Serum vitamin D, zinc, iron and copper levels in children with newly diagnosed coeliac disease

Priyanka Karnani[1], **Raghunath Barupal**[1], **S K Vishnoi**[1], **Sandeep Choudhary**[1], ***Rakesh Jora**[1]

Sri Lanka Journal of Child Health, 2022; **51**(4): 519-524
DOI: http://dx.doi.org/10.4038/sljch.v51i4.10364

Abstract

Objectives: To assess the occurrence of serum vitamin D, zinc, iron and copper deficiency in children with newly diagnosed coeliac disease (CD) as compared to healthy controls at a tertiary care centre in Western Rajasthan, India

Method: A case control study was conducted in the Gastroenterology Clinic, Department of Paediatrics, Dr S. N. Medical College, Jodhpur, India for a period of one year. Sixty consecutive newly diagnosed CD patients between 2-18 years of age, confirmed via serology and duodenal biopsy, who fulfilled the study criteria, were enrolled as cases in the study. Representative samples of 30 age-and-sex matched healthy subjects were taken as controls. Serum levels of vitamin D, zinc, iron and copper were assessed at diagnosis in cases and controls.

Results: Mean age of the cases in the study was 6.19 ± 3.42 years. Serum vitamin D, zinc, iron and copper deficiency were seen in 55%, 40%, 58.3% and 11.7% cases respectively. The median (IQR) of serum vitamin D ($p=0.002$), zinc ($p<0.001$) and iron ($p=0.003$) were significantly lower in cases as compared to controls. A statistically significant correlation was seen between serum tissue transglutaminase (TTG) and mean serum vitamin D levels of cases in the study group ($p<0.05$) and between vitamin D levels and short stature ($p <0.001$).

Conclusions: Vitamin D, zinc and iron deficiencies were more frequently observed in children with newly diagnosed CD as compared to healthy controls.

[1]*Department of Paediatrics, Dr S.N. Medical College and attached group of Hospitals, Jodhpur, Rajasthan, India*
**Correspondence:* jorarakesh@gmail.com
https://orcid.org/0000-0001-8875-9617

(Received on 13 February 2022: Accepted after revision on 25 March 2022)
The authors declare that there are no conflicts of interest
Personal funding was used for the project.

(Key words: Coeliac disease, Vitamin D, Zinc, Iron, Copper deficiency)

Introduction

Coeliac disease (CD) is an autoimmune disorder primarily affecting proximal small bowel secondary to permanent gluten intolerance in genetically susceptible individuals[1]. The classic presentation, commonly known as 'coeliac disease iceberg' involves failure to thrive, malnutrition, diarrhoea, abdominal pain and distension in the first two years of life[2,3,4]. Histopathologically, there is typically varying amounts of duodenal villous atrophy and crypt hyperplasia with inflammatory changes leading to malabsorption[5,6]. Consequently, micronutrient deficiencies may occur in undiagnosed CD.

Deficiencies in calcium, vitamin D, iron, folate, vitamin B12, copper and zinc have all been demonstrated in children with CD with varied results[7-10]. Iron deficiency anaemia is the most common form and may be the only finding in about 50% of patients with subclinical CD[11]. Vitamin D deficiency can also occur as a result of fat-soluble vitamin malabsorption. Lactose intolerance can lead to a decreased intake of food sources rich in calcium and vitamin D, which also contributes to deficiency of calcium and vitamin D[12]. Mineral and trace-element status of children with untreated CD has not been widely studied. There is even a lack of recent reports in the literature indicating which deficiencies should be checked in children with newly diagnosed CD.

Objectives

To measure the serum vitamin D, iron, zinc and copper levels in patients with newly diagnosed CD in order to assess the occurrence of these deficiencies as compared to healthy controls.

Method

A case control study was conducted in the Gastroenterology Unit of the Department of Paediatrics, Dr S. N. Medical College, Jodhpur, India, over a period of one year.

