

Original article

# Serum Zinc Levels in Celiac Paediatric Patients on Gluten Free Diet

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## ARTICLE INFO

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## ABSTRACT

Celiac disease is an autoimmune disorder with both genetic and environmental factors involved in its pathogenesis. It characterizes by small intestinal atrophy and malabsorption of essential nutrients, vitamins and trace elements due to ingestion of gluten. Zinc is an essential trace element for maintaining integrity of intestinal mucosa, immunity, and growth in children. Therefore, the aim of this study is to evaluate serum zinc levels in celiac paediatric patients on Gluten Free Diet (GFD) and compares it with healthy children. Sera of 22 celiac paediatric patients on GFD and 16 healthy children as a control group were obtained. The samples were prepared and zinc level was estimated via atomic emission spectrophotometer. Ninety one percent of celiac children on GFD had low zinc. The median plasma zinc concentration was significantly lower in celiac children on GFD (0.20 µg/ml) versus controls (0.86 µg/ml) with *p* value of < 0.0001. The current study shows that serum zinc concentrations are decreased significantly in celiac paediatric patients on GFD, suggesting zinc deficiency. Thus, good dietary guidance, zinc supplement therapy, and regular monitoring of zinc levels are highly recommended for celiac children on GFD.

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## INTRODUCTION

Celiac disease (CD) is a chronic immune-mediated enteropathy characterized by specific serological and histological findings that triggered by ingestion of gluten and related prolamines [1-2]. In recent years, there are significant changes in natural history, pathogenesis, and diagnosis of disease. The estimated prevalence is around 1% in general population; with female predominance [3-4]. Studies have demonstrated that prevalence is approximately 1:250 in Sweden, 1:524 in Denmark, 1:333 in Holland, 1:250 in USA, and 1:681 in Brazil [5-6]. Celiac disease is also common in Libyan children as in Europe, affecting around 1% of the general population [7]. High prevalence has been reported in first-degree relatives (10%), and high-risk groups, namely autoimmune disorders e.g type I diabetes mellitus (IDDM), selective IgA deficiency, addison disease, autoimmune thyroiditis, autoimmune hepatitis, and chromosomal diseases e.g. Down syndrome, Turner syndrome, and Williams syndrome [4,8-9]. CD results from environmental (gluten) and genetic factors, such as HLA class II: DQ2 or DQ8 and non-HLA genes in genetic susceptible individuals [10-13]. Gluten is a protein complex consisting of glutenin and gliadin proteins, which involves in T-cell mediated immune response [14]. It is found in wheat, barley, rye, and oat [15-16]. The clinical phenotypes are extremely variable, and include classic and non-classic gastrointestinal symptoms, extra-intestinal symptoms (atypical type), and asymptomatic (silent type) [17-19]. In children, symptoms usually appear between 4-24 months after ingestion of gluten containing grains [20]. The gastrointestinal symptoms include chronic diarrhea (steatorrhea), abdominal distension, vomiting, recurrent abdominal pain, and constipation. The extra intestinal or atypical symptoms, such as short stature, chronic iron deficiency anemia, osteoporosis, dermatitis herpetiformis, neurological problems, dental enamel defect, hypogonadism

and delayed puberty [21-22]. Diagnosis of CD depends on combination of clinical symptoms, positive celiac antibodies, presence of HLA-DQ2/DQ8, and duodenal histological findings [23]. Accordingly, the updated European Society for Pediatric Gastroenterology, Hepatology and Nutrition guidelines (ESPGHAN) for diagnosis of celiac disease in children and adolescents, 2020 has recommended that measurement of transglutaminase 2 (TGA-IgA), and total IgA antibodies is an initial screening test for suspected celiac disease. The TGA-IgA has a high sensitivity and specificity, and costly effective [24]. Analysis of deamidated gliadin peptide (DGP) IgA is recommended for children under 2 years of age, and measurement of TGA-IgG or EMA-IgG or DGP-IgG should be performed in children with IgA deficiency. If serological tests are negative for TGA-IgA and total IgA level is normal, so celiac disease is unlikely [24]. Furthermore, if TGA-IgA is positive, and less than 10 times upper limit of normal (ULN), gastroduodenoscopy and multiple duodenal biopsies should be performed to confirm the diagnosis. However, if TGA-IgA value is  $\geq 10$  times ULN, so diagnosis is confirmed and no need for duodenal biopsy, provided positive endomysial antibodies (EMA-IgA) in a second blood sample [24].

