An Innovative Effective Nutritional Therapy For Vitamin D Deficiency in Children with Celiac Disease

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Children with celiac disease (CD) are susceptible to reduced bone mineral density (BMD). Our target is to assess the severity of vitamin D deficiency in CD children on a gluten-free diet (GFD), and to evaluate the effectiveness of adding an innovative GF meal, on the clinical and bone biochemical indices of CD patients. 50 CD children who were diagnosed and followed up at Pediatric gastroenterology clinic, Specialized pediatric hospital, Cairo University; by serology and biopsy of the duodenum were included in this prospective study. CD children were on GFD for at least one year. As a control group, 40 healthy children were enrolled. Thorough clinical examination, anthropometric assessment, a complete history and 24 hours dietary recall were done for all the participants in this work. We introduced our innovative GF meal to CD patients twice/day, for 3 consecutive months. Venous blood samples were withdrawn from patients at the study beginning and after 3 months for detection of serum vitamin D, calcium, phosphorous and alkaline phosphatase levels. The anthropometric measurements, serum vitamin D, and calcium were markedly decreased in CD children than that of controls. In CD patients, a significant increase in anthropometric parameters, vitamin D and calcium were found. While there was a significant decline of serum alkaline phosphatase, and a slight decrease in serum phosphorus at the study end. The innovative gluten-free prepared meal confirmed to be of high nutritional value in the management of vitamin D deficiency and improvement of bone indices in CD patient.

Keywords: Celiac disease; gluten free meal; vitamin D deficiency.

Celiac disease (CD) is one of the immune-mediated chronic disorders principally affect the proximal small intestine absorptive surface. It occurs in susceptible individuals after exposure to gliadin and prolamin peptides found in wheat, rye and barley. The disease is characterized by atrophy of the villi and inflammatory changes of the mucosa of the small intestine, from the duodenum to the distal ileum. Pepsin–trypsin-resistant gliadin (PT-G) is an undigested gliadin fragment that substantially contributes to the pathogenesis of CD by altering intercellular tight junctions (TJs). In CD, loss of the intestinal barrier function occurs due to
exposure to environmental triggers in the presence of a genetic predisposition. Gliadins and gluten peptides permeabilize the gut, which initiates an immunomodulatory cytotoxic effect and opens TJ s. Subsequently, the deregulated traffic of macromolecules, due to the "leaky gut," severely damages the intestine, thus fueling the chronic inflammatory process.

The only known modulator of intercellular TJ s is zonulin, which regulates intestinal permeability. The zonulin pathway is upregulated, during the acute phase of CD.

It is known now that CD is the most common genetically predetermined condition in humans, with a prevalence ranging from 0.53% to 6.4% among Egyptian children and 14.2% in adult Egyptian CD patients.

It includes a wide variety of clinical manifestations ranging from the classical severe symptoms of diarrhea and growth failure, shortly after the intake of gluten, to the subclinical or even silent form of the disease diagnosed later in life.

Vitamin and mineral deficiencies have been noted to be common in a population of children with recently diagnosed celiac disease.

Current literature suggests that patients with untreated celiac disease are at increased risk of developing low BMD, osteoporosis, and bone fractures. CD children have low BMD at diagnosis either in symptomatic or asymptomatic patients. The recent evidence-informed expert opinion recommends screening for vitamin D status in children at the time of initial diagnosis for celiac disease.

There is no recent treatment except the gluten-free diet (GFD) strict adherence, which reverses and alleviates clinical manifestations and biopsy alterations in the intestine. Exacerbation of the disease can be accurately detected by Positive serologic indicators as tissue transglutaminase antibodies (TTG) and endomysium antibodies (EMA), which also denote sporadic ingestion of gluten.

Two fundamental mechanisms are potentially involved in developing the bone disease in these patients. First, small intestinal mucosal damage may lead to defective intestinal absorption of nutrients, including calcium and vitamin D. The second mechanism is that chronic intestinal inflammation may lead to proinflammatory cytokines release associated with increased bone loss. Treatment with a strict gluten-free diet promotes significant improvement of bone mineralization in celiac disease children.

As decreased BMD is commonly detected in CD patients, dietary guidelines recommended supplementation of vitamin D for celiac disease patients either children or adults.

A gluten-free diet might be limited and restrictive, in spite of supplying enough nutrition. The North American Society of Pediatric Gastroenterology, Hepatology, and Nutrition recorded that GFD compliance range from 45% to 81% in children. Gluten-free diet strict adherence is more challenging in children and adolescents than in adults.

