**ORIGINAL ARTICLE**

# Evaluation of Zinc and Copper Levels in Sudanese Patients with Celiac disease in Red Sea State

HAIFA IDRIS[1], AMNAO.M. ELZEIN[2], HUSHAM O ELXEIN[3]
[1]*Faculty of Medical laboratory Sciences, Al zaiem Al Azhari University, Khartoum, Sudan*
[2]*Department of clinical chemistry, faculty of Medical laboratory Sciences, Al zaiem Al Azhari University-Sudan.*
[3]*Faculty of medical laboratory science, University of Science and Technology, Khartoum, Sudan*
*Correspondence to Dr. Haifa Idris, Email: Email: hushamelzein@hotmail.com, Tel: 00966544450313*

## ABSTRACT

**Background:** Celiac disease (CD) is a small bowel enteropathy, aspects about 1% of the total population. It is initiated by immune response to gluten. Untreated treated celiac disease may lead to serious complications. Copper and Zinc are a crucial trace elements serve as cofactor for several oxidase enzymes, and plays a key role in cellular metabolism.
**Aim:** To evaluate the association of Copper and Zinc with the improvement of celiac disease.
**Methods:** This is a case control study was conducted in Red sea State, Sudan. From September 2018 to September 2019. The study enrolled 40 patients with celiac disease (Case group) and 40 healthy individual (control group). Zinc and copper measured by using flame Atomic Absorption Spectrometer. Anti-tissue Trans glutamate IgA has been determined by using Enzyme-linked Immunosorbent assay (ELISA) according to the manufacturer's protocol. Data were entered and analyzed by means of SSPS(version 16.0).
**Results:** The results showed a significant decrease in zinc level in case group 0.285±.1776 than 1.0±0.245 in control group, p. value = 0.000. The study also revealed a significant decrease in copper levels in case group 0.612±.279 in comparison with control 0.7 ±0.279, P .value = 0.010. It was also found that there were negative correlation between Zinc, Copper levels and anti-tissue trans gludine IgA (r = – 0.970, r =– 0.870, P=0.000 respectively.
**Conclusion:** This study revealed that serum zinc and copper levels are decreased in patients with celiac disease.
**Keywords:** Celiac disease; gluten; Zinc; Copper; Sudan

## INTRODUCTION

Celiac disease (CD) is a small bowel enteropathy initiated by an immune reaction in response to gluten intake[1]. The regular exposure to gluten can cause dual effects, through activating both innate and adaptive immune responses, which mayinitiate various patterns of symptoms at the intestinal and extra-intestinal levels [2]. Untreated or incompletely treated celiac disease may lead to serious complications including loss of absorptive surface area and secondary nutritional deficiencies, osteoporosis, infertility, increased malignancies; also result in abundant cases of neurological disorders. Beside that CD can increase the mortality rate; therefore, it has been recommended that patients with CD have to visit the clinic regularly to avoid substantial complications [3-10]. The cases of CD are relatively common and can affects about 1% of the population universally; the prevalence has been increasing in the last years, with noticeable rates over the previous 50 years[11,12,13,14,15]. Copper is a crucialtrace element it serves as a cofactor for several vital oxidase enzymes, so it plays an essential role in the cellular transporters. Copper also has an important role in the normal growth and development of the human body. Copper is a vital element for the cellular physiology and metabolism including hemoglobin synthesis, antioxidant activity, cellular respiration, pigment formation, and hematopoiesis. [16-19] .Copper deficiency may take place in the cases of zinc poisoning, gastric bypass surgery, and malabsorption conditions. It can be apparent in various neurologic and hematologic defects [20]. Zinc is an important mineral required by the human body. It's naturally occurring in some foods, and also available in the form of dietary supplements[21]. Zinc plays a key role in several parts of cellular metabolism in our bodies, therefore it's vital for the catalytic activity of nearly 100 enzymes also zinc plays a key role in growth development and immune system function[19,21]. Transglutaminase (TG) is an enzyme of nine different types, it plays a role in the pathological and physiological changes of the tissues through keratinocyte differentiation process, hence contribute to the cutaneous barrier function. TG2 has a role in the deamination of the bonds in gluten. Antibodies against other TGs types have been identified in extra intestinal forms of gluten associated conditions[22,23,24]. .In Sudan, there's rarely published data concerning trace element particularly copper and zinc levels and their association with celiac disease. So within the current study, we are tried to evaluate and highlight the particular role of those trace elements in the development of celiac disease among Sudanese patients.

## MATERIALS AND METHODS

This is a case control study was conducted in Red sea State, Sudan. During the length of September 2018 to September 2019.The total measured sample size was 80 which sub divided into two groups (Case group which included 40 patients with celiac disease and control group involved 40 healthy individual).

