**Original Research Article**

**BLOOD HEAVY AND TRACE METAL PROFILES AND THEIR ASSOCIATION WITH HIRSUTISM IN YOUNG ADULT FEMALES IN NIGERIA**

**ABSTRACT**

**Background:** Hirsutism poses serious concern, embarrassment, poor self-esteem, and psychological distress for its young adult-female sufferers in an African population like Nigeria. **Aims and Objectives:** This study aimed to assess the blood levels of trace metals; Zinc (Zn), Copper (Cu), Selenium (Se) and heavy metals; Lead (Pb), Cadmium (Cd), and Arsenic (As) in young adult females with Hirsutism in Owerri, Nigeria. **Materials and Methods:** The study population consisted of fourty (40) young adult females with Hirsutism and 40 age-matched female controls. Venous blood samples from all participants were analyzed using Flame Atomic Absorption Spectrophotometric method for blood trace metals and heavy metals. SPSS version 23 was employed in the statistical Analysis of the data obtained. **Results:** There were significantly higher mean blood levels of Pb [9.93 ± 0.49 vs. 8.13 ± 2.03µg/dl, p= 0.001], Cd [0.22 ± 0.04 vs. 0.20 ± 0.01 µg/dl, p=0.001] and As [0.32 ± 0.04 vs. 0.10 ± 0.06 µg/dl, p=0.000] in young adult females with hirsutism compared to the controls. While there were significantly lower mean blood levels of Zn [70.32 ± 13.84 Versus 87.54 ± 29.83 µg/dl, p=0.013], Cu [52.40 ± 16.74 versus 121.50 ± 17.21 µg/dl, p=0.000] and Se [11.60 ± 8.42 versus. 29.83 ± 7.92 µg/dl, p=0.000] in young adult females with hirsutism compared to controls. There were significant positive correlation of blood Cu with blood Se [r = 0.621, p=0.003] and Zn [r = 0.538, p= 0.014] in young adult females with hirsutism. While there were significant negative correlation of blood Cu with Pb [r = -0.472, p= 0.036], As [r= -0.610, p=0.004] and Cd [r = -0.724, p=0.000] in young adult females with hirsutism. There was a significant positive correlation of blood Cd with blood Pb [r = 0.842, p = 0.000], but significant negative correlations of blood Cd with Se [ r = -0.936, p = 0.000]. **Conclusion:** High blood levels of Pb, Cd and As parallels low blood levels of Zn, Cu and Se in young adult females with hirsutism. The alterations in the blood levels of these heavy and trace metals may in part be associated with the development of hirsutism in this environment, via endocrine disruption, metabolic dysregulation, and oxidative stress.

 **Key Word:** Hirsutism, Heavy metals, Trace Metal, Nigeria.

**INTRODUCTION**

**Hirsutism** is the occurrence of excessive terminal hair in androgen-dependent areas of the female body **[1]**, in a male-like distribution. It is a clinical manifestation of hyperandrogenemia, and a common reason of cosmetic embarrassment, poor self-esteem, and psychological distress for young adult females **[2]**. It affects around 5-10% of women and is a common presenting complaint in the dermatological outpatient department (OPD) for cosmetic reasons **[3]**.

**Heavy metals** are elements that cause toxic effects when its exposure exceeds the higher tolerable limits **[4]**. The potential routes of entry of heavy metals into the body include either natural environmental exposure or other means such as mining, soil erosion, industrial waste, air pollution, or pesticides. Although Occupational exposure is possible in some individuals, diet represents the major source of exposure **[5]**.

Most heavy metals; Lead (Pb), Cadmium (Cd), Arsenic (As) and Mercury (Hg) are noxious to human beings when their blood levels surpass a certain threshold or even at very low concentrations **[6]**,

**Trace elements** are natural, essential, homogenous, inorganic substances which are taken with food or drink and are present in minor amounts in the human body, but also have a unique role in a number of important structures and processes **[7]**. Trace elements play several important roles in the maintenance of a healthy state of an organism, some are essential for enzyme reactions where they attract and facilitate conversion of substrate molecules to specific end products. Moreover, some of them donate or accept electrons in redox reactions that are of primary importance in the generation and utilization of metabolic energy. Some of them have structural roles and are responsible for the stability of important biological molecules. Consequently, disturbances in trace element homeostasis may result in the development of pathologic states and diseases. Therefore, their role in the maintenance of undisturbed biological functions is highly important **[8]**.