Sample size calculation: $n = \frac{Z_{\frac{\alpha}{2}} \times P \times (1-P)}{e^2}$ Where, n= sample size, $z_{\alpha/2}$ = critical appraisal = 3.98 (confidence interval -95%, power= 80%) P = 0.82

(As Iron deficiency was seen in 81.6% cases in a study done by Kuloglu Z, et al[15] in children with coeliac disease) (1-P) = 1-0.82 =0.18 e = precision error (which is taken as 0.10 i.e., 10% for the study). Sample size as calculated by above formula comes out to be 59. We have evaluated a total of 60 patients of CD in our study

In this study, 60 consecutive newly diagnosed CD patients, aged 2-18 years, who attended the outpatient departments (OPDs) and wards of attached hospitals during the study period were included. A representative sample of 30 healthy age-and-sex matched subjects who visited our OPD for immunization or routine health check-up, and siblings of patients were included as controls. Children who had any suspected or confirmed gastrointestinal anomaly other than CD or received any vitamin/mineral supplementation in the past 3 months were excluded from the study.

Diagnosis of CD was established according to the ESPGHAN guidelines 1990[13]. Biopsy specimens were taken from the second part of the duodenum and histological evidence of CD was reported according to the Modified Marsh-Oberhuber classification[14]. All patients testing positive for both serology and biopsy and fulfilling study criteria were enrolled and their serum vitamin D, zinc, iron and copper levels were measured. A age-and-sex matched healthy control population was simultaneously screened for the same deficiencies to study their prevalence in the general population. Anthropometric assessment was done using WHO growth charts based on the WHO Multicentre Growth Reference Study (MGRS) standards for weight, height/length for children up to 5 years of age and WHO reference data based on National Centre of Health Sciences (NCHS) data on weight, height and Body Mass Index (BMI) for children 5-19 years of age. Serum iron was measured by a modification of the automated AAII-25 colorimetric method while serum zinc and copper were measured by mass spectrometry at the biochemistry laboratory of Dr S.N. Medical College, Jodhpur. Trace element free vials (6 ml capacity) were used and 100µl serum was taken for estimation.

Operational definitions for vitamin D and mineral deficiency were pre-defined.

- Vitamin D deficiency was defined as 25 OH vitamin D levels <20ng/dl.
- Zinc deficiency was defined as <74 µg/L in males, <70 µg/L in females.
- Iron deficiency was defined as <70 µg/dl.
- Copper deficiency was defined as <70 µg/dl in males, <70 µg/dl in girls <12 years and <80 µg/dl in girls >12 years

Ethical issues: Study received approval from the Ethics Committee of Rajasthan University of Health Sciences (RUHS) (approval number ACAD/MC/JU/17/17538). Written informed consent was obtained from the parents of the children enrolled in the study.

Statistical analysis: Data were entered into a Microsoft Excel sheet and analysed using Statistical Package for the Social Sciences (SPSS) software version 2009. Continuous variables were presented as means and standard deviations. Histogram was drawn to assess the normality of variables. Mann Whitney test was used for analysis of continuous variables. Categorical variables were presented as percentages. Categorical data were compared using Chi-square test. The correlation between two variable was compared by Spearman correlation test and correlation coefficient was 0.8 (strong association). A p value of <0.05 was considered statistically significant. We also plotted the Receiver Operating Characteristic (ROC) curves for determining cut off value of serum TTG for predicting vitamin D deficiency in study population.

Results

Baseline demographic characteristics of cases and controls are shown in Table 1. Ages ranged from 1-17 years with a male female ratio of 0.82:1.

Table 1: ***Baseline demographic characteristics of cases and controls***

Parameter	Cases *(n=60)*	Controls *(n=30)*
Age (in years) - Mean ± SD	6.19 ± 3.42	5.88 ± 3.01
Height (cm) - Mean ± SD	103.94 ± 19.30	105.4 ± 18.85
Weight (kg) - Mean ± SD	15.56 ± 7.19	15.82 ± 7.32
BMI (kg/m^2) - Mean ± SD	13.74 ± 2.25	13.46 ± 2.30

Table 2 shows the clinical presentation of cases.

Table 2: ***Clinical presentation of Cases (n=60)***

Sign and symptoms	n (%)
Failure to thrive/ Not gaining weight	53 (88.3)
Anaemia	50 (83.3)
Diarrhoea	40 (66.7)
Chronic pain in abdomen	37 (61.7)
Short stature	34 (56.7)
Abdominal distension	32 (53.3)
Anorexia	19 (31.7)
Constipation	04 (06.7)
Clubbing	03 (05.0)
First degree relatives with CD	02 (03.3)

CD: Coeliac disease

In our study 43.3% cases were underweight (Z score <-3SD) at diagnosis. BMI was <-2SD in 48.3% cases while one case was obese (BMI >+3SD). The most common clinical presentation was failure to thrive/not gaining weight (88.3%). Two patients presented with type 1 diabetes mellitus while one each presented with hypothyroidism and alopecia with thalassaemia minor.

