Original Article

# Serum Copper, Zinc and Selenium Levels in Women with Unexplained Infertility in Ibadan Nigeria: A Cross-sectional Analytical Study

**Adewale Ayodeji Adeniyi, Olayinka Oladunjoye Ogunbode[1], Ayodeji Sylvester Adeyanju, Adesina Oladokun[1]**

Department of Obstetrics and Gynaecology, University College Hospital, [1]Department of Obstetrics and Gynaecology, College of Medicine, University of Ibadan, Ibadan, Nigeria

## Abstract

**Background:** Infertility is a global public health issue affecting couples. Trace metals have been implicated in effective reproductive functions in males but less studied in females. **Objective:** To compare the serum levels of copper (Cu), zinc (Zn), selenium (Se) and copper/zinc ratio in women with unexplained infertility and fertile women. **Subjects and Methods:** This was a cross-sectional analytical study that compared 75 consenting women who had unexplained infertility with 74 fertile women that were controls. Both groups were seen within 1 year of delivery and were recruited from the family planning unit, at the University College Hospital, Ibadan. Data were obtained through a semi-structured questionnaire, after which 10 mL of venous blood was collected. Analysis of selected trace elements were done by atomic absorption spectrophotometry. IBM SPSS version 23 was utilized for data analysis and the levels of statistical significance was set at <0.05. **Results:** The mean (± SD) serum concentrations of Cu (93.11 ± 16.55 µg/dL), Zn (72.04 ± 15.03 µg/dL) and Se (28.28 ± 8.33 µg/dL) amongst the women with unexplained infertility were lower when compared to the control group (all with $P < 0.001$). The serum Cu/Zn ratio was higher among the fertile women, though not statistically significant ($P < 0.62$). Age of <35 years was associated with normal serum levels of Cu ($P < 0.01$), while women with normal body mass index had low serum concentrations of Cu ($P = 0.04$), amongst the fertile group. **Conclusion:** Serum copper, zinc and selenium concentrations are significantly lower in women with unexplained infertility, therefore diets or supplements containing these trace elements may be helpful in their management.

**Keywords:** Copper, selenium, trace elements, unexplained infertility, zinc

## Introduction

Microelements are substances needed in small quantities for effective functioning of the body. They play a central role in the metabolism and maintenance of tissue functions.[1-4] Microelements comprises of vitamins and minerals such as calcium, zinc, selenium, copper and iron.[5,6]

Zinc (Zn) and copper (Cu) are the second and third most abundantly distributed trace elements in the body, respectively,[7] though not synthesised in the body, but are obtained from dietary sources.[8,9] Copper plays a major role in the biochemistry of living organisms through enzymatic activities both as a cofactor and as an integral component of many metalloenzymes, while Zn functions by multiple pathway, serving as a cofactor in DNA transcription, protein synthesis and regulating gene expression.[7,10] Selenium is an essential micronutrient found in soil, water, and some foods and is vital for functioning of many body processes.[9,11] It plays a vital role in the oxidative processes in the humans and a few researchers have reported low selenium level in women with infertility.[9,11-13]

Infertility, which is often a disturbing issue for couples, had been documented to be more prevalent in developing countries, especially secondary infertility, where an estimated one in every four couples are affected,[14,15] though primary infertility

**Address for correspondence:** Dr. Olayinka Oladunjoye Ogunbode, Department of Obstetrics and Gynaecology, College of Medicine, University of Ibadan, Ibadan, Nigeria.
E-mail: jamchose@yahoo.co.uk

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is more common in males in some studies in Nigeria.[16] The term unexplained infertility is used when no identifiable cause is found on evaluation of the infertile couple and is dependent on the available facilities at the place of care.[17]

Copper, zinc and selenium deficiencies have been associated with infertility and a few other studies have reported low serum levels of zinc and higher serum levels of copper amongst women with unexplained infertility.[1,9,18-20] The Cu/Zn ratio was also higher suggesting a role in the pathogenesis of unexplained infertility.[19] However, most of these studies are in developed countries and very few in Nigeria, where infertility is the most common presentation in our gynaecological outpatient departments,[21] so this study was designed to evaluate the serum levels of copper, zinc and selenium in women with unexplained infertility and compare these with fertile women.

## Subjects and Methods

Ethical approval was obtained on 15 August 2019 from the University of Ibadan, Ibadan, and University College Hospital, Ibadan, Institution Research Board (UI/UCH/IRB). The ethical committee's assigned number is UI/EC/19/0233 and informed consent was obtained from all participants before enrolment into the study.