Dong et al. reported that VD3 has the most powerful influence on restraining zonulin release and suppressing upregulating TJ protein expression. Moreover, it inhibits intestinal MyD88 expression, a factor promoting zonulin release. Thus, VD3 has a protective effect on the intestinal mucosal barrier from PT-G and can act as the principle for new efficient treatments for CD.

Vitamin D monitoring and supplementation advice are not included in the 2013 British Society of Pediatric Gastroenterology, Hepatology and Nutrition (BSPGHAN) guideline. Current adult celiac guideline advice is to monitor and/or supplement as required.

In the current work, we investigated vitamin D serum levels in CD populations in order to challenge its routine supplementation in the form of ready novel healthy balanced easily supplied meal.

We prepared a balanced gluten-free meal (GFD) and aimed to assess its effect on celiac disease (CD) children and on serum biochemical bone health indices. We compared serum vitamin D, phosphorous, calcium, and alkaline phosphatase level in children and adolescents on strict GFD patients before and after supplementation of our novel balanced meal.

Participants and methods

We studied patients with CD who regularly attend the gastroenterology clinic at Specialized Pediatric Hospital (Abo-El Rish), Cairo University. Participants diagnosed according to the criteria of the European and North American Society.
for Pediatric Gastroenterology, Hepatology and Nutrition (i.e. demonstration of villous atrophy in the duodenal mucosa, positive serological markers of disease activity at diagnosis and negative EMA with GFD)\(^{13,37}\).

Children aged 3–15 years with CD \((n = 50)\) were selected and compared with non-CD age and sex-matched children as controls \((n = 40)\). Patients were excluded if they had diseases of calcium or vitamin D metabolism, or had other autoimmune diseases. All patients had been on GFD for at least 1 year and had received calcium and vitamin D supplementation therapy. Duodenal biopsy specimens were done to detect the grade of villous atrophy due to gluten-sensitive enteropathy. CD-associated antibodies, i.e., anti-tissue transglutaminase (tTG) antibodies, were measured. Demographic data (sex, age), disease characteristics (age at diagnosis, disease duration, GFD duration) and symptoms of vitamin and mineral deficiencies were recorded for every participant.

Informed consents were obtained from the parents of all of the participants and the study was approved by both the National Research Center and Cairo University ethics committees (no: 15093).

### Table 1. Demographic Data and Disease Characteristics

<table>
<thead>
<tr>
<th>Total cases:</th>
<th>50 case</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of patients (mean)</td>
<td>8 years</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
</tr>
<tr>
<td>65%</td>
<td>35%</td>
</tr>
<tr>
<td>Residence</td>
<td>Rural</td>
</tr>
<tr>
<td>57.5%</td>
<td>42.5%</td>
</tr>
<tr>
<td>Age of onset(mean)</td>
<td>4.2 years</td>
</tr>
<tr>
<td>Mean Duration of disease</td>
<td>3.8 years</td>
</tr>
<tr>
<td>Serology</td>
<td>+ve serology</td>
</tr>
<tr>
<td>82.5%</td>
<td>17.5%</td>
</tr>
<tr>
<td>Biopsy</td>
<td>Atrophy of the villi</td>
</tr>
<tr>
<td>95%</td>
<td>5%</td>
</tr>
<tr>
<td>Gastrointestinal manifestations</td>
<td>Present</td>
</tr>
<tr>
<td>72.5%</td>
<td>27.5%</td>
</tr>
<tr>
<td>Poor weight gain</td>
<td>Present</td>
</tr>
<tr>
<td>97.5%</td>
<td>2.5%</td>
</tr>
<tr>
<td>Manifestations of vitamin deficiency</td>
<td>Present</td>
</tr>
<tr>
<td>63.2%</td>
<td>36.8%</td>
</tr>
<tr>
<td>pallor</td>
<td>Present</td>
</tr>
<tr>
<td>35%</td>
<td>65%</td>
</tr>
</tbody>
</table>

All participants continued on their gluten-free diet and a special meal was added to them a twice/day (breakfast and lunch supplementation). The nutritional status of these patients was determined by anthropometric measurement and by estimation of haematological and biochemical indices in blood samples before the beginning and at the end of the study (after 3 months).

