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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2018 15(1): 207-214. **OPEN ACCESS**

A novel gluten-free meal as a nutritional therapy for Iron deficiency anemia in children with celiac disease

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Celiac disease (CD) is a genetically determined autoimmune disease characterized by a long lasting intolerance to gluten. The gluten-free diet (GFD) is currently the only treatment for celiac disease; however, unbalanced selection of gluten-free products may lead to nutritional deficiencies. We aim in this study to explore the extent of iron deficiency anemia in children with celiac disease, and to assess the efficacy of introduction of a newly designed healthy, balanced, gluten-free meal on their clinical and biochemical parameters. A prospective study enrolling 50 children with CD diagnosed at out-patient clinic of pediatric gastroenterology, specialized pediatric hospital, Cairo University; by duodenal biopsy and serology, they were on gluten-free diet for at least one year. 40 healthy subjects were taken as a control group. Full History taking including symptoms of celiac disease and dietary history, anthropometric assessment and full clinical examination were performed for all the study population. We gave our balanced gluten-free meal to CD patients twice per day, for 3 successive months. Venous blood samples were withdrawn from patients once before the meal and another time after 3 months for assessment of hemoglobin concentration (Hb), serum iron, and ferritin and lipid profile. The mean values of anthropometric measurements, Hb level and iron indices were significantly lower in CD patients compared to controls. There was marked improvement in anthropometric measurements, increasing Hb (from 10.3±1.2 to 11.7±1.1) (P=0.02), MCV (from 68.2± 6.5 to 76.5±3.7) (P=0.02), ferritin (from 45.7±10.6 to 90.4±16.1) (P=0.01), serum iron (from 68.7±10.4 to 83.4±15.2) (P<0.001), HDL (from 64.5±15.1 to 81.6±19.3) (P=0.02), while there was a significant reduction of serum cholesterol (from 153.7±38.2 to 119.5 ±29.8) (P<0.001), triglyceride (from 111.7 ± 25.3 to 85.4 ± 21.1) (P=0.005) after 3 months of ingestion of the designed meal. The designed meal proved to be of high nutritional value in management of iron deficiency anemia in CD patient

Keywords: Celiac disease, Iron deficiency, Anemia, Gluten-free diet

INTRODUCTION

Celiac disease (CD) is defined as a chronic immune-mediated inflammation of the small intestine precipitated by introduction of gluten in diet to hereditarily predisposed subjects **(**Ludvigsson et al., 2013). This reaction prompts variable degrees of mucosal affection extending from mild lymphocytic infiltration to severe villous

atrophy (Hill et al., 2005), (Rubio-Tapia et al., 2013).

The small intestinal villous atrophy leads to malabsorption which in turn results in various nutritional deficiencies of macro- and micronutrients. Previous studies found that a significant percentage of CD patients were suffering from symptoms of one or more nutritional deficiency such as minerals, vitamins, calories and dietary fiber (Wierdsma et al., 2013), (Leffler and Schuppan, 2010).

There is wide clinical spectrum of CD, ranging from asymptomatic cases with only positive serological tests to symptomatic cases with intestinal symptoms (e.g. Diarrhea, abdominal pain, weight loss) or extra intestinal manifestations (e.g. osteoporosis, anemia, neurological manifestations) (Di Sabatino and Corazza, 2009).

Gluten-free diet is the mainstay of management of CD which prompts vanishing of the symptoms and sign (Caruso et al., 2013). However, clinical manifestations related to vitamins and minerals deficiency (i.e. Ca, iron, vitamins D and B12) may still present even the patients were on GFD (Abdulkarim and Murray, 2002). A growing number of gluten-free foodstuff products from companies in the USA, Canada and Europe are available in stores, conversely, there are no such foodstuffs or diets in Egypt.