Patients with any history of diseases that could affects the levels of serum copper or zinc such as malignant diseases, hepatitis, renal failure, burn, rheumatoid arthritis, tuberculosis, genetic disease were excluded from the study. After all the participants signing the informed

consent, medical history was taken from each group (cases and controls) using structured questionnaire.

5ml of venous blood was taken under septic condition from the case and control groups, the whole blood samples allowed to clot, centrifuged for 3 minutes at 3000 rpm to obtain serum and then stored at (−20 C) till the assay of zinc and copper by using flame Atomic Absorption Spectrometer (Buck Scientific Model 210 VGP, 601 E Main St, Collegeville, PA 19426, United States).Anti-tissue Trans glutamate IgA has been determined for the participants by using

Enzyme-linked Immunosorbent assay (ELISA) according to the manufacturer's protocol. Sample size of 40 women in each group of the study was calculated using a formula for the difference in the mean of (Zinc and copper concentrations) that would provide 80% power to detect a 5% difference at α = 0.05 and which assumed that 10% of the participants would not respond.

**Statistical analysis:** Data have been entered, analyzed by means of the usage of SSPS for windows (version 16.0).Data were compared between the two groups (cases and controls) using a *t*-test. The mean and standard deviation of zinc, copper levels were showed in form of tables. A *P value* of <0.05 was considered significant.

**Ethics:** Ethical approval was obtained from the Research Board at the Faculty of medical laboratory science, Alzaiem Alazhari University, Khartoum, Sudan.

## RESULTS

The study enrolled 40 patients (22 (55%) males, and 18 (45%) females) with celiac disease as case group and 40 individual, (healthy and gender matched) as control. In this study there was significant decrease of zinc level in case group than in control group (Table. 1). The study also revealed a significant decrease in copper levels in case group in comparison to control group as shown in Table 1. Regarding anti-tissue Trans glutamate IgA test the study showed a significant increase in case group than in control group (Table 1). Table 2 revealed significant decrease in zinc and copper levels in Celiac disease male patient than females (p. value = 0.00) in both groups. It was also found that there was negative correlation between zinc levels and anti-tissue transgludine IgA (Scatter. 1). There was a negative correlation in case group between copper level and levels of anti-tissue transgludine IgA (Scatter. 2). Negative correlation(r = –0.760, P = 0.000) also occurred between copper level and duration of disease per years.

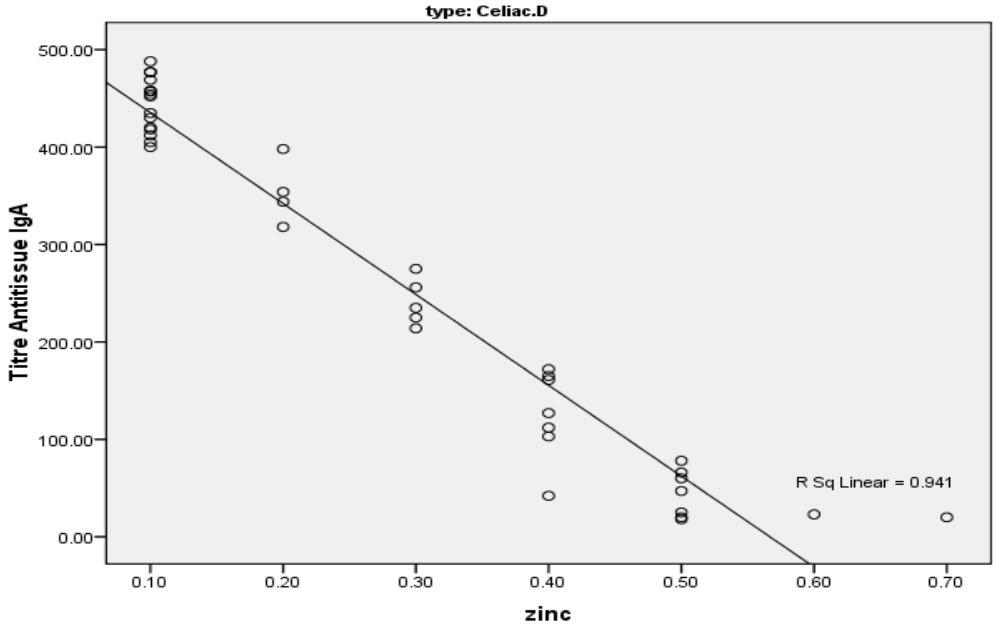
Table 1: Comparison of mean ± stander deviation in case and control group. For level of zinc and copper

| Parameters | Test group (n=40) | Control group (n=40) | P value |
|---|---|---|---|
| Zinc (mg/l) Mean ±SD | 0.285±0.1776 (0.1-0.7) | 1.0±0.245 (0.7-1.6) | 0.000 |
| Copper (mg/l) Mean ±SD | 0.612 ± 0.279 (0.3—1.4) | 0.7 ±0.279 (0.3—1.6) | 0.01 |
| Anti-Tissue-Transglutaminase (IgA) Mean ±SD | 170.82 ±170EU/mL | 5.35±2.75 EU/mL | < 0.05 |