At present, the only effective treatment is a lifelong gluten-free diet (GFD). There is a significant improvement in symptoms, quality of life, and normalization of biochemical tests on a strict GFD [25]. CD affects mainly the proximal small intestine. The small intestine has a crucial role for maintaining zinc homeostasis. Recently, zinc has emerged as an important micronutrient that supports immunity, and normal growth during childhood. Short stature, poor appetite, delay sexual maturation and hypogeusia have been demonstrated in children with zinc deficiency [26]. In CD children, zinc deficiency may result from poor oral intake, a cumulative loss of insoluble zinc complexes with fat and phosphate, exudation of zinc protein complexes into the intestinal lumen and massive loss of intestinal secretions or impaired zinc absorption because of damaged intestinal epithelial cell membrane [27-28]. Studies have showed that zinc deficiency is an important issue in children with CD [29]. Additionally, literatures have demonstrated that serum zinc levels are low in newly diagnosed celiac patients and rise with GFD irrespective of zinc supplementation [30]. Thus, the aim of present study is to investigate serum zinc levels in celiac paediatric patients on GFD and compared it with healthy children.

## METHODS

### *Study population and design*

The study was conducted as a clinical case control study at Medical Misurata Center (MMC). It was carried out on pediatric celiac patients on GFD, who aged up to 18 years old, and came the pediatric gastroenterology follow-up clinic, over a period of nine months from 1st December 2016 to 31st August 2017. Twenty-two celiac children on GFD were compared with sixteen healthy children as a control group. All celiac children on GFD were enrolled for this study. For each patient, demographics and clinical information were collected from parents by means of an interviewer-administered questionnaire. The data included age, sex, height, weight, initial presenting symptoms, onset of symptoms, duration on GFD treatment, family history of celiac disease and autoimmune diseases. Ethical clearance was obtained from the institutional ethics committee, and verbal consent to use the results in research was provided from parents. Exclusion criteria included inability of a parent to provide verbal consent, and celiac children, who are poor adherence to GFD or newly diagnosed celiac cases.

### *Measurement of plasma zinc levels*

Standard solutions containing 0.2  $\mu\text{g/ml}$ , 0.4  $\mu\text{g/ml}$ , 0.8  $\mu\text{g/ml}$ , 1.2  $\mu\text{g/ml}$ , and 1.6  $\mu\text{g/ml}$  of zinc were used for standardization and calibration. Standard reference material (SRM) of Zn (998+5 $\mu\text{g/ml}$ ), 2% HNO<sub>3</sub> (V/V) was used. The venous blood samples 2 ml were collected in morning non-fasting state via zinc-free vials under aseptic technique, centrifuged immediately for 2 minutes at a speed of 4500 rpm. The plasma was stored in a freezer at -20 C until further analysis. Subsequently, 0.3 ml of plasma, and 1.5% of nitric acid were diluted via 10 ml of distilled water, filtered through 70 mm a filter paper, then via 0.22  $\mu\text{m}$  a filter paper under a vacuum pressure. Finally, zinc concentration in filtrate was measured by atomic emission spectrophotometer (Agilent 4100 Microwave Plasma) in Pharmacy Faculty of Misurata University. All samples were analyzed in triplicates in serial order and mean values were reported for each sample. The reference normal range was 0.72–1.15  $\mu\text{g/ml}$  [31].