Iron deficiency is a common presentation in children with CD, a finding that can be explained by that iron is absorbed primarily in the proximal part of the small bowel which is typically most severely affected by CD. The prevalence of CD in patients referred for endoscopy to evaluate iron deficiency anemia ranges from 3 to 12% (Theethira et al., 2014). It has also been reported that iron deficiency anemia refractory to oral supplementation is a major consequent in CD and that may help to exclude individuals with iron deficiency due to dietary factors or bleeding (Hershko and Patz, 2008). Yet another possible cause of iron deficiency is through loss of duodenal enterocytes, which are a storage site of ferritin (Wang and Pantopoulos, 2011).

The aim of the present prospective study was to investigate the prevalence of iron deficiency anemia in children with celiac disease and to assess the clinical and biochemical improvement in these patients following introduction of newly designed healthy, balanced, gluten-free meal.

MATERIALS AND METHODS

The present study enrolling fifty children with age range between (3-15 years) who were diagnosed as CD and followed up in the out-patient clinic of pediatric gastroenterology, Specialized Pediatric Hospital, Cairo University. The selected patients regularly attend the gastroenterology clinic and were on gluten-free diet for at least one year and were treated with mineral/iron supplementation therapy for (50 mg iron/ 5ml, once daily). Duodenal biopsy specimens were harvested to determine the grade of histological damage due to gluten-sensitive enteropathy. CD-associated antibodies, *i.e,* anti-tissue transglutaminase (tTG) antibodies, were determined. The diagnosis of CD was based on these histopathological and serological criteria. A representative sample of 40 healthy subjects (comparable for sex, age and BMI) was added as control. Informed written consent was obtained from all patients' parents and the protocol was approved by the Ethical Committee of National Research Centre and of Cairo University.

Clinical Evaluation

The clinical presentation of celiac disease was recorded and categorized into gastrointestinal symptoms (e.g. diarrhea, stomach pains, constipation, bloating) and extra-intestinal symptoms (e.g. neurologic symptoms, rash, poor growth, anemia, fatigue, arthralgia). Anthropometric assessment of body weight and height were performed. Body weight to the nearest 0.01 Kg was measured using Seca scale balance adult type, with minimal clothes for which no correction was made. Body height was measured to the nearest 0.1 cm using Holtain anthropometer. The body mass index (BMI) was calculated as weight/ (height in meters)^2 (Kg/m2).

Nutritional workup

24-hour Dietary Recall Questionnaires were taken from all patients, their analysis was done using NutriSurvey 2007 software

In this work, we gave Healthy, nutritional, balanced, safe and gluten-free meal (HNB-GFM) for CD patients twice per day. In brief, the HNB-GFM was free of gluten and hidden gluten. The energy derived from the package of one of the HNB-GFM manufactured from the six groups of food is 600 calories and balanced because 50-60, 10-25, and 10-30% of them are derived from carbohydrates, protein and fat, respectively. The package of one of the HNB-GFM provides children with the daily needs of the internationally vitamins and mineral Dietary Recommended Intake (Dietary Reference Intakes, 2001), and is

rich in dietary fiber as an effective food ingredient according to dietary recommendations. Finally, the package of one of the HNB-GFM contains 126.38 % and 157.97% of iron dietary recommended intake for children 4-8 years and 9-13 years, respectively.

Laboratory Workup

Three milliliters of fasting (8 h) venous blood samples were taken from each child participating in the study once before taking the meal and another time 3 months after introducing the meal, then the samples were divided into two parts: the first part was added to tube containing EDTA for Complete blood count determination including Hb concentration by cation-exchange resin and the second part was put in a serum separator tube. The separated serum was stored at -20C until the time of assay. Quantitative determination of serum iron was performed according to the method of Perrotta, 1984 (Perrotta, 1984). The kit was supplied from chemelex, Espana. Serum ferritin was measured by ferritin ELISA coated microtiter strips (Cat. No.:EK-310-25), Phonix Pharmaceuticals, INC. 330 Beach Road, Burlingame CA. Serum levels of IgA anti-tTG were determined by ELISA by use of a kit based on human recombinant antigen supplied by E-lab science Biotechnology Co., Ltd, WuHan, China-Catalog No (E-EL-H1815) in accordance with the method described by **Tosco et al, 2008**

Anemia was diagnosed following Hb levels according to (WHO values of Hemoglobin concentrations for the diagnosis of anemia and assessment of severity): (WHO, 2001).