Thirty percent of cases had serum Tissue Transglutaminase Immunoglobulin A (TTG IgA) values between 21-70 (IU/ml) while 36.7% of cases had Marsh stage IIIC on histopathological examination. The correlation between TTG and Marsh staging was statistically significant (p <0.0001) (Table 3).

Table 3: ***Correlation between Marsh staging and serum TTG in Cases***

Marsh staging	Number of cases (%)	Mean serum TTG (IU/ml)
Sage I	08 (13.3)	25.19 ± 11.02
Stage II	11 (18.3)	40.60 ± 59.70
Stage IIIA	09 (15.0)	65.32 ± 54.71
Stage IIIB	10 (16.7)	118.48 ± 59.19
Stage IIIC	22 (36.7)	219.40 ± 80.54
Total	60	120.79 ± 101.99

TTG: tissue transglutaminase, ***p <0.0001***

Serum vitamin D, zinc, iron and copper deficiencies were seen in 55%, 40%, 58.3%, 11.7% cases respectively. The median (IQR) of serum vitamin D, iron and zinc levels were significantly low in cases as compared to controls (Table 4). Vitamin D deficiency was more frequently observed in patients who presented with short stature (p <0.0001). A statistically significant correlation was seen between serum TTG IgA antibody levels and mean vitamin D levels in cases. The ROC analysis cut off value of serum TTG IgA for predicting vitamin D deficiency (<20ng/ml) in CD was 21.7 IU/ml with a sensitivity of 85% and a specificity of 85% (Figure 1). Vitamin D and mineral deficiencies were not statistically significantly correlated with nutritional status and BMI at presentation (p >0.05) and to the severity of Marsh staging (p>0.05).

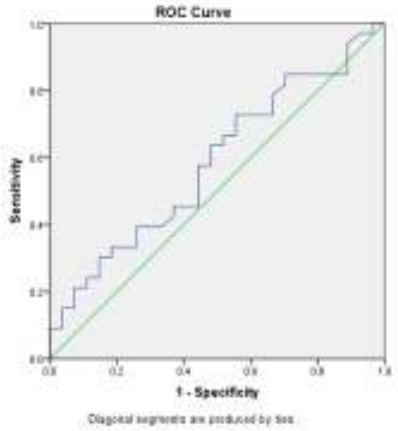

Figure 1: Receiver Operating Characteristic (ROC) curve between tissue transglutaminase (TTG) and Vitamin D levels

Table 4: Vitamin D and mineral levels in Cases and Controls

Serum parameter	Deficient Cases *(n=60)* n (%)	Mean serum levels in Cases (Mean ± SD)	Deficient Controls (n=30) n (%)	Mean serum levels in Controls (Mean ± SD)
Serum vitamin D (ng/ml)	33 (55.0)	20.29 ± 8.97	02 (06.7)	33.30 ± 10.94
Serum zinc (µg/L)	24 (40.0)	80.63 ± 21.18	02 (06.7)	102.13 ± 16.52
Serum copper (µg/L)	07 (11.7)	90.95 ± 17.62	01 (03.3)	90.95 ± 17.62
Serum iron (µg/L)	35 (58.3)	58.24 ± 29.63	10 (33.3)	80.63 ± 21.18

Discussion

There are very few studies depicting the serum levels of vitamin D and minerals in children with newly diagnosed CD and their association with TTG IgA and Marsh staging. Moreover, no definite results on the association of these deficiencies with Marsh staging or TTG levels are known till date. We have shown in our study that there is vitamin D and

iron deficiencies in around 50% cases at the time of diagnosis of CD. The main differences in the clinical features of CD in our study and many other studies from northern India[16,17,18] as compared to West[19] are the higher incidence of failure to thrive (88.3%) and anaemia (83.3%) in the Indian population.