This was a prospective cross-sectional analytical study conducted at the UCH Ibadan, Nigeria, which is a tertiary health facility in Ibadan, the largest city in Oyo state. UCH Ibadan is Federal Government-owned and provides inpatient and outpatient care to people in the South-West, Nigeria, though many patients also come from the other regions of Nigeria. It is a 1000 bedded hospital and comprises of over 80 departments among which is the department of Obstetrics and Gynaecology where the study was conducted.

The study included women presenting at the gynaecological out-patients department of the hospital with infertility for at least one year duration and with diagnosis of unexplained infertility. The control group were non-pregnant women attending the family planning clinic at the study site before the uptake of any contraception of their choices. Non-consenting women and non-pregnant women in the puerperium or who are still breast feeding were excluded from the study.

The sample size was calculated using the formulae for comparison of two means.[22]

$$N = \frac{(1+r)}{r}\frac{(Z_{1-\alpha/2}+Z_{1-\beta})^2}{d^2}+\frac{Z^2_{1-\alpha/2}}{2(1+r)}$$

$$d=\frac{\mu_2-\mu_1}{\sigma}$$

where $N$ is the estimated sample size for each group, $\alpha$ is the confidence level = 0.05, $Z_{1-\alpha/2}$ is the abscissa of the normal curve that cuts off an area $\alpha$ at the tails = 1.96, $\beta$ is the power of the test at 80% = 0.2, effect size ($d$) is 0.5 and $r$ is the ratio (Group 2/Group 1).

$$N = \frac{(1+1)}{1}\frac{(1.96+0.84)^2}{0.5^2}+\frac{1.96^2}{2(1+1)}$$

$=2\,(2.8)^2/0.25 + 3.8416/4$

$=2\,(7.84)/0.25 + 0.9604$

$=15.68/0.25 + 0.9604$

$=62.72 + 0.9604$

$=63.68$

Approximately: 64 participants per group.

10% Attrition = 10/100 × 64 = 0.1 × 64 = 6.4

Total = 63.68 + 6.40 = 70.08

The minimum estimated number of participants in each group was 70 after adding a 10% rate for attrition.

The non-probability purposive consecutive sampling technique was used in the selection of study participants. Women with infertility and normal hormonal profile, normal hysterosalpingograph or laparoscopic findings and normal findings on seminal fluid analysis were classified as unexplained infertility. There were two groups of participants, with the cases consisting of 75 participants and 74 women as controls.

Relevant information was obtained from the women using interviewer-administered semi-structured questionnaire. Ten millilitres of venous blood sample was collected once from the consenting participants at the point of recruitment to participate in the study. No other invasive procedure was carried out on the participants and the blood samples were stored at in a refrigerator at −20°C until they were analysed.

Determination of copper, zinc and selenium was the atomic absorption spectrophotometry (AAS) method.[23,24] Frozen serum bottles were placed in bottle racks and allowed to thaw; 0.1 N hydrochloric acid (1:20) was then added to the thawed sample to release bound trace metals. The digested samples were aspirated directly into the AAS. Working standard solutions were prepared in part per million and used for the standardisation of the corresponding trace elements. These analyses were performed using 210/211 VGP and 220 GF AAS (Buck Scientific, USA). Copper level was read at wavelength of 324.8 nm, zinc at 213.9 nm and selenium at 196 nm. The normal level serum copper, zinc and selenium for this study was 85–180 µg/dL, 66–110 µg/dL and 50–120 µg/dL, respectively.

All information obtained and data generated were imputed to Microsoft Excel sheet and exported into the IBM Statistical Package for Social Sciences (SPSS) Statistics for Windows, Version 23.0, (Armonk, NY, USA: IBM Corp.), Descriptive statistics was performed using frequency tables and calculation of means and standard deviations. Chi-square and *t*-test for associations were performed as appropriate, and with the statistical significance level set at $P < 0.05$.

## Results

Most (57.0%) of the participants were in the age group 30–39 years. Majority of the participants were Christians (77.6%) and had at least the tertiary level of education (80.5%). The participants with an overweight body mass index (BMI) (38.3%) were the highest, with an equal proportion (29.5%) in the normal and obese BMI. The mean age in the fertile and infertile group was 36.28 ± 6.13 and 34.15 ± 5.14, respectively. The mean BMI amongst the fertile and infertile group was 28.72 ± 5.52 and 26.51 ± 6.11, respectively. Amongst the infertile group, forty-eight participants (64.0%) had secondary infertility, while slightly over half of them (51.6%) were in a union of <5 years. This is shown in Table 1.