### *Statistical analysis*

All variables were entered, and analyzed using the statistical software (SPSS, version 18). Descriptive statistics were utilized to summarize demographics and clinical profile of celiac group. Two types of hypotheses were statistically tested. Mann-Whitney U test was used to examine the difference in plasma zinc concentrations between two groups. P value of  $\leq 0.05$  was considered statistically significant. Pearson's correlation and univariate linear regression model were run to measure the relationship between plasma zinc levels and duration on GFD among celiac group.

## RESULTS

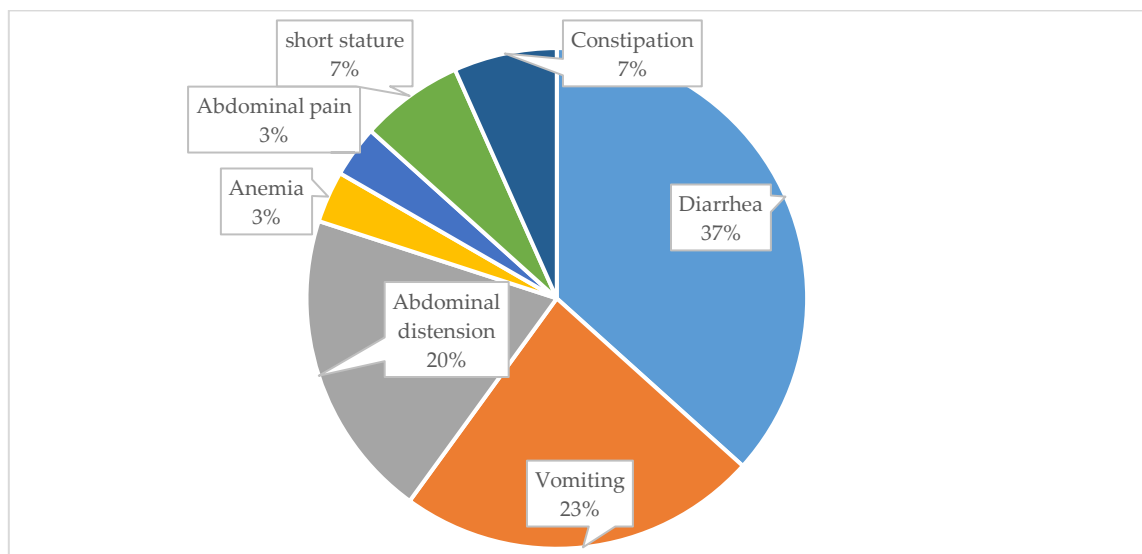
### Clinical characteristics of CD group

In this study, zinc concentrations in twenty-two celiac children on GFD were compared with sixteen normal children. Regarding celiac group, the mean age was 8.8 years with median duration on GFD was one year (range = 10.9 year). The disease onset had two peaks with median age of onset was 5 years. In 36.3% of patients, the symptoms developed early in the 1st 2 years of age and 63% of symptoms were non-classic. Diarrhea was found in 37%, while other gastrointestinal symptoms included vomiting 23%, abdominal distension 20%, constipation 7%, and abdominal pain 3%. Extraintestinal manifestations were short stature 7% and anemia 3% of celiac group. Moreover, a large proportion of patients had no history of celiac disease in family and around 27.2% of patients showed autoimmune diseases, such as diabetes mellitus type I (IDDM) in 22.7% of celiac cases, as displayed in table 1, figure 1&2.

**Table 1. Clinical characteristics of CD group**

Variables	N=22
<b>Sex, n (%)</b>	F=7 (31.8%), M=15 (68.2%)
<b>Age (years), mean ± SD</b>	8.795 ± 4.638
<b>Weight (kg), mean ± SD</b>	32.383 ± 15.37
<b>Height (cm), mean ± SD</b>	125.194 ± 22.35
<b>Age of onset (years), median (IQR)</b>	5 (10)
<b>Duration on GFD (years), median (IQR)</b>	1 (2.9)
<b>F/H of CD, n (%)</b>	Yes = 4 (18.2%), No = 18 (81.8%)
<b>Autoimmune diseases, n (%)</b>	IDDM = 5 (22.7%), Hypothyroidism = 1 (4.5%)