Despite numerous previous studies reporting on the health effects of heavy metals, the impact of certain heavy metals and trace elements on the hair remain unknown. Heavy metals have a major impact on endocrine health **[9]**, and thus may be consequential to Hirsutism. Altreations in heavy and trace metals has been associated with certain disorders like pre-eclampsia **[10, 11]**, acne vulgaris **[12]** and heart failure **[13]**, in African-Nigerian population. It has been reported that low level of trace element have a potential role of causing endocrine disorder **[14]**, and that Heavy metal exposures in Polycystic Ovarian Syndrome (PCOS) may be related to insulin resistance and hirsutism through oxidative and inflammatory mechanisms in a Caucasian population in Turkey **[15]**. Yet the role of heavy and trace metals in the development of hirsutism in an African population like Nigeria has not received adequate attention. Thus, this study is aimed to bridge the gap in knowledge.

 **MATERIALS AND METHODS**

#  Study Design

The study comprising of participants selection, assessment/classification of subjects, sample collection, determination of blood heavy/trace metals and data generation lasted from February 2023 to August, 2023. This study was conducted at Imo State University, Owerri, Nigeria. Owerri is the capital and main town of Imo State. The University is highly populated, as it attracts students from the state and other five neighboring states, due to the location in town, subsidized tuition fee by the state government and highly dedicated University staffs that ensure the smooth training of the students.

# Ethical Consideration

This study Protocol was approved by the institutional Research Ethics Committee of the Faculty of Health Sciences, Imo State University, Owerri. Only subjects who gave informed consent, approval and signed a consent form after the procedure and implications were explained to them participated in the study. Voluntary participation was ensured as each participant could withdraw from the study at any time.

#  Study Population

Using random sampling method, a total of 80 young adult females who were students of Imo state University Owerri, Nigeria were selected for this study. The study population consists of fourty (40) young adult females with Hirsutism within the age range of 18 to 30 years. They were aged-matched with fourty young adult females without Hirsutism, who served as controls.

**Selection Criteria**

***Inclusion Criteria for Hirsutism Subjects***

[i] The Ferriman-Gallwey score for hirsutism as modified by Swingler et al., **[16]** was employed in assessment of eleven androgen-sensitive areas of the body (chin, upper lip, chest , legs , buttocks , arm , forearms , upper back, lower back, upper abdomen, lower abdomen) as the inclusion criteria for hirsutism. A score of 1 to 4 is given for eleven areas of the body, where: score 0 (complete lack of terminal hairs), score 1 (minimal presence of terminal hairs), score 2 (more than minimal terminal hairs), score 3 (not too large hairs) and score 4 (presence of terminal hairs).

A total score less than 8 is considered normal; a score of 8 to 15 indicates mild hirsutism; and a score greater than 15 indicates moderate to severe hirsutism. A score of 0 indicates absence of hirsutism.

[ii] A detailed physical scrutiny with precise emphasis on signs of virilization (including frontal baldness, loss of female body contours, increased muscularity, acne, clitoromegaly, and atrophy of breast) was performed in all the young adult females so as to rule out ***Virilism***.

[iii] Young adult female students within the age of 18 – 30 years.

[iv] Apparently healthy young adult female students, who were mentally and physically fit.

***Exclusion Criteria for Hirsutism Subjects***

[i] Manifestation of signs of Virilism (including frontal baldness, loss of female body contours, increased muscularity, acne, clitoromegaly, and atrophy of breast)

[ii] Females not within the age range of 18 to 30 years

[iii] Manifestation of any chronic disease.

**Inclusion Criteria for Control subjects**

[i] Young adult females with Ferriman-Gallwey score for hirsutism less than 4

[ii] Young adult female students within the age of 18 – 30 years

[iii] Apparently healthy young adult female students, who were mentally and physically fit

**Exclusion Criteria for Control subjects**

[ii] Females not within the age range of 18 to 30 years

[iii] Manifestation of any chronic disease.

# Sample Collection and Preparation

Two (2ml) of venous blood was collected aseptically from all participants using sterile needle and syringes, it was dispensed into Lithium Heparin tube and mixed gently. It was stored refrigerated as 2-80C until analysis.