The parameters were similar amongst the two groups in terms of level of education, religion and BMI. Amongst the infertile group, most were nulliparous (49.4%) and 64 (85.3%) had no living children alive, while amongst the fertile group, most had at least two parous experiences (78.3%) and nearly all of them (97.7%) had at least a child alive. These observations were statistically significant with $P < 0.001$ and <0.001, respectively.

The mean serum copper, zinc and selenium levels in µg/dL amongst the infertile group were 93.11 ± 16.55, 72.04 ± 15.03 and 28.28 ± 8.33, respectively, and these were statistically significantly lower than the control group ($P \leq 0.001$). Regarding the copper-to-zinc ratio amongst the two groups, there were a slightly higher mean level (1.39 ± 1.37) amongst the control group; however; this is not statistically significant ($P = 0.62$). This is shown Table 2.

Based on whether the participants had primary or secondary infertility, the mean serum level of copper, zinc and selenium was lower amongst those with primary infertility and with values of 91.13 ± 15.93, 71.52 ± 15.20 and 27.08 ± 8.26, respectively, compared with 94.16 ± 16.94, 72.32 ± 15.09 and 28.92 ± 8.38 in those with secondary infertility, respectively. However, these differences in serum levels of copper, zinc and selenium were not statistically significant, with $P = 0.45$, 0.83 and 0.37, respectively.

Table 3 shows the associations between age and BMI with the serum levels of copper, zinc and selenium amongst the fertile and infertile participants. The statistically significant associations were amongst the fertile group and with serum copper. More participants in the age group <35 years had

**Table 1: Sociodemographic characteristics of the study participants**

| Variable | Fertile group (*n*=74) | Infertile group (*n*=75) | Total | $\chi^2$ | *P* |
|---|---|---|---|---|---|
| Age (years) | | | | | |
| <20 | 1 (1.4) | 0 | 1 (0.7) | 11.81 | 0.008 |
| 20–29 | 6 (8.1) | 20 (26.7) | 26 (17.5) | | |
| 30–39 | 43 (58.1) | 42 (56.0) | 85 (57.0) | | |
| ≥40 | 24 (32.4) | 13 (17.3) | 37 (24.8) | | |
| Highest educational status | | | | | |
| None | 3 (4.1) | 1 (1.3) | 4 (2.7) | 1.99 | 0.574 |
| Primary | 3 (4.1) | 2 (2.7) | 5 (3.4) | | |
| Secondary | 8 (10.8) | 12 (16.0) | 20 (13.4) | | |
| Tertiary | 60 (81.0) | 60 (80.0) | 120 (80.5) | | |
| Religion | | | | | |
| Christianity | 62 (83.8) | 54 (72.0) | 116 (77.6) | 3.63 | 0.162 |
| Islam | 11 (14.8) | 17 (22.7) | 28 (18.8) | | |
| Others | 1 (1.4) | 4 (5.3) | 5 (3.4) | | |
| BMI (kg/m$^2$) | | | | | |
| <18.0 | 1 (1.4) | 3 (4.0) | 4 (2.7) | 2.65 | 0.452 |
| 18.0–24.9 | 19 (25.7) | 25 (33.3) | 44 (29.5) | | |
| 25.0–29.9 | 29 (39.2) | 28 (37.4) | 57 (38.3) | | |
| ≥30.0 | 25 (33.7) | 19 (25.3) | 44 (29.5) | | |
| Parity | | | | | |
| 0 | 1 (1.4) | 37 (49.4) | 38 (25.5) | 55.57 | <0.001 |
| 1 | 15 (20.3) | 19 (25.3) | 34 (22.8) | | |
| 2–4 | 48 (64.9) | 18 (24.0) | 66 (44.3) | | |
| ≥5 | 10 (13.5) | 1 (1.3) | 11 (7.4) | | |
| Number of living children | | | | | |
| 0 | 2 (2.7) | 64 (85.3) | 66 (44.3) | 104.67 | <0.001 |
| 1 | 27 (36.5) | 7 (9.3) | 34 (22.8) | | |
| 2 | 24 (32.4) | 1 (1.3) | 25 (16.8) | | |
| 3 | 13 (17.6) | 2 (2.7) | 5 (10.1) | | |
| ≥4 | 8 (10.8) | 1 (1.3) | 9 (6.0) | | |

BMI: Body mass index

normal serum copper ($P < 0.01$) and those with normal BMI had low serum copper ($P = 0.04$).

