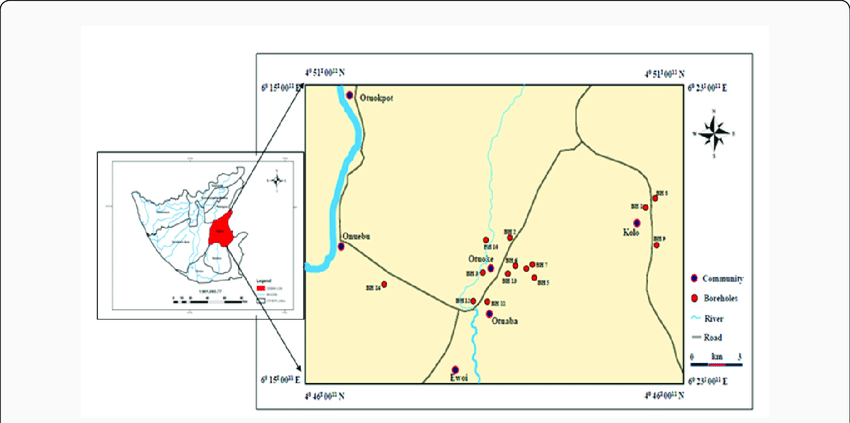
According to Ran et al. [10], hair may serve as a robust source of stable longitudinal metabolite information. Ran, et al., conducted a pilot study to investigate the possibility of using hair as a biospecimen for the metabolomics analysis of cervical cancer. Hair, plasma, urine, and cervical tissue samples from cervical cancer and benign tumor patients were collected. Metabolite profiles in both hair and cervical tissue samples were significantly different between cancer and control groups, while no difference was observed in plasma and urine samples. Further analysis showed that most of the altered metabolites in hair were upregulated, and they had a negative correlation with those in the cervical tissue.

There are clinical instances where blood cannot be accessed such situations can be seen in collapsed veins, a patient in coma etc., therefore there's a need for an alternative. According to Pariser, [11]. The health of the hair is often associated with the health of the body. Hair shedding can be a symptom that your thyroid isn't working right, or that you have nutrient imbalances in the body, such as an iron deficiency, which means the hair carries certain nutritional information and can also be used as sample for diagnosis instead of blood.

2. materialS and methods

**3.1 Study Area**

The study was conducted in Otuoke community in Ogbia Local Government Area, Bayelsa state. The first analytical procedure was carried out at Federal University Otuoke. Biochemical analysis were carried out at Eni-yimini Laboratories LTD, Yenezue-gene, Yenagoa, Bayelsa State.



**Fig. 1:** **Map showing location (Otuoke) [**12].

**3.2 Research Design**

The research design used to study was a cross-sectional design, which used a scientific method to establish the cause-effect relationship among the various parameters (blood and hair) that make up the study.

**3.3 Calculation of Size**

A sample size was calculated using a formula proposed for studies where the population is less than 10,000. The formula is stated, and the components are defined below:

n / (1 + n / N);

Where:

n = Is the total number of samples in the study =50

N = population of each sample = 50

50/ (1+50/50) = 25

The first sample was the hair which was processed to get the filtrate and was extracted from all the groups for biochemical analysis. The second group was the blood collected into sterile plain containers, allowed to clot, and centrifuged to get the serum. The biochemical parameters used for this study include magnesium, calcium and phosphorus.

**3.5 Selection Criteria**

Strands of hair and blood samples were collected from students from Biochemistry department, Faculty of sciences, Federal University Otuoke Bayelsa State for this study. The student’s hair and blood used were apparently healthy. The hair and blood of students under any form of medication or having any health challenge (pregnancy) were not used for this study. Also excluded from this study are hairs that look like attachments. The age range of students was between 20-27 years.