## Method

## *Determination of Trace and Heavy Metals by Flame Atomic Absorption Spectrophotometry*

Heavy metal analysis was conducted using Varian AA240 Atomic Absorption Spectrophometer according to the method of American Public Health Association (APHA) **[17]**

***Working principle:*** Atomic absorption spectrometer's working principle is based on the sample being aspirated into the flame and atomized when the AAS's light beam is directed through the flame into the monochromator, and onto the detector that measures the amount of light absorbed by the atomized element in the flame. Since metals have their own characteristic absorption wavelength, a source lamp composed of that element is used, making the method relatively free from spectral or radiation interferences. The amount of energy of the characteristic wavelength absorbed in the flame is proportional to the concentration of the element in the sample.

**Preparation of reference solutions**

A series of standard metal solutions in the optimum concentration range are prepared, the reference solutions were prepared daily by diluting the single stock element solutions with water containing 1.5 ml concentrated nitric acid/litre. A calibration blank was prepared using all the reagents except for the metal stock solutions. Calibration Curve for each metal was prepared by plotting the absorbance of standards versus their concentrations.

**Sample Digestion [18]**

One (1ml) of blood sample was measured and added 1ml of nitric acid. It was mixed properly and boiled at 1000c for 30mins. It was then made up to 10ml for analysis. Trace metals and heavy metals were then determined by atomic absorption spectroscopy.

# Statistical Analysis

# IBM SPSS version 23 was employed in statistical analysis. All values were expressed as mean ± standard deviation. The test of significance was determined by student t-test. Pearson correlation was also determined. Values with p < 0.05 were considered statistically significant.

 **RESULTS**

**Blood levels of heavy metals and trace metals in young adult females with hirsutism**

There were significantly higher mean blood levels of Pb [9.93 ± 0.49 vs. 8.13 ± 2.03, p= 0.001], Cd [0.22 ± 0.04 vs. 0.20 ± 0.01, p=0.001] and As [0.32 ± 0.04 vs. 0.10 ± 0.06, p=0.000] in young adult females with hirsutism compared to the controls. While there were significantly lower mean blood levels of Zn [70.32 ± 13.84 Versus 87.54 ± 29.83, p=0.013], Cu [52.40 ± 16.74 versus 121.50 ± 17.21, p=0.000] and Se [11.60 ± 8.42 versus. 29.83 ± 7.92, p=0.000] in young adult females with hirsutism compared to controls (Table 1).

**Correlation of blood Cu with heavy metals and trace metals in young adult females with hirsutism.**

There were significant positive correlation of blood Cu with blood Se [r = 0.621, p=0.003] and Zn [r = 0.538, p= 0.014] in young adult females with hirsutism. While there were significant negative correlation of blood Cu with Pb[r = -0.472, p= 0.036], As [r= -0.610, p=0.004] and Cd [r = -0.724, p=0.000] in young adult females with hirsutism (Table 2).

**Correlation of Blood Cd with Heavy Metals and Trace Metals in Young Adult Females with Hirsutism.**

There was a significant positive correlation of blood Cd with blood Pb [r = 0.842, p = 0.000]. There were significant negative correlations of blood Cd with Cu [ r = -0.724, p = 0.000] and Se [ r = -0.936, p = 0.000]. However, there was a non-significant negative correlation of blood Cd with blood Zn [r = -0.434, p = 0.056] and non-significant positive correlation blood Cd with blood As [ r = 0.361, p = 0.118] (Table 3).