## Discussion

In this study, the sociodemographic characteristics of the participants were comparable. The infertile participants had lower serum level of copper compared to the fertile participants and this difference is significant statistically. This finding is similar to a case–control study in Ireland and another in India where the researchers reported a significantly lower level of copper amongst the infertile participants.[18,25] However, a few other studies in some other had reported higher serum levels of copper amongst infertile women, though many of them had smaller sample sizes.[4] Since the women were recruited within 1 year of birth, their nutritional status may be better, as diet is a major source of copper in the body and this may not be sustained later on in life. Furthermore, though the mean copper level of the infertile women was still within the normal limit of 85–180 µg/dL, it is possible that the minimal threshold of copper necessary for effective and optimal enhancement of fertility is above the mean for this studied group and this may be the reason for the infertility.

This study also reported a significantly lower serum levels of zinc amongst infertile women than in the fertile control group, which is similar to findings in a study in Iraq.[1] Though another study in India reported a different findings of higher

**Table 2: Comparison of serum copper, zinc and copper/zinc ratio with the fertility status among the study participants**

| Variable | Mean±SD (µg/dL) | | *t*-test | *P* |
|---|---|---|---|---|
| | Fertile group | Infertile group | | |
| Copper | 144.70±18.83 | 93.11±16.55 | 17.77 | <0.001 |
| Zinc | 103.8±13.71 | 72.04±15.03 | 13.46 | <0.001 |
| Copper/zinc | 1.39±1.37 | 1.29±1.10 | 0.49 | 0.621 |
| Selenium | 55.41±7.42 | 28.28±8.33 | 20.93 | <0.001 |

SD: Standard deviation

**Table 3: Associations of age and body mass index with serum levels of copper, zinc and selenium among the fertile and infertile group**

| Variable | Fertile group | | | Infertile group | | |
|---|---|---|---|---|---|---|
| | Low copper, *n* (%) | Normal copper, *n* (%) | $\chi^2$ (*P*) | Low copper, *n* (%) | Normal copper, *n* (%) | $\chi^2$ (*P*) |
| Age (years) | | | | | | |
| <35 | 7 (21.1) | 26 (78.9) | 9.23 (<0.01) | 22 (66.7) | 11 (33.3) | 0.05 (0.83) |
| >35 | 23 (56.1) | 18 (43.9) | | 27 (64.3) | 15 (35.7) | |
| BMI | | | | | | |
| <25.0 | 6 (30.0) | 14 (70.0) | 6.49 (0.04) | 19 (67.8) | 9 (32.2) | 3.94 (0.14) |
| 25–29.99 | 17 (58.6) | 12 (41.1) | | 21 (75.0) | 7 (25.0) | |
| >30 | 7 (28.0) | 18 (72.0) | | 9 (47.4) | 10 (52.6) | |
| Total | 30 (40.5) | 44 (59.5) | | 49 (65.4) | 26 (34.6) | |

| Variable | Fertile group | | | Infertile group | | |
|---|---|---|---|---|---|---|
| | Low zinc, *n* (%) | Normal zinc, *n* (%) | $\chi^2$ (*P*) | Normal zinc, *n* (%) | High zinc, *n* (%) | $\chi^2$ (*P*) |
| Age (years) | | | | | | |
| <35 | 15 (45.5) | 18 (54.5) | 2.07 (0.15) | 24 (72.7) | 9 (27.3) | 0.97 (0.32) |
| ≥35 | 12 (29.3) | 29 (70.7) | | 26 (61.9) | 16 (38.1) | |
| BMI | | | | | | |
| <25.0 | 7 (35.0) | 13 (65.0) | 1.00 (0.61) | 20 (71.4) | 8 (28.6) | 1.85 (0.40) |
| 25–29.99 | 9 (31.0) | 20 (69.0) | | 16 (57.1) | 12 (42.9) | |
| >30 | 11 (44.0) | 14 (56.0) | | 14 (73.7) | 5 (26.3) | |
| Total | 27 (36.5) | 47 (63.5) | | 50 (66.7) | 25 (33.3) | |

| Variable | Fertile group | | | Infertile group | | |
|---|---|---|---|---|---|---|
| | Low selenium, *n* (%) | Normal selenium, *n* (%) | $\chi^2$ (*P*) | Low selenium, *n* (%) | Normal selenium, *n* (%) | $\chi^2$ (*P*) |
| Age (years) | | | | | | |
| <35 | 4 (12.1) | 29 (87.9) | 1.79 (0.18) | 32 (97.0) | 1 (3.0) | 0.03 (0.86) |
| >35 | 10 (24.4) | 31 (75.6) | | 42 (100.0) | 0 | |
| BMI | | | | | | |
| <25.0 | 4 (20.0) | 16 (80.0) | 0.09 (0.96) | 28 (100.0) | 0 | 0.11 (0.95) |
| 25–29.99 | 5 (17.3) | 24 (82.7) | | 27 (96.4) | 1 (3.6) | |
| >30 | 5 (20.0) | 20 (80.0) | | 19 (100.0) | 0 | |
| Total | 14 (18.9) | 60 (81.1) | | 74 (98.7) | 1 (1.3) | |