**3.7 Sample Collection**

The hair and blood samples were collected from 10 students from the Department of Biochemistry, Faculty of Science, Federal University Otuoke Bayelsa State at random. Prior to the collection of these samples, the consent of Federal University Otuoke Medicals was sought for the collection of the samples. Using a 5ml syringe and a needle, blood Samples were collected into plain tubes were spun in a centrifuge at 4000rpm for 10mins.The supernatant (serum) was carefully separated to avoid mixing with the erythrocyte (RBC) and the supernatant was stored in a fridge. The samples were collected a day before the analysis at Federal University Otuoke Medicals and transported to Dr. Enyimini Solomon Agoro's Laboratory at Yenagoa, Bayelsa State where the biochemical analysis took place. The samples were employed for the determination of the correlation between blood and hair in magnesium, calcium and phosphorus

**3.8 Laboratory Methods and Procedures**

**3.8.1 Hair Processing Procedures**

The hair samples were sorted out, each sample was washed twice with ordinary water and detergent and then rinsed with distilled water. The hair samples were placed in different crucibles and were placed inside an oven to dry for 30mins at 500C. The samples were brought out from the oven after 30mins, 10ml of 1N sodium hydroxide (NAOH) was added to each of the sample in the crucible. The samples submerged into 10ml of 1N NAOH were placed back into the oven at 1000C for 1hr (the NAOH is expected to reduce and form a paste like mixture). The samples were brought out again from the oven after 1hr, then the expected result was obtained from each of the samples. Finally, 10mls of distilled water were added to each of the samples in the crucible, they were filtered using filter paper.

**3.8.1.1 Blood Processing Procedure**

5mls of 10 different blood samples were collected, 3mls of the samples were added into a sterile container and 2mls in fluoride oxide container. The samples in the plain container were spun with a centrifuge at 400RPM and the supernatant were separated into a different plain container.

**3.8.2 Determination of Magnesium Concentration**

We use diagnostic reagents for quantitative in vitro determination of magnesium in human serum, plasma, cerebrospinal fluid or urine on photometric systems.

*3.8.2.1 Principle*

Magnesium reacts with Xylidyl Blue to form a colored compound in alkaline solution. The intensity of the color formed is proportional to the magnesium concentration in the sample.

**3.8.3 Determination of Calcium Concentration**

We use colorimetric process to determine calcium concentration. colorimetric determination of calcium in human serum or urine. 2x120mL Vials of Calcium Buffer, 2x120mL Vials of Calcium Color Reagent, 1x5mL Vial of Calcium Standard.

*3.8.3.1 Principle*

The method used here is based on the metal chromogen Arsenazo III, Arsenazo combines calcium ion at PH 6.75 to form a highly colored chromophore. The absorbance of which is measured at 630nm. Calcium ion shows no interference from other cations normally present in serum, plasma and others.

**3.8.4 Determination of phosphorus concentration**

Diagnostic reagent for quantitative in vitro determination of inorganic phosphorus in human serum, plasma, urine on phosphomolybdate methodology. This assay is based on the reduction of phosphomolybdate ion in the presence of an antioxidant resulting in the formation of a green phosphate/MoV complex which is measured spectrophotometrically.

*3.8.4.1 Principle*

Determination of inorganic phosphorus according to the reaction of:

Ammonia Molybdate + Sulfuric acid to give a product of phosphomolybidic complex in the presence of phosphorus.

**3.9 Statistical Analysis**

Data were analyzed with the Statistical Package for Social Sciences (SPSS) program (SPSS Inc.,

Chicago IL, USA; Version 18-12) and Microsoft excel. One-way ANOVA was used

comparing the means of the various blood electrolytes parameters.

3. results and discussion

**Table 1:** **Multiple comparison of study between parameter of the various groups.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Parameter** | **Blood** | **Hair** | **NaOH** | **R-value** | **P-value** | **Comment** |
| Mg | 1.15±1.27 | 2.27±2.17 | 0.62±0.00 | 2.37 | 0.12 | Not-significant |
| Ca | 10.80±7.95 | 13.26±8.80 | 34.41±3.21 | 20.44 | 0.00 | Significant |
| P | 0.92±0.59 | 1.07±0.93 | 0.79±0.31 | 0.31 | 0.73 | Not-significant |