Their height and weight were measured following the recommendations of the International Biological Program\(^{18}\). BMI standard deviation score (BMI SDS) was calculated as weight/height\(^2\) (expressed as z value). Three consecutive measurements were taken and the mean was recorded.

Height was measured to the nearest 0.01 cm using a wall-mounted stadiometer (Holtain Ltd, Crymych, Dyfed, UK) that was calibrated daily. Weight was measured to the nearest 0.01 kg using Seca scale balance adult type, with minimal clothes for which no correction was made.

All patients or their parents filled out questionnaires about adherence to GFD. 24-hours weighted food records were obtained by means of a self-completed questionnaire of total food and beverage intake. Patients and their parents were carefully instructed by the clinical dietician to record the exact amount of all foods and drinks consumed.

The electronic version of the USDA National Nutrient Database, which includes 5062 food items, was used to determine the usual nutritional vitamin D and calcium intake of foods and beverages consumed by participated CD children’s\(^{19}\). The food items were specifically adapted and validated for the celiac population.

#### Balanced GF meal description

Our healthy novel balanced gluten-free (HNB-GF) Meal was manufactured without hidden gluten, directly before use. According to the WHOD FAO commission Codex Alimentarius, ‘gluten–free foods’ are dietary foods “consisting of or made only from one or more ingredients which do not contain wheat (i.e., all Triticum species, such as durum wheat, spelt, and kamut), rye, barley, oats or their crossbred varieties, and the gluten level does not exceed 20 mg D kg in total, based on the food as sold or distributed to the consumer\(^{20}\).

The source of vitamin D in HNB-GF meal are cereals ready-to-eat, fortified corn flakes (28
IU/serving, 1 cup, 28 g) and Milk, dry, instant, with added vitamin A and vitamin D (299 IU/serving, 1 cup, 68 g). Those reflect 265.9 IU/one HNBGF meal (6.6 µg). Dietary Reference Intakes (DRIs), Recommended Dietary Allowances and Adequate Intakes for vitamin D are 10, 15 and 15 µg/d for children aged 4–8 y, Males and Females 9–13 y, respectively. Consumption of one HNBGF meal/day represents 60% from daily vitamin D DRI for children aged 4–8 y and 40% from daily DRI for Males and Females children aged 9–13. Our CD children consumed two HNBGF meal/day, that means that the HNBGF meal extends the body with 120% from daily vitamin D DRI for children aged 4–8 y and 80% from daily DRI for Males and Females children aged 9–13.

No one of our patient’s subjects consumed fish or milk or other products fortified with vitamin D (ex. Milk, yogurt…..ext), the highest source of vitamin D.

The main source of calcium in HNB-GF meal is milk, dry, instant, with added vitamin A and vitamin D in whole and nonfat forms. The calcium content of one HNBGF meal was 425.68 mg calcium, it represents 53.21% and 38.70% of recommended Dietary Reference Intakes (DRIs) for children aged 4–8 y and both sexes aged 9–13 y. The Recommended Dietary Allowances and Adequate Intakes for calcium are 800 mg/d for children aged 4–8 y and 1100 mg/d for both males and females aged 9–13 y. Our CD children consumed two HNBGF meal/day, that means that the HNBGF meal extends the body with 106.42% from daily calcium DRI for children aged 4–8 y and 77.40% from daily DRI for Males and Females children aged 9–13 y. The mean dietary intake of calcium for our patients ranged from 99.00 to 1088.40 mg calcium/day with mean value 515.16 mg calcium/day, depending on 24-h dietary recall. That represents 64.39% from the Dietary Reference Intake of calcium for children aged 4–8 y (ranged from 12.38 to 136.05%) and 46.83% from the Dietary Reference Intake of it for both of males and females aged 9–13 y (ranged from 9.00 to 98.95%).

Supplementation

The daily vitamin D supplement consumed by children and adolescent CD children was 400 IU/day, and 50 mg calcium/day, it continued through the study period.

Laboratory work

Three millilitres of fasting venous blood sample (12 hrs.) was collected from each patient enrolled in the study and divided into two parts: the first part was added to a tube containing EDTA for Complete blood count determination by cation-exchange resin. Serum was separated from the second part of the sample and was stored at – 20C for determination of albumin, urea, creatinine, vitamin D, calcium, phosphorous and alkaline phosphatase, Anti-endomysial antibodies and anti-tissue transglutaminase antibody, TTG IgA.