Mineral and trace-element status of children with untreated CD has not been widely studied. There is even a lack of recent reports in the literature indicating which deficiencies should be checked in children with newly diagnosed CD. The present study showed that the majority of children with CD has a deficiency of vitamin D and micronutrients at the time of presentation. Botero-Lopez JE, *et al*[20] concluded that mineral deficiency is present in both typical and atypical forms of CD, that active search of mineral deficiency should be conducted in all cases and that patients with CD should receive mineral supplements as part of their treatment. Wierdsma NJ, *et al*[21] stated that deficiencies of vitamins or minerals are frequently observed in untreated adult Dutch CD-patients using a Western diet although they are currently diagnosed earlier than in the previous century.

In our study, serum 25 hydroxy-vitamin D deficiency (values <20ng/ml) was seen in 55% of the cases with CD. Moreover, the mean serum values of 25 OH vitamin D in cases were significantly low as compared to controls (20.59 ± 8.98ng/ml compared to 33.30 ± 10.94ng/ml, p <0.0001). Turner J, *et al*[22] reported vitamin D deficiency in 60% of children with CD. Erdem T, *et al*[23] screened children with newly diagnosed CD and found vitamin D deficiency in 51.9% of the children. In our study, a statistically significant correlation was seen between serum TTG IgA and mean vitamin D levels in cases with deficient vitamin D levels (p <0.05). Dankers W, *et al*[24] suggested a possible correlation between vitamin D and autoimmunity because of its immunoregulatory properties. Tavakkoli A, *et al*[25] studied the prevalence of other autoimmune diseases in patients with CD and vitamin D deficiency and reported a higher prevalence of psoriasis in CD patients who presented with low serum vitamin D levels compared to patients with normal vitamin D levels. The serum deficiencies of these minerals were independent of serum TTG levels and nutritional status except serum vitamin D in this study[25]. Maximum number of patients with serum vitamin D deficiency had serum TTG values between 211-300 IU/ml. However mean vitamin D levels were found to be lowest (9.96+2.38 ng/ml) in the cases with TTG values between 141-210 IU/ml. Vitamin D deficiency (<20ng/dl) was seen in 50% cases who presented with short stature (p value <0.0001). These findings may suggest that atypical presentation of CD with short stature might be attributed to the presence of vitamin D deficiency secondary to malabsorption in CD and warrants that we should go for screening of serum vitamin D levels in patients with CD presenting with short stature.

Zinc deficiency may arise secondary to both malabsorption and endogenous zinc secretion into the intestine lumen. CD is associated with a wide array of skin lesions and manifestations, which may be partly ascribed to zinc deficiency; 40% cases had serum zinc deficiency while 6.7% healthy controls had serum zinc deficiency in our study. The mean serum zinc levels in cases were significantly low compared to controls (80.63 ± 21.18 µg/L compared to 102.5 ± 15.84 µg/L, p <0.0001). Fathi F, *et al*[26] compared the zinc levels in CD patients to healthy controls and reported similar results as that of our study. Rawal P, *et al*[27] reported zinc deficiency in 72% cases in CD patients at diagnosis.

Primary dietary copper deficiency is infrequent. It occurs mainly secondary to malabsorption syndromes. In our study, 7 (11.7%) cases were found to have deficiency in serum copper while 3.3% in the control group had serum copper deficiency. Botero-Lopez JE, *et al*[20] reported copper deficiency in 15% cases (p <0.05) with CD while 20% had zinc deficiency in this study. Copper deficiency has been described in patients with CD and it is a known cause of anaemia and thrombocytopenia. Copper and iron share the transporters and a number of proteins in their metabolism and copper is needed for the formation of haemoglobin molecules; the low number of cases with copper deficiency may explain at least part of this result[20].

A study by Wierdsma NJ, *et al*[21] in adult CD population to document the presence of vitamin and mineral deficiencies in CD had at least one value below the lower limit of reference. Zinc deficiency was observed in 67% of the CD-patients, 46% had decreased iron storage, and 32% had anaemia. They found no correlation between the serum deficiencies of vitamin and minerals and serum TTG IgA values and Marsh staging. However, no such correlation is reported in any of the studies done in the paediatric population till date.

There were some limitations in this study. Firstly, the study sample size was small and secondly controls were not in equal numbers to the cases which would have strengthened the study. Vitamin or mineral supplement use, whether at the patient's own initiative or prescribed by the general practitioner, was based on self-reported information and could therefore have been underestimated.