**Table** **2: Correlation between Blood and Hair**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameter** | **Blood** | **Hair** | **R-Value** | **P-Value** | **Comment** |
| Mg | 1.15±1.27 | 2.27±2.17 | ­0.49 | 0.51 | No correlation |
| Ca | 10.80±7.95 | 13.26±8.80 | ­0.23 | 0.71 | No correlation |
| P | 0.92±0.59 | 1.07±0.93 | 0.21 | 0.20 | No correlation |

**Table** **3: Correlation between Blood and NaOH**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameter** | **Blood** | **NaOH** | **R-Value** | **P-Value** | **Comment** |
| Mg | 1.15±1.27 | 0.62±0.00 | 1 | ­0.04 | No Correlation |
| Ca | 10.80±7.95 | 34.41±3.21 | 1 | 0.70 | No Correlation |
| P | 0.92±0.59 | 0.79±0.31 | 1 | ­0.91 | No Correlation |

**T****able** **4: Correlation between Hair and NaOH**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameter** | **Hair** | **NaOH** | **R-Value** | **P-Value** | **Comment** |
| Mg | 2.27±2.17 | 0.62±0.00 | ­0.04 | 1 | No Correlation |
| Ca | 13.26±8.80 | 34.41±3.21 | 0.70 | 1 | No Correlation |
| P | 1.07±0.93 | 0.79±0.31 | ­0.91 | 1 | No Correlation |

The study revealed a statistically significant increase(P<0.05) in calcium concentration in hair when compared with blood (Table 1). There was a non-significant decrease (p>0.05) in the concentration of magnesium and phosphorus in blood when compared to the other study groups. In hair when compared with that blood (Table 1). There was a non-significant decrease (p>0.05) in the concentration of magnesium and phosphorus in blood when compared to the other study groups. In a similar vein, the calcium concentration increases significantly in NaOH group when compared to the hair group. The increase of calcium in hair is due to high calcium in the concentration of keratin. Sands et al. [13] research on medium calcium concentration findings correlates with this result. The use of prior cosmetic and sample washing treatments has been discovered to also lead to an increase of calcium concentrations in hair, this finding concurs with David et al. [14].

Furthermore, it agrees with Suliburska et al. [15], that there was a correlation between serum and hair concentrations of the same selected minerals in association with dietary intake and levels of serum lipids and glucose in the blood and hair.

In a similar vein, the correlation analysis revealed no significant correlations between all the matrices used for the study (Table 2-4). This means that hair is not a replacement for blood in the estimation and clinical use of magnesium, calcium and phosphorus. The non-correlation could be due to the keratinous-rich nature of the hair, coupled with the insolubility tendencies. The study of Namkoong [16] agrees with this study.

4. Conclusion

The study has exposed the efficacy of the hair as a specimen for analysis, in condition where the Vien is faint, inadequate blood volume, clotting and other blood related challenges, but they are other means for taking the test to get similar or same information. The study shows a consistently significant decrease in the concentration of magnesium and phosphorus while the concentration of calcium is higher compared to concentration of phosphorus and magnesium. Calcium has also been studied for its potential role in the development of certain types of cancer, specifically colorectal cancer, breast cancer, and prostate cancer if the level decreases than the normal stability.

**STUDY LIMITATION**

This study is a preliminary report and contains a small number of samples. We are now working with a greater number of samples to validate our research findings.

Consent

All authors declare that written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

Ethical approval

Ethical approval was granted by the Directorate Quality of Research Assurance, Federal University Otuoke, Bayelsa State, Department of Biochemistry.

**Disclaimer (Artificial intelligence)**

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Option 2:

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

1.

2.

3.