Quantitative determination of serum vitamin D: It was done by ELISA technique using Kit provided from Glory science co., Ltd, USA. While the determination of serum Alkaline phosphatase was measured using a colorimetric kit (Biodiagnostic) according to Belfield and Goldberg method21.

Determination of serum inorganic phosphorous was measured by colorimetric monoreagent kit (Centronic Gmb H- Germany) according to Henry 1974. Determination of serum calcium: was measured using o-Cresolphthalein Direct

<table>
<thead>
<tr>
<th>Table 2. Anthropometric data of the study subjects and controls</th>
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<tbody>
<tr>
<td>CD cases at the beginning</td>
</tr>
<tr>
<td>N=50</td>
</tr>
<tr>
<td>Group I-A (mean± SD)</td>
</tr>
<tr>
<td>HAZ score</td>
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<tr>
<td>WAZ score</td>
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<tr>
<td>BMI</td>
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</table>

P1: I-A Vs I-B P2: I-A Vs II
Statistical Analysis

Statistical analyses were performed using SPSS (Statistical Package for Social Sciences Inc., Chicago, IL, USA—Windows version 20.0). Data were tested for normal distribution and presented as means ± SD. A Student’s t-test was applied to compare between two groups, while paired t-test was used to compare continuous variables at the beginning and at the end of the study. Analysis of variance (one way ANOVA), was used to compare more than two groups. Chi-square test was performed for qualitative data and the results were presented as numbers and percentages. The level of statistical significance was determined at P < 0.05.

RESULTS

The control subjects had significantly higher weight and BMI z-score compared to the study subjects. There is marked increase in weight Z score (p= 0.02) and BMI (p=0.03) of patients at end in comparison to the start of the study. At the study beginning, 71% of patients had BMI<18.5, while at the end only 36% of patients had BMI<18.5.

There were a marked increase in serum level of vitamin D and calcium at the study end in comparison to their level at the beginning (p<0.001). While marked decrease in serum alkaline phosphatase (ALP) level was detected.

Table no (4): illustrate the daily nutritional intake in our patients with CD and their comparison to the recommended value/day, using 24-hour dietary recall questionnaires, analyzed by NutriSurvey 2007 program.

<table>
<thead>
<tr>
<th>Table 3. Biochemical bone indices at the start and end of study</th>
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<tr>
<td>CD casesat the beginning</td>
</tr>
<tr>
<td>N=50 Group I-A (mean± SD)</td>
</tr>
<tr>
<td>N=50 Group I-B (mean± SD)</td>
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<tr>
<td>(mean± SD)</td>
</tr>
<tr>
<td>Vit D (µg/l)</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
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<tr>
<td>Alkaline phosphatase (ALP) (mg/dl)</td>
</tr>
</tbody>
</table>

P1: I-A Vs I-B P2: I-A Vs II

<table>
<thead>
<tr>
<th>Table 4. Difference between daily consumption of nutrients and dietary reference intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily nutrients consumption</td>
</tr>
<tr>
<td>Carbohydrate</td>
</tr>
<tr>
<td>Fat</td>
</tr>
<tr>
<td>Protein</td>
</tr>
<tr>
<td>Energy</td>
</tr>
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</table>
protein (P < 0.01) and total protein (P < 0.01), but a markedly increased ingestion of vegetable (P < 0.01) and total (P < 0.01) lipid compared to controls.

**DISCUSSION**

The celiac disorder is one of the chronic, immune-associated enteropathies of the small intestine\(^\text{23}\). It has been recorded that following GFD strictly improves nutritional status\(^\text{24, 25}\). Although adherence to a GF diet for life leads to significantly improved or totally recovered intestinal mucosa, literature suggests that complete normalization of nutritional deficiencies does not happen following GFD. While this treatment is highly effective, strict following to GFD causes difficulties to patients in social, family and working contexts, worsen his/her quality of life\(^\text{26}\). Moreover dropping gluten-containing foods from the diet may lead to an unbalanced diet lacking in certain nutrients\(^\text{27}\). GF-diet was found to be defective in alimentary fiber and micronutrients\(^\text{28}\).

In general celiac disease, patients do not ingest the advisable amount of complex carbohydrates or fiber and have a decreased intake of different vitamins and minerals as reported by many researchers. These patients likely to have an increased supplement of energy from saturated, total fats and protein\(^\text{29, 30, 31}\).