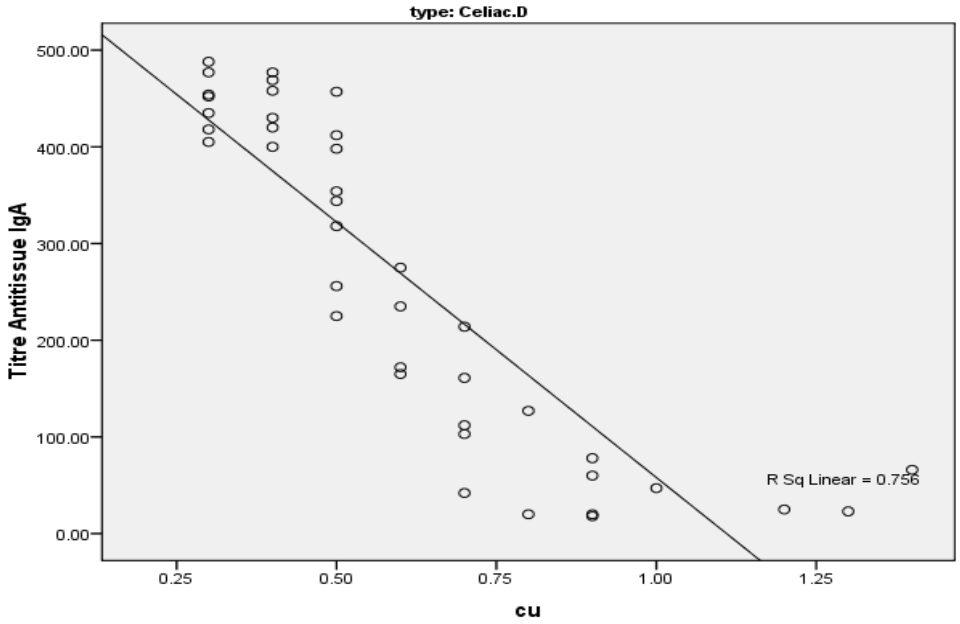
Table 2: Statistics and mean differences of Zn and Cu among gender in case groups

| Parameter | Male (22) | Female (18) | P .value |
|---|---|---|---|
| Zn mg/l | 0.15 ± .08 | 0.45±0.11 | 0.00 |
| Cu mg/l | 0.43±.12 | 1.70±.33 | 0.00 |

Scatter 1: Shows the correlation between the serum zinc level and the level of Anti tissue transglidine IgA (*r* = -0.970, P = 0.000)



Scatter 2: Shows negative correlation between the serum copper level and the level of Anti tissue transglidine IgA (*r* = - 0.870, P = 0.000)




## DISCUSSION

Copper and zinc are important elements for the human body. Therefore, their deficiency may lead to complications such as hemoglobin synthesis defects, abnormal hematopoiesis, immune system defects and growth retardation [25]. In the current study, we found a significant decrease in the mean levels of zinc in patients with celiac disease (0.285±0.1776) in comparisonwith the mean level of control group (1.0±0.245, P-value = 0.000), this finding is consistent with other previous studies such as the study of FaribaFathi et al in Tehran 2013[26] who reported that the mean levels of Zinc in patients with celiac disease were significantly lower than control group (75.97±12 compared with 92.83±18, P-value < 0.0001).Another study matching our finding was performed by Singhal N et al 2008 [27] who revealed that serum zinc levels of newly diagnosed celiac disease were significantly low (Cases 0.64±0.34 m*g/mL* versus controls (0.94±0.14 mg*/mL*, 95% CI -0.44 to -1.4). The variations of zinc level are reported in another previous study performed by Adam C et al study 2019 [28] who revealed that Zinc level was decreased in 59.4% (126/212) of cases with celiac disease compared with 33.2% (205/618) of controls *P*<.001 and this result agrees with our finding. Regarding copper levels. The study confirmed that the level of copper is significantly decreased in the case group (0.612±.279) when compared with the control group (0.7 ±0.279, P-value = 0.000). An equivalent results were obtained by Adam C. Bledsoe et al 2019 [28] who showed that copper concentration was significantly reduced in celiac patients 6.4% (13/204) compared with the control group 2.1% (13/618) P =.003. Although the present study determined the copper and zinc deficiency in celiac disease, the results still controversial and there have beensome limitations such as small sample size that should be amplified to achieve recommended results. Besides that the current study is hospital-based so its results don't reflect the overall population level; therefore further researches are needed to clear up any issues.

## CONCLUSION

This study revealed that serum zinc and copper levels are decreased in patients with celiac disease, therefore patients should be monitored continuously to verify sufficient control for the disease.

**Conflict of Interest:** The authors declare that they have no conflict of interest

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