**Table 1: Blood levels of heavy metals and trace metals in young adult females with hirsutism versus controls.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Variables****[mean+SD]**  | **Hirsutism Subject****[n=40]** | **Controls****[n=40]** | **t-value** | **p-value** |
| **Zn [µg/dl]**  | 70.32 ± 13.84 | 87.54 ± 29.83 | 2.743 | 0.013 |
| Lower 95% C.L  | 63.83 | 73.57 |  |  |
| Upper 95% C.L  | 76.80 | 101.50 |  |  |
| **Cu [µg/dl]**  | 52.40 ± 16.74 | 121.50± 17.21 | -16.513 | 0.000 |
| lower 95% C.L  | 44.56 | 113.44 |  |  |
| Upper 95% C.L  | 60.23 | 129.55 |  |  |
| **Se [µg/dl]**  | 11.60±8.42 | 29.83± 7.92 | -28.492 | 0.000 |
| Lower 95% c.l  | 7.65 | 26.12 |  |  |
| Upper 95% c.l  | 15.54 | 33.53 |  |  |
| **Pb [µg/dl]**  | 9.93± 0.49 | 8.13± 2.03 | 3.741 | 0.001 |
| Lower 95% c.l  | 9.70 | 7.18 |  |  |
| Upper 95% c.l  | 10.16 | 9.09 |  |  |
| **Cd [µg/dl]**  | 0.22 ± 0.04 | 0.20± 0.01 | 4.105 | 0.001 |
| Lower 95% c.l  | 0.20 | 0.19 |  |  |
| Upper 95% c.l  | 0.24 | 0.21 |  |  |
| **As [µg/dl]**  | 0.32 ± 0.04 | 0.10± 0.06 | 24.28 | 0.000 |
| Lower 95% c.lUpper 95% c.l  | 0.300.34 | 0.070.13 |  |  |

**Table 2: Correlation of blood Cu with heavy metals and trace metals in young adult females with hirsutism.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Dependent Variables**  | **n**  | **r-values**  | **p-values**  |
| SeZn | 4040  | 0.621\*\* 0.538\* | 0.0030.014 |
| Pb  Cd As  | 40 40 40  | -0.472\*-0.724\*\*-0.610 \*\* | 0.036 0.000 0.004  |

**Table 3: Correlation of Blood Cd with Heavy Metals and Trace Metals in Young Adult Females with Hirsutism.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Dependent Variables**  | **n**  | **r-values**  | **p-values**  |
| Zn  | 20  | -0.434  | 0.056  |
| Cu Se Pb As  | 20 20 20 20  | -0.724\*\*-0.936\*\* 0.842 0.361  | 0.000 0.000 0.000 0.118  |

**DISCUSSION**

Significantly lower blood levels of Zn, Cu and Se were observed in young adult females with Hirsutism compared to controls in the study. This raise questions about the potential protective role of these trace metals. ***Zinc***helps to maintain integrity of skin and mucosal membrane. It is a co-factor for metallo-enzymes required for cell membrane repair **[19]**. Zn has antioxidant effect that protects against ROS, Reactive Nitrogen species **[20]** and influences the activity of antioxidant proteins **[21].**

Zinc acts as an anti-androgen by inhibiting 5α-reductase and therefore reducing the synthesis of dihydrotestosterone **[22, 23]**. Zn participates in the metabolism of androgens by inhibiting aromatase; thereby reducing testosterone conversion into estradiol and increasing conversion of androstenedione to testosterone **[22,23, 24]**. Furthermore, it has been documented that zinc deficiency disrupted the action of angiotensin converting enzyme, which appears to participate in the synthesis of adrenals androgens **[25]**. Because DNA-binding site of androgen receptor is a zinc finger protein, zinc can affect the action of androgens; therefore zinc deficiency may to suppress activity of this receptor **[26, 25]**. One of the effects of hyperandrogenism is hirsutism, which was also observed in women with PCOS [22]. Thus, it seems that the significantly lower blood Zn observed in this present study may have contributed to hyperandrogenism and thus hirsutism as one of its manifestation in the young adult females examined in this study.

**Copper** functions as a crucial cofactor for various enzymatic reactions, and redox chemistry for proteins to carry out vital biological functions **[27]**. It may have implications for hormone metabolismand thus in Hirsutism. Low blood Copper can result to impaired energy production and increased oxidative damage. Alteration in serum copper level has been associated with oxidative stress initiation; in which copper acts as a catalyst **[27]**]. The alterations whether low or high copper levels are connected to the progression of oxidative stress as low serum copper level decreases antioxidant enzyme activity, while higher serum copper level causes development of Fenton reaction**[28,29].** Copper plays an essential role in the proliferation and differentiation of dermal papillary cells, which have significant function in the development of the hair follicle **[30]**. On the other hand, it seems that alteration in copper levels may impair the ability of the body to form hair shafts through covalent bond formation with the sulfhydryl groups in keratin **[31]**. Hence alteration in blood Copper level may lead to oxidative stress and inflammatory reactions, which may be associated with a variety of health consequences including Hirsutism.