Excluding gluten from their diet may cause a low intake of iron, fiber, niacin, folate, zinc and phosphorus possibly because CD patients usually feed grain foods prepared essentially from processed GF grains and starch. That is why it is essential to encourage that the patient adds enriched and whole gluten-free grains and products in the diet\(^\text{32}\).

The low quality of packaged GF foods is the cause of this defective nutritional value of the diet. These foods are generally identified as being of low nutritional value than their gluten-containing counterparts, also decreased the delicious taste of these dietary products forces the food producers to use an increased amount of lipids and salt in their formation. In addition, individuals simply making incorrect food choices. It is valuable to clarify that the metabolic hazards of consuming GF foods on metabolic indices, like the lipid profile and the glucose/insulin ratio, either on a short term or long term\(^\text{30, 33}\). Our results appear to mirror this hypothesis.

In other words, the GF food considered as a treatment that is easy in theory but complicated in a real application. The management of many of CD-linked features may need extra supplementation\(^\text{34, 35, 36}\).

An increase in celiac users leads to rising importance to generate high effective GF diet which is highly nutritious. An increasing number of GF foodstuff products from companies in the Europe, USA and Canada are accessible in health food and grocery shops that help to increase compliance so that the celiac disease children will have decreased morbidity and helps to reach their normal growth potential. Unfortunately, there are no such foodstuffs in Egypt.

So we designed and tested this diet that is healthy, in addition, to be free of gluten to prevent nutrient, vitamins and minerals deficiencies or excess.

Our gluten-Free meal was designed to be balanced diets, containing the six food groups with the proposed proportions of protein, carbohydrates and fats, so as to evaluate its effect on celiac disease patients’ nutritional status.

As regards anthropometric evaluation, marked improvement in weight of CD cases occurred, more than that their weight at the beginning. However, the height is comparable at the start and end of the study and this is an anticipated result due to the short period of the study. Some researches on young CD patients found different findings. Aurangzeb et al., examined 25 CD children and found that overweight was found in more than 20% of them\(^\text{37}\). Controversially, malnourishment was detected in less than 9% of these CD patients. A different work on 150 CD children reported decreased obesity and decreased overweight and increased underweight compared to normal individuals\(^\text{38}\).

There are few pediatric studies which assess the state of vitamin D in celiac disease patients. Even though subdermal vitamin precursors activation by sun-UVB rays is considered to be the fundamental basis for vitamin D metabolic status, the small bowel compromise may affect vitamin D absorption and activity\(^\text{39}\).

Celiac disease is a condition at high risk for secondary osteoporosis. osteopenia or
Osteoporosis are typically found in untreated adult symptomatic celiac disease with severe malabsorption syndrome but is detected in about 50% in suboptimal treated celiac patients, asymptomatic adult celiac patients and subclinical patients, too. So the cause of pathologic bone changes in celiac disease is multifactorial; however, two fundamental mechanisms are participating: chronic inflammation and intestinal malabsorption40.

Our 24-hour dietary recall questionnaires illustrated that no one of our children and adolescents CD patient’s subjects consumed fish or vitamin D fortified foodstuffs (ex. cereal, milk, and yogurt…etc) through the study, the highest source of vitamin D. Therefore, our findings increase argument regarding the dietary selections of celiac patients, recommending the demand to promote them to select right dietary choices, which would enhance their nutritional status and better defend them against non-communicable diseases (NCDs) at long term. The same results were found previously by Morreale et al., who highlighted the importance of encouraging patient with CD to select better food choices in agreeing with a Mediterranean diet that can help in primary and secondary prevention of major NCDs in the overall population41.

Bone health can be adversely affected in patients with CD. These patients need enough vitamin D and calcium supplementation, in addition to the detection of vitamin D levels and bone mineral density with regular follow-up to help prevent osteoporosis and fractures42. In our CD patients, it appeared that supplementation of vitamin D and calcium was not efficient enough, as our CD patients had vitamin D and calcium deficiency at the beginning of the study. This may be because the real food contains a whole range of minerals, vitamins, co-factors and enzymes that permit optimal use by the body in comparison to consuming single nutrients. Therefore, CD patients should get functional food ingredients and nutrients from a wide variety of whole foods for optimal nutrition and health well-being, not from dietary supplements43. For CD patients, our work concludes that it’s not what they eat, but what they absorb. Nutrient absorption may vary among foods. For example, calcium from broccoli and kale may be absorbed almost twice as well as calcium from milk; they are loaded with highly absorbable calcium and a host of other healthful nutrients44.