BMI: Body mass index

numerical serum level of zinc in fertile than in infertile women, there was no significant statistical difference, and this could have been because of the small sample size.[18] Furthermore, other studies have also reported that there was no significant difference in zinc level between fertile and infertile women; nonetheless, the finding of a significant difference in this study may be because this study has a larger sample size than these other studies, thus giving it a higher power. Furthermore, all these authors studied women in different population compared to Nigerian women in this study and nutritional diversity may be an important factor to consider as possible explanation for the dissimilarities of the research finding. In addition, the absorption and metabolism of trace elements may significantly vary from person to person.

In this study, in addition, infertile women had significantly lower serum levels of selenium compared with fertile women, and low level of micronutrients including selenium in women with unexplained infertility has been reported, similar to the finding in this study.[2] Szkodziak *et al*. also reported low serum levels of selenium in the blood and follicular fluid of women with infertility, especially unexplained infertility and these findings are in keeping with the findings in this study.[26] Apart from the infertile women having low mean selenium level, their average selenium level is lower than the lower limit of 50–120 μg/dL, the normal range reported in humans.

It is well known that diet is the main source of micronutrients for the body; however, it is a little challenging to conclude that low dietary intake accounted for the lower levels of the studied elements in the infertile respondents because they live in the same geographical area with the fertile women who had higher micronutrients level.[27] Therefore, there are possibly other factors that could explain low micronutrient levels amongst the infertile women which may include poor absorption in the gut amongst other factors. However, very obvious in this study is the fact that copper, zinc and selenium levels are significantly lower in infertile women with unexplained infertility than in fertile women.

Amongst the cohort of women with infertility, when separated to primary and secondary infertility, there was no statistically significant difference in the serum levels of copper ($P = 0.45$), zinc ($P = 0.83$) or selenium ($P = 0.37$), though Soltan and Jenkin reported a significantly lower level of copper amongst women with secondary infertility than those with primary infertility.[18]

Copper/Zinc ratio (Cu/Zn) has been used frequently in assessing infertile women, with normal ratio said to be about 1.0. The Cu/Zn ratio in the two groups studied were slightly raised, 1.29 for infertile women and 1.39 for fertile women ($P = 0.60$) but not statistically significant, however Khulood *et al*. reported a much higher Cu/Zn ratio in infertile women than in fertile women among their studied population.[1]

In our study, the statistically significant associations were with serum levels of copper and only amongst the fertile participants. More participants in the age group <35 years had normal serum copper ($P < 0.01$) and this is the age group that has been documented with the peak age of fertility as fertility declines from around the age >35 years.[28,29] This may imply that the effect of age on fertility may be due to the serum levels of copper amongst other factors. This study further showed that serum copper level was significantly lower in participants with normal BMI ($P = 0.04$), though the reason for this was not known but it is hoped that in future studies with larger sample size, the reason may be explained.

This study has some limitations in that it is cross-sectional, was conducted over a short period and the assays were taken once per patient. It would have been useful to assay their serum copper and zinc level again to see if it has normalised, but this study could not achieve this purpose. Having limited studies of assay of serum levels of copper, zinc and selenium amongst Nigerian infertile women makes it difficult to discuss the findings in this study by lack of comparison with similar populations. However, the strength of this study is that it will contribute to the data on levels of micronutrients amongst women with unexplained infertility in a low-resource setting like Nigeria.

# Conclusion

This study has demonstrated that there is significantly reduced levels of copper, zinc and selenium in women with unexplained infertility than in their fertile counterparts, therefore diets or supplements containing copper zinc and selenium is recommended for women with unexplained infertility.

## Data availability statement

Most of the data supporting the results had been published as a dissertation for Fellowship of the Faculty of Obstetrics and Gynaecology of the National Postgraduate Medical College and we are willing to share the data on request.

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## Conflicts of interest

There are no conflicts of interest.

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