**Selenium** is a main constituent of [selenoproteins](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/selenoprotein) which plays a critical role in [human health](https://www.sciencedirect.com/topics/medicine-and-dentistry/public-health)  **[32]**, and is important for host defense system. It stimulates anti-carcinogenic factors, prevents cardiovascular diseases, and performs anti-proliferative and anti-inflammatory activities **[33]**. It affects reproductive by strengthening fertility **[34]**. Also, selenium has vital [enzymatic functions](https://www.sciencedirect.com/topics/medicine-and-dentistry/enzyme-activity) including [thyroid hormone metabolism](https://www.sciencedirect.com/topics/medicine-and-dentistry/thyroid-hormone-metabolism) **[34]**, insulin-like action, protection from [oxidative stress](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/oxidative-stress) and redox [homeostasis](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/homeostasis) **[35]**.

Selenium, along with selenocysteine and selenomethionine, have antioxidant properties and anti-inflammatory effects **[36]**. Inflammation is a strong risk factor for PCOS  **[37]**, and thus Hirsutism. Se, as an intense antioxidant, can reduce ROS **[35]**, thus selenium deficiency may lead to androgen disorders and consequential to hirsutism as observed in this present study. Several studies, including one systematic review, have investigated the impact of Selenium Supplementation on biomarkers in women with Polycystic Ovarian Syndrom (PCOS) **[38]**; however, there is still a paucity of information in the blood levels of Se in young adult females with hirsutism. This present study seems to be the first report to bridge the gap in knowledge.

Data from this study reveals significantly higher blood levels of Cd, Pb, and As in young adult females with hirsutism compared to controls; an indication that these metals may in part contribute to the development of this disorder. These findings suggest a need for further research into the mechanisms by which these metals may influence Hirsutism development.

**Heavy metals** play an important endocrine-disrupting role in the health of humans **[39]**. Endocrine-disruption may lead to endocrine disorders manifesting as hypertrichosis and Hirsutism, **[40]**. Heavy metal exposures may lead to hirsutism through oxidative and inflammatory mechanisms. The mechanism underlying heavy metal toxicity in humans is mainly their interaction with the sulfhydryl groups in the non-enzymic antioxidant system (e.g., replacing a hydrogen atom on the reduced GSH moieties), resulting in the formation of organo-metallic complexes which deactivate further biochemical reactions **[41]**.

Besides, the buildup of heavy metals will act together and influence the activity of enzymes, proteins, and metabolism, consequently causing biochemical, morphological, and functional changes. **[42]**

Sources of Pb and Cd exposure in this environment includes batteries, cables, pigments, chemical additives, paints, cosmetics pigments, hair dyes, cements , petrol, mining products **[10, 12,13]** and agricultural products for Arsenic. The Nigerian population is exposed to these heavy metals via the ingestion of contaminated food and water, inhalation of airborne Pb and skin penetration. Actions of Pb include depleting GSH and protein-bound sulfhydryl groups and enhancing lipid peroxidation **[43]** which might contribute to the pathogenesis of hirsutism. Pb has been associated with endocrine disruption **[44]**, possibly affecting hormone levels in Hirsutism patients

The observed correlations between blood metal concentrations highlight complex interactions among these elements in Hirsutism subjects. Positive correlations of blood Cu with blood Se and Zn suggest potential synergistic effects or shared metabolic pathways. Conversely, the negative correlations of blood Cu with blood Pb, Cd and As hint at antagonistic relationships that might influence androgenic hormone regulation and thus hirsutism. These intricate associations underscore the need for comprehensive studies investigating the interplay of multiple metals in Hirsutism development. These alterations in trace metals and heavy metals levels may contribute to a combination of endocrine (androgen) disruption and metabolic dysregulation, and thus consequential to Hirsutism.

***Limitations:*** However, the authors acknowledge that the study sample size (n=40 cases, n=40 controls) is relatively small, and was due to absence of grant (sponsorship). A larger sample size should be applied in future studies.

**Conclusion**

High blood levels of Pb, Cd and As parallels low levels of blood Zn, Cu and Se in young adult females with hirsutism. The alterations in the blood levels of these heavy and trace metals may in part be associated with the development of hirsutism in this environment.

Disclaimer (Artificial intelligence)

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.

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