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 in case of continuous data, 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. A Mann-Whitney U test (Wilcoxon) or in case of more than 2 variables, a Kruskal-Wallis test was applied for variables not found in a normal distribution. The level of statistical significance was determined at $P \leq 0.05$.

RESULTS

The mean age of CD patients was 8 years (age range between 3 and 15 years) with male sex predominance 65%. Villous atrophy was present in 95% of CD cases at the time of diagnosis, while 82.5% of cases had positive serology (Anti- tTG antibodies) **(table 1)**

Table 1: Demographic and diagnostic data of CD children at the beginning of the study

Total cases:	50 case	
Mean age of children	8 years	
Mean age of diagnosis	4.2 years (1.5- 12 year)	
Mean Duration of disease	3.8 years (1-9 years)	
Sex	Males	65%
	Female	35%
Habitat	Urban areas	42.5%
	Rural areas:	57.5%
Biopsy	Villous atrophy	95%
	No atrophy	5%
Serology(anti-tTG antibodies)	Positive serology	82.5%
	Negative serology	17.5

There was a significant improvement in GIT symptoms and manifestations of vitamin deficiency after 3 months of introduction of the gluten-free meal **(Table 2).**

The mean values of BMI, weight z-score, Height z-score, cholesterol, TG, Hb, iron and serum ferritin were significantly lower in CD patients compared to controls. There was also a significant improvement in anthropometric and biochemical parameters in CD patients at the end of the study in comparison to their state at enrollment **(Table 3).** About 16 CD patients (40%) were considered anemic (Hb<11 g/dl) at the beginning of the study according to WHO, this percentage has declined to 15% of cases (6 patients) after 3 months of the gluten-free meal consumption.

Table 2: Clinical status of children with celiac disease before and after gluten-free diet intervention:

Symptom	% of cases before gluten-free meal	% of cases after gluten-free meal	P value
GIT symptoms	72.5%	53.2%	0.02*
Poor weight gain	97.5%	84.1%	0.07
Symptoms of vitamin deficiency	63.2%	48.7%	0.05*
pallor	35%	19.5%	0.04*

***P < 0.05 is significant.** ****P < 0.01 is highly significant**

Table 3: Clinical and Biochemical data of CD patients (at beginning and at the end of study) and controls

	CD patients at the beginning of the study	CD patients at the end of the study	Controls	P1	P2
	Group I_a	Group I_b	Group II		
	(N= 50)	(N= 50)	(N= 40)		
	(mean± SD)	(mean± SD)	(mean± SD)		
Weight Z score	-3.1± 0.8	-2.5 ±0.6	0.8±0.3	0.03*	<0.001
Height Z score	-3.5 ± 0.7	-3.1 ± 0.6	-0.2± 0.1	0.1	<0.001
Body mass index (BMI)	16.2±3.9	17.7±4.2	19.1±4.3	0.04*	0.02
Hb (gm/dl)	10.3±1.2	11.7±1.1	12.23 ± 0.55	0.02	0.006
MCV (fl)	68.2± 6.5	76.5±3.7	78.5±4.8	0.03	0.002
Ferritin (ng/ml)	45.7±10.6	90.4±16.1	122.8 ± 21.3	0.01	0.004
Serum iron(µg/dl)	68.7±10.4	83.4±15.2	86.8±8.9	<0.001	<0.001
Cholesterol	153.7±38.2	119.5 ±29.8	129.3±16.5	<0.001**	0.01
Triglyceride	111.7 ± 25.3	85.4 ± 21.1	82.5±7.1	0.005**	0.004
HDL	64.5±15.1	81.6±19.3	82.3±4.4	0.02*	0.02
Blood glucose	85±12.2	91±10.8	94.3±8.7	0.2	0.1

P1: Group Ia Vs Group Ib P2: Group Ia Vs Group I *P < 0.05 is significant **P < 0.01 is highly significant.