Conclusions

Vitamin D, zinc, iron and copper deficiencies were frequently observed in children with CD. These deficiencies were independent of the severity of disease and so we should screen all CD patients for these deficiencies at the time of disease presentation.

References

1. Ludvigsson JF, Leffler DA, Bai JC, Biagi F, Fasano A, Green PH *et al.* The Oslo definitions for coeliac disease and related terms. *Gut* 2013; **62**(1): 43-52. https://doi.org/10.1136/gutjnl-2011-301346 PMid: 22345659 PMCid: PMC3440559

2. Husby S, Koletzko S, Korponay-Szabó IR, Mearin ML, Phillips A, Shamir R *et al*. European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) guidelines for the diagnosis of coeliac disease. *Journal of Paediatric Gastroenterology and Nutrition* 2012; **54**(1): 136-60. https://doi.org/10.1097/MPG.0b013e31821a23d0 PMid: 22197856

3. Fasano A, Catassi C. Clinical practice. Coeliac disease. *New England Journal of Medicine* 2012; **367**(25): 2419-26. https://doi.org/10.1056/NEJMcp1113994 PMid: 23252527

4. Nenna R, Tiberti C, Petrarca L, Lucantoni F, Mennini M, Luparia RP *et al.* The coeliac iceberg: characterization of the disease in primary schoolchildren. *Journal of Paediatric Gastroenterology and Nutrition* 2013; **56**(4): 416-21. https://doi.org/10.1097/MPG.0b013e31827b7f64 PMid: 23149808

5. Niewinski MM. Advances in coeliac disease and gluten-free diet. *Journal of the American Dietetic Association* 2008; **108**(4): 661-72. https://doi.org/10.1016/j.jada.2008.01.011 PMid: 18375224

6. See J, Murray JA. Gluten-free diet: the medical and nutrition management of coeliac disease. *Nutrition in Clinical Practice* 2006; **21**(1): 1-15. https://doi.org/10.1177/011542650602100101 PMid: 16439765

7. Rawal P, Thapa BR, Nain CK, Prasad KK, Singh K. Changing spectrum of coeliac disease in India. *Iranian Journal of Paediatrics* 2010; **20**(4): 459-65.

8. Bode S, Gudmand-Hoyer E. Symptoms and histological features in consecutive adult coeliac patients. *Scandinavian Journal of Gastroenterology* 1996; **31***(*1*):* 54-60. https://doi.org/10.3109/00365529609031627 PMid: 8927941

9. McWhirter WR. Plasma tocopherol in infants and children. *Acta Paediatrica* 1975; **64**(3): 446-8. https://doi.org/10.1111/j.16512227.1975.tb03863.x PMid: 1155063

10. Hallert C, Grant C, Grehn S, Granno C, Hulten S, Midhagen G, *et al.* Evidence of poor vitamin status in coeliac patients on a gluten free diet for 10 years. *Alimentary Pharmacology and Therapeutics* 2002; **16**(7): 1333-9. https://doi.org/10.1046/j.13652036.2002.01283.x PMid: 12144584

11. Bottaro G, Cataldo F, Rotolo N, Spina M, Corazza GR. The clinical pattern of subclinical/silent coeliac disease: An analysis on 1026 consecutive cases. *American Journal of Gastroenterology* 1999; **94**(3): 691-6. https://doi.org/10.1111/j.15720241.1999.00938.x

12. Misra M, Pacaud D, Petryk A, Collett-Solberg PF, Kappy M. Drug and Therapeutics Committee of the Lawson Wilkins Paediatric Endocrine Society. Vitamin D deficiency in children and its management: review of current knowledge and recommendations. *Paediatrics* 2008; **122**(2): 398-417. https://doi.org/10.1542/peds.2007-1894 PMid: 18676559

13. Walker-Smith JA, Guandalini S, Schmitz J, Shmerling DH, Visakorpi JK. Revised criteria for diagnosis of coeliac disease. *Archives of Disease in Childhood* 1990; **65**: 909–11. https://doi.org/10.1136/adc.65.8.909 PMid: 2205160 PMCid: PMC1792502