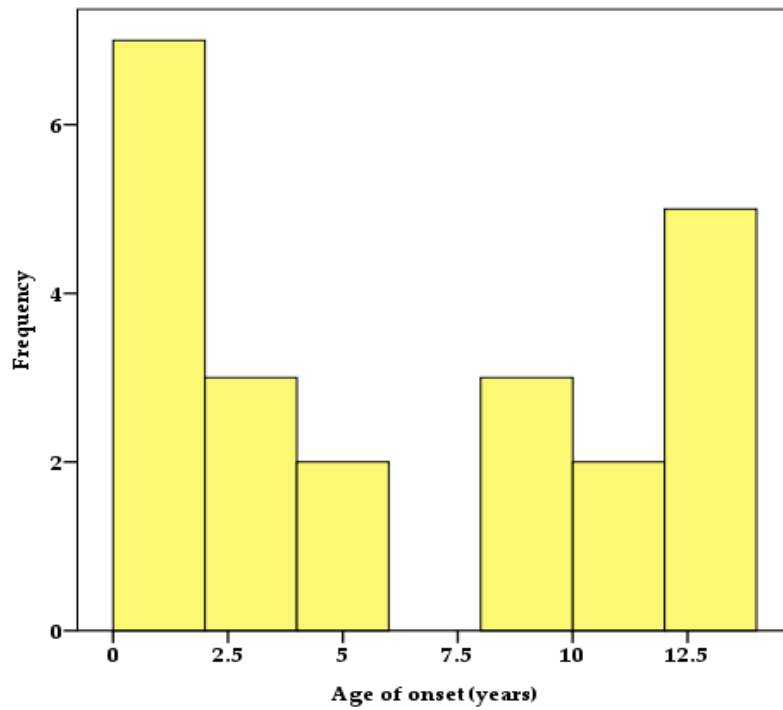
IDDM: Insulin Dependent Diabetes Mellitus (Type I D.M), F/H: Family History, IQR: Interquartile range, SD: Standard deviation, CD: celiac disease.



**Figure 1. Clinical phenotypes among celiac group**

### Zinc level information

Around 20 (91%) of celiac cases on GFD had low plasma zinc concentrations versus control group 5 (31.25%) in this study. The median plasma zinc concentration was significantly lower in celiac patients 0.20 µg/ml compared to control group 0.86 µg/ml with p value < 0.0001. Moreover, the lowest plasma zinc concentration was 0.03 µg/ml with two outliers in celiac children versus controls 0.48 µg/ml with one outlier, as shown in table 2, and figure 3. Concerning the relationship between plasma zinc levels and duration on GFD in celiac group, the correlation coefficient was weak, and negative ( $\rho = -0.177$ ) with a statistical nonsignificant linear relationship (P value = 0.431), as demonstrated in figure 4.