Table 4: Daily nutritional intake in CD patients in comparison to dietary reference intake (DRI)

	Daily consumption of nutrients	Meal nutrients intake in comparison to DRI, %	HNB-GFM content
Energy	1138.75 kcal (389.10-1737.50)	55.92 (19.10-85.30)	629.76 kcal
Protein	54.23 g (10.80-91.00)	90.17 (17.90-151.40)	16.59g
Fat	45.66 g (16.10-87.90)	66.11 (23.40-127.30)	17.56 g
Carbohydrate	124.86 g (41.60-223.10)	42.96 (14.30-76.70)	93.04 g
Iron	7.98 mg (2.40-13.60)	77.56 (30.40-143.60)	12.64 mg

Mean (min.-Max.)

Our CD patient's 24-hour Dietary Recall Questionnaires analysis, using NutriSurvey 2007 software, illustrated low recommended nutrient daily intake for energy, protein, carbohydrates, dietary fiber, fat and several other nutrients include: poly-unsaturated fatty acids (PUFA), cholesterol, Vit. A, Vit. E (eq.), Vit. B1, Vit. B2, tot. folic .acid, Vit. C, sodium, potassium, calcium, magnesium, iron. For our CD patient's, The mean daily iron intake was 7.98 mg (ranged from 2.40-13.60 mg). It represents 77.56% (30.40-143.60%) (Dietary Reference Intakes, 2001). (Table 4)

DISCUSSION

Celiac disease (CD) is a chronic enteropathy, which affects approximately 1% of the general population (Fasano et al., 2003), (Maki et al., 2003). In childhood, CD is characterized with classical symptoms, such as diarrhea and malabsorption. Some children do not have obvious diarrhea, but only show weight loss and nutritional deficiencies with a consequent iron deficiency or macrocytic anemia, due to folate or vit. B12 deficiency (Leffler and Schuppan, 2010).

While treatment with life-long GFD causes a marked improvement or a complete restoration of the intestinal mucosa, the nutritional deficiencies do not completely normalize after GFD (Bascuñán et al., 2016), (Theethira and Dennis, 2015). Nutritional deficiencies were still described in patients with celiac disease who had adhered to GFD. Overcoming or minimizing these is of great importance for patients' health (Kovacev-zavisic et al., 2015), (Farnetti et al., and 2014).

Alimentary fiber was found to be deficient in GF diet due to avoidance of addition of natural foods rich in fiber (e.g. grain) and low content of fiber in GF products that's is made with refined flour or starches. Several vitamins and minerals are found to be poor in GF products as Vit. D, folate, Vit. B12, iron, zinc and calcium (Vici et al., 2016), (Caruso et al., 2013).The invention of good quality gluten free food with high nutritive value becomes an urgent need to face the high prevalence rate of celiac disease

Fifty children with CD who attended the gastroenterology clinic were enrolled in this study. As regards anthropometric evaluation, Weight and height were significantly lower in cases compared to controls, the height was comparable before and at the end of the study and this was an expected finding due to the short duration of the study, whereas there was a significant increase in weight and BMI of patients at the end of the study compared to their weight at the beginning. Studies in young patients with CD show divergent results. Aurangzeb et al, 2010 evaluated a group of twenty five children with CD and demonstrated that over 20% of these patients were overweight while less than 9% of them were malnourished (Aurangzeb et al., 2010).. On the opposite side, a study done on 150 children with CD showed less overweight and more wasting in contrast with healthy children (12% vs. 23.3% and 16% vs. 4.5%, respectively) (Brambilla et al., 2013).

The main finding of the present study was the presence of anemia in about 40 percent of cases with a significant decrease in Hb levels compared to normal subjects. The majority of CD patient group had iron deficiencies at enrollment in the study and its levels were significantly lower (68.7±10.4) compared to controls (86.8±8.9). No statistically significant difference in serum iron was found between males and females.