In the present study, a large number of our CD patients had vitamin D deficiency. There are marked rise in serum level of vitamin D, serum calcium and phosphorous levels at the study end in comparison to their levels at the beginning. While there is a significant decrease in the level of alkaline phosphatase.

Reduced levels of vitamin D, serum calcium and phosphorous are common in untreated celiac disease (CD) patients probably as a result of a loss of brush border proteins and enzymes needed for these nutrients absorption11.

Not only the removal of gluten from the diet but also insufficient ingestion of calcium-rich foods such as milk and dairy products are found in CD children dietary choices. This may be a result of associated intolerance to lactose in several CD cases at the start of therapy, in addition to the fact that CD patients give the priority to exclude gluten from the diet and as a result forget sufficient ingestion of calcium2.

Similarly, bone mineral density does not always normalize after exclusion of gluten from the diet; supplementation of vitamin D and calcium is recommended in these cases45. Impaired secretion of cholecystokinin due to villous atrophy, which is responsible for the absorptive mechanism of fat-soluble vitamins and substances, results in the reduction in vitamin D levels46.

Similar to our results Kalayci et al., found that in newly diagnosed CD patients, the mean calcium level was detected to be less than the patients who follow their diet strictly. Strict gluten avoidance enhances a significant rise in BMD. However, bone indices values still remained markedly decreased after 1 year of follow-up in some patients47.

Several Pediatric retrospective studies as Imam et al., and Villanueva et al., found vitamin D deficiency in celiac disease patients1,48. However, the small retrospective study by Villanueva et al. showed no difference in the average 25-OH D level in children with recently diagnosed celiac disease versus controls, although both groups demonstrated insufficient levels of 25-OH D.

Also in the cross-sectional study by Lerner et al., insufficient but comparable 25-OH D levels were noted in Israeli CD children as well as those
with nonspecific abdominal pain. Also Wessels et al., in a recent case-control study reported that about 27% (8/30) of children were vitamin D deficient (<20 ng/ml (50nmol/L)] at diagnosis and 25% (7/28) remained vitamin D deficient at 5-year follow up 

In agreement with our results Setty-Shah et al., who examined the effect of CD, on the state of vitamin D in prepubertal children reported a higher incident of vitamin D deficiency in CD children (27.3%) compared to the controls (18.4%), On the other hand, the normal-weight subjects showed no marked difference in their serum 25(OH) D concentration between the subgroups.

However, the prevalence of vitamin D deficiency (50 nmol/L) among healthy adolescents recorded in other studies range from 16% to 54%.

In contrast to our finding, Villanueva and his colleagues reported that there was no diversity in mean 25(OH) D level between non-obese normal children and non-obese CD patients. Similarly, no difference could be detected in BMI SDS between non-obese normal children and non-obese CD patients.

We recommend that CD patients should adhere to GFD strictly to permit adequate bone mineralization. Also, patients on strict GFD might be at danger of decreased BMD due to vitamin D insufficiency and decreased calcium ingestion, therefore strict gluten-free diet with recommended vitamin D supplementation and calcium ingestion should be encouraged, also we recommend the standard management of CD children should include dual x-ray absorptiometry to evaluate BMD.

There is no current guidance on the frequency of monitoring vitamin D levels for pediatric patients, but our practice reflects that monitoring serum vitamin D levels in the first year of diagnosis is essential as these patients are most vulnerable to developing mineral and vitamin deficiencies in this period. We suggest that Vitamin D serum levels should be investigated at diagnosis, and annually thereafter.

Also, those who were discovered to be deficient should be treated to maintain a normal vitamin D level higher than 30 ng/ml but less than 100 ng/ml.

In conclusion, in spite of our results require to be proved in greater population studies even at a national level, our findings increase awareness to the better dietary selections of CD individuals and to improve the nutritional value of their GF diet. These issues are serious because there are some opinions that the celiac disease may be accompanied by a more risk of major NCDs.

As our designed gluten-free meal were balanced, healthy diets, contain the six food groups with the proposed proportions of protein, carbohydrates and fats, and that the satisfaction of the diet was amazing, also both calcium and vitamin D levels rise significantly on treatment by the provided diet, so it proves to be efficient towards a better nutritive value, but more researches are required to understand the cause of this nutritional improvement and if it is related to change in gut flora or there are other causes.

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