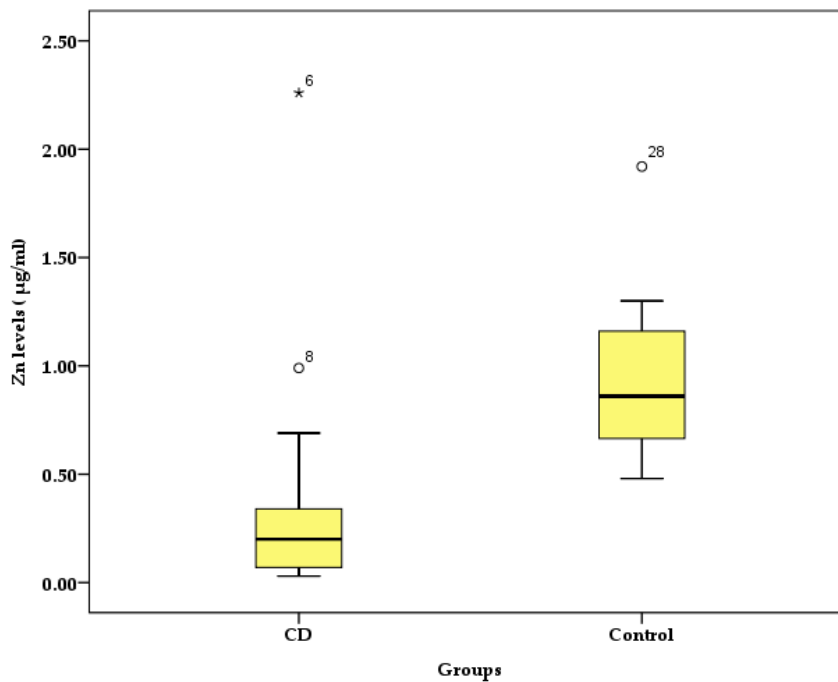


**Figure 2. Age of onset distribution among celiac group**

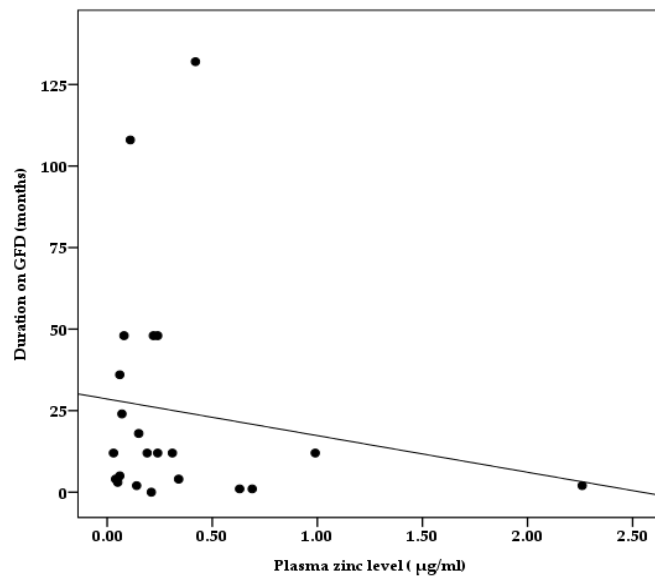
**Table 2. Zinc concentrations ( $\mu\text{g/ml}$ ) in two groups**

Group	Median (IQR)	P value
CD	0.20 (0.29)	<b>&lt; 0.0001</b>
Control	0.86 (0.53)	

*P value: ( $P < 0.05$ ) indicates the differences between two groups is statistically significant. Mann-Whitney U test.*



**Figure 3. Box plot: Plasma zinc levels between two groups**



**Figure 4. Relationship between zinc levels and duration on GFD among celiac group**

## DISCUSSION

CD patients exhibit micronutrient deficiencies e.g., vitamins and minerals, including zinc [32]. In addition, zinc deficiency is an important issue in children with CD. Therefore, the primary concern of current study was to assess plasma zinc levels in celiac children on GFD. In this study, 91% of celiac children on GFD were found to be deficient in zinc. Other authors have made similar findings - according to Altuntal et al. (2000), low plasma zinc levels were demonstrated in 54.2% of celiac children with short stature [29], and Rawal et al. (2010) stated that 71.6% celiac children had zinc deficiency [30]. Other studies also have demonstrated similar results [28,33-34]. There are several reasons that may lower plasma zinc concentrations in celiac patients, such as decrease oral intake, zinc malabsorption because of enteropathy, low serum protein-binding capacity, desaturation of plasma zinc binding sites, and loss through damaged intestinal mucosal epithelium [34]. In CD, gluten withdrawal was associated with improved zinc turnover and reduced loss of endogenous zinc. Henker et al. (1985) stated that low zinc values were demonstrated only in children with acute CD (50% at  $< 2$  SD), and not in children on GFD [33]. Also, Rawal and co-authors (2010) evaluated the effects of GFD with or without zinc supplementation on plasma zinc levels in CD patients with low plasma zinc levels. The authors concluded that zinc levels rise with GFD irrespective of zinc supplementation [30]. Similarly, other authors concluded that children with untreated celiac disease and enteropathy exhibited significantly low plasma zinc levels, which returned to normal upon adopting of GFD [28, 35]. In contrast to our study, the median baseline zinc levels were statistically lower in celiac group on GFD compared to normal group. This finding supports previous results of studies as GFD is usually associated with persistent micronutrient deficiencies, and around 40% of patients experiencing zinc deficiency [36-38]. Additionally, previous studies concluded that the intake of minerals e.g., Ca, Fe, Mg, and Zn was insufficient in subjects on a GFD [39]. Other observation of our study is low plasma zinc concentrations are negatively associated with duration on GFD. However, the relationship was weak and non-significant. Accordingly, there are other factors than duration on GFD that may affect zinc levels in celiac children on GFD, such as GFD itself, zinc homeostasis, presence of enteropathy or not, and nutritional adequacy should be evaluated.