Iron is absorbed in the proximal small intestines and the absorption depends upon several factors, including an intact mucosal surface and intestinal acidity. Iron deficiency primarily results in the CD patients, with consequent iron-deficiency anemia, for its impaired absorption as a result of the villous atrophy of the intestinal mucosa [(Fernández-bañares et al., 2009). However, the CD patients do not often respond well to iron supplementation treatment. Many gluten-free products containing phytic acid (myo-inositol hexakisphosphate) and its salts (phytates) are the main storage form of phosphate in seeds and grains that reduces the bioavailability and chelates certain nutrients, such as iron, calcium, manganese, and zinc (Petry et al., 2010). Reports have also appeared in celiac disease with occult gastrointestinal bleeding as a cause of iron deficiency anemia (Fine, 1996). In a study of young males presenting with iron deficiency anemia, peptic ulcer disease was the most common finding in 30% (Mant et al., 2006), (Carter et al., 2013), meanwhile, celiac disease was subsequently diagnosed in 4% of these cases, adding a common cause of gastro-intestinal blood loss associated iron deficiency.

Generally, there are several different substitutes for gluten-containing cereals and derived products include: soybeans, millet, buckwheat, amaranth, quinoa, green banana flour, sorghum, teff and derived products (Fric et al., 2011), but they are not available in Egypt. Our nutritional questionnaire illustrates that our CD patients have been used three different wheat substitutes in their daily meals include: rice, potatoes, maize and derived products. As to the

nutritional profile, our CD patient's 24-hour Dietary Recall Questionnaires analysis, using NutriSurvey 2007 software, illustrated low recommended nutrient daily intake for energy, protein, carbohydrates, fat and several other nutrients (eg. Iron), the daily intake did not reach the recommended level according to the age and sex nutrients recommended tables, (table no 4).

Our studied CD patients were on gluten-free diet as well as mineral/iron supplementation therapy for at least 1 year. Even so, they had statistically significant iron deficiency anemia, which can be explained by that nutrient absorption can be impaired in case the patient does not follow a strict diet and presents with damaged intestinal mucosa (Simpson and Thompson, 2012), (Roma et al., 2010).

For our CD children and adolescents, on introducing our HNB-GFM, the average daily intake was 5.32 mg iron per day, in addition to 50 mg iron as iron supplementation and 12.64 mg from HNB-GFM.

Iron Dietary Reference Intakes (DRIs) are 10 mg/d for children (4–8 y) and 8 mg/d males and females (9–13 y) (Dietary Reference Intakes, 2001). During our experimental period, the total daily iron intake for the CD children and adolescents patients was 55.32 and consuming our HNB-GFM increase it to 67.96 mg iron/day, it represents 679.6 and 849.5 % from the dietary reference intake (DRI) for children aged (4–8 y) and males or females aged (9–13 y), respectively. Iron in excess of daily needs is stored in ferritin molecules, which hold up to 4,500 iron atoms each. Normally, dietary intake offsets daily loss iron loss (about 1 to 1.5 milligrams per day), therefore, one gram of stored iron (1,000 milligrams) is usually adequate to meet all foreseeable needs (Smith et al., 2015).

CD children and adolescents in our study were found to have mean serum ferritin 45.7±10.6 ng/mL at the beginning of the study and 82.5% and 40% of them had positive serologic screening for CD and anemia, respectively (Table 4). Clinically, it is recommended to check serum ferritin in the work-up of CD and to supplement with oral iron if the ferritin level is below 45–50 ng/mL. The lower serum ferritin can predict anemia or iron deficiency anemia (Earley, 2003), (Silber et al., 2004). Our data before HNB-GFM intervention had found that serum iron, ferritin and Hb are 68.7±10.4 (µg/dl), 45.7±10.6 (ng/ml) and 10.3±1.2 (gm/dl), respectively. It improved significantly after HNB-GFM intervention to 83.4±15.2 (µg serum iron /dl), 90.4±16.1 (ng ferritin /ml) 11.7±1.1(gm Hb/dl). So it seems that CD patients were not able to benefit from tolerate supplemental iron alone. That raises an interesting possibility that the HNB-GFD might have helped CD children and adolescents' gut to regain its absorptive function that leads to increase in serum iron, ferritin and Hb. There was no change in medication or other medical conditions that could explain this improvement.