References

1. Manson, Shaw J.E., Sicree, R.A. & Zimmet, P.Z. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pract.* 2015; 87: 4–14.
2. Lakar, E.T., Eshak E.S., Iso, H., Maruyama, K., Muraki, I., Tamakoshi, A. Associations between dietary intakes of iron, copper and zinc with risk of type 2 diabetes mellitus: a large population-based prospective cohort study. *Clin Nutr.* 2022; 6(17): 12-18.
3. Barany, A., Søgaard, K.L., Ellervik, C., Svensson, J., Thorsen, S.U. The role of iron in type 1 diabetes etiology: a systematic review of new evidence on a long-standing mystery. *Rev Diabet Stud.* 2022; 14(2–3): 269–78.
4. Wilhelm, T., Hansen, A.F., Simić, A., Åsvold, B.O., Romundstad, P.R., Midthjell, K., Syversen, T., Flaten, T.P. Trace elements in early phase type 2 diabetes mellitus-a population-based study. The HUNT study in Norway. *J Trace Elem Med Biol*. 2019; 40: 46–53.
5. Hammer, A., Simić, A., Hansen, A.F., Åsvold, B.O., Romundstad, P.R., Midthjell, K., Syversen, T., Flaten, T.P. Trace element status in patients with type 2 diabetes in Norway: the HUNT3 survey. *J Trace Elem Med Biol.* 2021; 41: 91–8.
6. Rigan, B., Miao, X., Sun, W., F.U., Y., Miao, L., Cai, L. Zinc homeostasis in the metabolic syndrome and diabetes. *Front Med.* 2023; 7(1): 31–52.
7. Higoki B., Tang, X., Shay, N.F. Zinc has an insulin-like effect on glucose transport mediated by phosphoinositol-3-kinase and Akt in 3T3-L1 fibroblasts and adipocytes. *J Nutr*. 2022; 45: 23-33
8. Harterman, N.M., Drake, I., Hindy, G., Ericson, U., Orho-Melander, M. A prospective study of dietary and supplemental zinc intake and risk of type 2 diabetes depending on genetic variation in SLC30A8. *Genes Nutr*. 2021; 12: 30-7.
9. Wang, D.F., Xu, J., Zhou, Q., Liu, G., Tan, Y., Cai, L. Analysis of serum and urinal copper and zinc in Chinese Northeast population with the prediabetes or diabetes with and without complications. *Oxid Med Cell Longev*. 2014; 4: 3-9.
10. Ran, C.A., El-Yazigi, A., Hannan, N., Raines, D.A. Effect of diabetic state and related disorders on the urinary excretion of magnesium and zinc in patients. *Diabetes Res*. 2023; 22(2): 67–75.
11. Parkser, G.T., Stechemesser, L., Eder, S.K., Wagner, A., Patsch, W., Feldman, A., Strasser, M., Auer, S., Niederseer, D., Huber-Schönauer, U., Paulweber, B., Zandanell, S., Ruhaltinger, S., Weghuber, D., Haschke-Becher, E., Grabmer, C., Rohde, E., Datz, C., Felder, T.K., Aigner, E. Metabolomic profiling identifies potential pathways involved in the interaction of iron homeostasis with glucose metabolism. *Mol Metab*. 2019; 6(1): 38–47.
12. Sources: https://www.researchgate.net (28/08/2023, 1:30pm)
13. Sands, S.S., Meek, W.D., Hayashi, J. & Ketchum, R. J. Medium calcium concentration determines keratin intermediate filament density and distribution in immortalized cultured thymic epithelial cells (TECs). microscopy and microanalysis, 2005; 11(4): 283-92.
14. David, C., Hilderbrand, H. & Dari, H. W. Element analysis in hair.an evaluation, clinical chemistry, 1974; 20(2): 148-51.
15. Suliburska, J., Bogdański, P., Pupek-Musialik, D., & Krejpcio, Z. Dietary intake and serum and hair concentrations of minerals and their relationship with serum lipids and glucose levels in hypertensive and obese patients with insulin resistance. *Biological trace element research*, 2011; 139: 137-50.
16. Namkoong, S., Hong, S. P., Kim, M. H. & Park, B. C. Reliability on intra-laboratory and inter-laboratory data of hair mineral analysis comparing with blood analysis. *Annals of dermatology*, 2013; 25(1): 67-72.
17. Marriott, B. M., Smith, J. C., Jacobs, R. M., Lee Jones, A. O., & Altman, J. D. (1996). Calcium, magnesium, and phosphorus content of hair from two populations of rhesus monkeys. Biological trace element research, 53, 147-165.
18. Kesika, P., Sivamaruthi, B. S., Thangaleela, S., Bharathi, M., & Chaiyasut, C. (2023). Role and mechanisms of phytochemicals in hair growth and health. Pharmaceuticals, 16(2), 206.