14. Corazza GR, Villanacci V. Coeliac disease. *Journal of Clinical Pathology* 2005; **58:** 573-4. https://doi.org/10.1136/jcp.2004.023978 PMid: 15917404 PMCid: PMC1770677

15. Kuloglu Z, Kirsaçlioglu CT, Kansu A, Ensari A, Girgin N. Coeliac disease: presentation of 109 children. *Yonsei Medical Journal* 2009; **50**(5): 617-23. https://doi.org/10.3349/ymj.2009.50.5.617 PMid: 19881963 PMCid: PMC2768234

16. Poddar U, Thapa BR, Singh K. Clinical features of coeliac disease in Indian children: Are they different from the West? *Journal of Paediatric Gastroenterology and Nutrition* 2006; **43**(3): 313-7. https://doi.org/10.1097/01.mpg.0000231589.32114.9d PMid: 16954952

17. Patwari AK, Anand VK, Kapur G, Narayan S. Clinical and nutritional profile of children with coeliac disease. *Indian Paediatrics* 2003; **40**(4): 337-42.

18. Rawal P, Thapa BR, Nain CK, Prasad KK, Singh K. Changing spectrum of coeliac disease in India. *Iranian Journal of Paediatrics* 2010; **20**(4): 459-65.

19. Tursi A, Brandimarte G, Giorgetti GM. Prevalence of antitissue transglutaminase antibodies in different degrees of intestinal damage in coeliac disease. *Journal of Clinical Gastroenterology* 2003; **36**(3): 219-21. https://doi.org/10.1097/00004836200303000-00007 PMid: 12590232

20. Botero-López JE, Araya M, Parada A, Méndez MA, Pizarro F, Espinosa N *et al.* Micronutrient deficiencies in patients with typical and atypical coeliac disease. *Journal of Paediatric Gastroenterology and Nutrition*. 2011; **53**(3): 265-70. https://doi.org/10.1097/MPG.0b013e3181f988fc PMid: 21865972

21. Wierdsma NJ, van Bokhorst-de van der Schueren MA, Berkenpas M, Mulder CJ, van Bodegraven AA. Vitamin and mineral deficiencies are highly prevalent in newly diagnosed celiac disease patients. *Nutrients* 2013; **5**(10): 3975-92. https://doi.org/10.3390/nu5103975 PMid: 24084055 PMCid: PMC3820055

22. Turner J, Pellerin G, Mager D. Prevalence of metabolic bone disease in children with coeliac disease is independent of symptoms at diagnosis. *Journal of Paediatric Gastroenterology and Nutrition* 2009; **49**(5): 589-93. https://doi.org/10.1097/MPG.0b013e31819ca18e PMid: 19644400

23. Erdem T, Ferat Ç, Nurdan YA, Halime E, Muhammed Selçuk S, Hamza K *et al.* Vitamin and mineral deficiency in children newly diagnosed with coeliac disease. *Turkish Journal of Medical Sciences* 2015; **45**(4): 833-6. https://doi.org/10.3906/sag-1408-94

24. Dankers W, Colin EM, van Hamburg JP, Lubberts E. Vitamin D in autoimmunity: Molecular mechanisms and therapeutic potential. *Frontiers in Immunology* 2017; **7**: 697. https://doi.org/10.3389/fimmu.2016.00697 PMid: 28163705 PMCid: PMC5247472

25. Tavakkoli A, DiGiacomo D, Green PH, Lebwohl B. Vitamin D status and concomitant autoimmunity in coeliac disease. *Journal of Clinical Gastroenterology* 2013; **47**(6): 515-9. https://doi.org/10.1097/MCG.0b013e318266f81b PMid: 23328299 PMCid: PMC3640651

26. Fathi F, Ektefa F, Tafazzoli M, Rostami K, Rostami Nejad M, Fathi M, *et al.* The concentration of serum zinc in coeliac patients compared to healthy subjects in Tehran. *Gastroenterology and Hepatology from Bed to Bench* 2013; **6**(2): 92-5.

27. Rawal P, Thapa BR, Prasad R, Prasad KK, Nain CK, Singh K. Zinc supplementation to patients with coeliac disease--is it required? *Journal of Tropical Paediatrics* 2010; **56**(6): 391-7. https://doi.org/10.1093/tropej/fmq011 PMid: 20176568