CONCLUSION

HNB-GFM positive effects were observed in CD patients enrolled in the study: increase in body mass index, higher energy intakes, reducing cholesterol, triglyceride and increasing HDL, blood glucose, Hb, MCV, ferritin, serum iron, moderates the risk of the associated complications. The designed meal proved to be novel, healthy, nutritional, balanced, gluten-free meal and that the overall acceptability of the diet was fabulous as it proved to be tasty and accepted by the participated patients

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CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

Authors are thankful to the National Research Centre, Giza, Egypt and The Specialized Pediatric Hospital, Cairo University for their support to carry out this study

AUTHOR CONTRIBUTIONS

MAE, MAZ, GME did clinical examination to the CD patients. AEE invented the healthy, nutritional gluten-free meal. AAE, MAE, NAM wrote the manuscript, AAE, NAM reviewed the manuscript. MA performed the laboratory work. All authors read and approved the final version.

REFERENCES

Abdulkarim AS, Murray JA, 2002. Celiac disease. Curr Treat Options Gastroenterol. 5: 27– 38.

-Aurangzeb B, Leach S T, Lemberg D A, et al., 2010. Nutritional status of children with coeliac disease. Acta Paediatr. 99, 1020–1025.

-Brambilla P, Picca M, Dilillo D et al., 2013. Changes of body mass index in celiac children on a gluten-free diet. Nutr. Metab. Cardiovasc. Dis. 23, 177–182.

-Bascuñán KA, Vespa MC, Araya M., 2017. Celiac disease: understanding the gluten-free diet. Eur J Nutr. Mar; 56(2):449-459

Carter D, Levi G, Tzur D, Novis B, Avidan B., 2013. Prevalence and predictive factors for gastrointestinal pathology in young men evaluated for iron deficiency anemia. Dig Dis Sci. 58: 1299-1305.

Caruso R, Pallone F, Stasi E, Romeo S, Monteleone G. 2013 Dec. Appropriate nutrient supplementation in celiac disease. Ann Med. 45(8):522-31.

Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc (2001), Food and Nutrition Board, Institute of Medicine, National Academies. Retrieved from https://goo.gl/Xshf48

Di Sabatino A, Corazza GR., 2009. Coeliac disease. Lancet. 373: 1480 – 93.

Earley CJ., 2003. Clinical practice. Restless legs syndrome. N Engl J Med. 348(21):2103–9.

Farnetti S, Zocco MA, Garcovich M, Gasbarrini A, Capristo E., 2014 Nov. Functional and metabolic disorders in celiac disease: new implications for nutritional treatment. J Med Food. 17(11):1159-64.

Fasano A, Berti I, Gerarduzzi T, Not T, Colletti RB, Drago S, et al., 2003. Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: a large multicenter study. Arch Intern Med. 163: 286 – 92.

Fernández-bañares F, Monzón h, forné M., 2009. A short review of malabsorption and anemia. World J Gastroenterol. 15: 4644-4652.

Fine KD, 1996. The prevalence of occult gastrointestinal bleeding in celiac sprue. N Engl J Med. 334: 1163-1167

Fric P, Gabrovska D, Nevoral J., 2011. Celiac disease, gluten-free diet, and oats. Nutr Rev. 69: 107-115.

Hershko C, Patz J., 2008 Dec. Ironing out the mechanism of anemia in celiac disease. Haematologica. 93(12):1761-5.

-Hill ID, Dirks MH, Liptak GS, et al., 2005. Guideline for the diagnosis and treatment of celiac disease in children: recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. J Pediatr Gastroenterol Nutr. 40:1-19.