## CONCLUSION

The study concluded that serum zinc concentrations are decreased significantly in pediatric celiac patients on GFD, suggesting zinc deficiency. This highlighted the necessity for good dietary guidance, zinc supplement therapy, and regular monitoring of zinc levels, and not only focusing on gluten elimination in celiac children. In addition, factors that may affect plasma zinc levels in celiac children on GFD should be evaluated thoroughly in the future.

## Acknowledgments

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## Conflicts of Interest. Nil

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## مستويات الزنك في مصل الدم لدى مرضى سيلياك الأطفال الذين يتبعون نظامًا غذائيًا خاليًا من الجلوتين

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### المستخلص

داء السيلياك (داء حساسية الغلوتين) هو اضطراب مناعي ذاتي تساهم العوامل الوراثية والبيئية في إصابته وتطوره. يتميز بضمور الأمعاء الدقيقة وسوء امتصاص العناصر الغذائية الأساسية والفيتمينات والعناصر النزرة نتيجة تناول الغلوتين. يُعد الزنك عنصرًا نزرًا أساسيًا للحفاظ على سلامة الغشاء المخاطي للأمعاء، ودعم المناعة، والنمو لدى الأطفال. لذلك، يهدف هذا البحث إلى تقييم مستويات الزنك في البلازما لدى الأطفال المصابين بداء السيلياك الذين يتبعون نظامًا غذائيًا خاليًا من الغلوتين، ومقارنتها بالأطفال غير المصابين بالداء. تم جمع عينات مصل الدم من 22 طفلًا مصابًا بداء السيلياك ويتبعون نظامًا غذائيًا خاليًا من الغلوتين، بالإضافة إلى 16 طفلًا غير مصاب كمجموعة مرجعية. تم تحضير العينات وقياس مستوى الزنك باستخدام مطياف الانبعاث الذري. تبين أن 91% من الأطفال المصابين بداء السيلياك والذين يتبعون نظامًا غذائيًا خاليًا من الغلوتين يعانون من نقص الزنك. وكان متوسط قيمة تركيز الزنك في البلازما لدى هؤلاء الأطفال (0.20 ميكروغرام/مل)، وهي أقل بكثير مقارنة بالأطفال غير المصابين (0.86 ميكروغرام/مل)، مع قيمة احتمالية  $p < 0.0001$ . تُظهر الدراسة الحالية أن مستويات الزنك في البلازما منخفضة لدى الأطفال المصابين بداء السيلياك الذين يتبعون نظامًا غذائيًا خاليًا من الغلوتين، مما يشير إلى انتشار نقص الزنك لديهم. لذا، يُوصى بشدة بتقديم إرشادات غذائية مناسبة، وتوفير مكملات الزنك، ومراقبة مستويات الزنك بشكل دوري لدى هؤلاء الأطفال.

**الكلمات المفتاحية:** داء السيلياك، النظام الغذائي الخالي من الغلوتين، الزنك، مطياف الانبعاث الذري.