Kovacev-zavisic B, Icin T, Novakovic-paro J., 2015. Osteoporosis reversibility in a patient with celiac disease and primary autoimmune hypothyroidism on gluten free diet--a case report. Vojnosanit Pregl. 72: 72-76.

-Leffler DA, Schuppan D., 2010. Update on serological testing in celiac disease. Am J Gastroenterol; 105: 2520-2524.

-Ludvigsson JF, Leffler DA, Bai JC, BIAGI F, et al., 2013. The Oslo definitions for coeliac disease and related terms. Gut; 62: 43-52.

Maki M, Mustalahti K, Kokkonen J, Kulmala P, Haapalahti M, Karttunen T, et al., 2003. Prevalence of celiac disease among children in Finland. N Engl J Med. 348: 2517– 24.

-Mant MJ, Bain VG, Maguire CG, Murland K, Yacyshyn BR., 2006. Prevalence of occult gastrointestinal bleeding in celiac disease. Clin Gastroenterol Hepatol. 4: 451-454

Perrotta G. Iron – binding capacity. Clin Chem 1984:1063-1065.

Petry N, Egli I, Zeder C, Walczyk T, Hurrell R., 2010. Polyphenols and phytic acid contribute to the low iron bioavailability from common beans in young women. J Nutr. 140: 1977-1982.

Roma E, Roubani A, Kolia E, Panayiotou J, Zellos A, Syriopoulou VP., 2010. Dietary compliance and life style of children with coeliac disease. Journal of Human Nutrition and Dietetics. 23: 176-182.

Rubio-Tapia A, Hill ID, Kelly CP, et al., 2013. ACG clinical guidelines: diagnosis and management of celiac disease. Am J Gastroenterol.108:656-76.

Silber MH, Ehrenberg BL, Allen RP, Buchfuhrer MJ, Earley CJ, Hening WA, et al., 2004. An algorithm for the management of restless legs syndrome. Mayo Clin Proc.79 (7):916–22

Simpson S, Thompson T., 2012. Nutrition assessment in celiac disease. Gastrointest Endoscopy Clin N Am. 22: 797-809.

Smith JW, Holmes ME1, McAllister MJ., 2015. Nutritional Considerations for Performance in Young Athletes. J Sports Med (Hindawi Publ Corp). 2015:734649.

Theethira TG, Dennis M., 2015. Celiac disease

and the gluten-free diet: consequences and recommendations for improvement. Dig Dis. 33(2):175-82.

Theethira TG, Dennis M, Leffler DA., 2014 Feb. Nutritional consequences of celiac disease and the gluten-free diet. Expert Rev Gastroenterol Hepatol. 8(2):123-9

Tosco A, Maglio M, Paparo F, Rapacciuolo L, Sannino A, Miele E, et al., 2008 Sep. Immunoglobulin A anti-tissue transglutaminase antibody deposits in the small intestinal mucosa of children with no villous atrophy. J Pediatr Gastroenterol Nutr. 47(3):293-8.

Vici G, Belli L, Biondi M, Polzonetti V., 2016 Dec. Gluten free diet and nutrient deficiencies: A review. Clin Nutr. 35(6):1236-1241

Wang J, Pantopoulos K., 2011. Regulation of cellular iron metabolism. Biochem J. 434(3):365-81

WHO, UNICEF, UNU. Iron deficiency anemia: assessment, prevention and control, a guide for programme managers. Geneva, World Health Organization, 2001. Available at http://www.who.int/nutrition/publications/micronutrients/anaemia_iron_deficiency/WHO_NHD_01.3/en/index.html

Wierdsma NJ, van Bokhorst-de van der Schueren MA, Berkenpas M, Mulder CJ, van Bodegraven AA., 2013 Sep. Vitamin and mineral deficiencies are highly prevalent in newly diagnosed celiac disease patients. Nutrients. 30; 5(10):3975-92.