Serum homocysteine levels in children with coeliac disease

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Abstract

Background: Vitamin B status evaluation of patients with coeliac disease reveals that, compared to healthy controls, these patients are more prone to develop deficiencies of vitamin B_1, B_2, B_6, folate and vitamin B_{12}, all of which play an important role in homocysteine metabolism.

Objectives: To study the serum homocysteine levels in children with coeliac disease and to analyse in comparison to a few related markers.

Method: A cross-sectional study was done among 36 newly diagnosed children (44.4 % females) with coeliac disease (mean age: 7.72 ± 3.26 years); and 36 age-and-sex-matched controls. Serum concentrations of homocysteine, C-reactive protein (CRP), vitamin B_{12}, and folic acid were determined after overnight fasting. Comparisons between the two groups were performed by Student's t-test or Mann–Whitney U-test, for continuous variables. Pearson's Chi-square test or Fisher's exact test was used for categorical variables. $p<0.05$ was considered significant.

Results: Biochemical assessment of the two groups revealed significantly increased serum levels of homocysteine (mean 14.1 ± 1.35 µmol/L) and CRP levels (mean 7.3 ± 2.21 mg/dL) and decreased vitamin B_{12} levels (mean 127.11± 16.16 pg/dL) and folic acid levels (mean 5.96 ± 4.17 ng/dl) as compared to controls. There was a negative correlation of statistical significance between vitamin B_{12} levels, folic acid levels and homocysteine levels. Furthermore, there was a statistically significant positive correlation between CRP and homocysteine levels.

Conclusions: There were significantly decreased levels of vitamin B_{12} and folic acid in children with coeliac disease as compared to controls. There were also significantly increased levels of CRP and serum homocysteine levels along with correlation between vitamin B_{12} levels, folic acid levels, CRP levels and homocysteine levels.

(Key words: Coeliac disease, Homocysteine, Vitamin B_{12}, Folic acid, CRP)

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Introduction

Coeliac disease is characterized by gluten dependent clinical manifestations, specific autoantibodies, HLA DQ-2 and DQ-8 haplotypes and enteropathy[1]. Usual age of onset coincides with weaning with wheat products and that occurs around 6-24 months of age[1]. Treatment is undoubtedly lifelong strict gluten restriction i.e. diet free of wheat, barley and rye[2]. Coeliac disease occurs concurrently with various comorbidities of which type-I diabetes mellitus is an important one[3]. Children with coeliac disease also show a four-fold risk for development of systemic hypertension[3]. Thus, they are more prone to develop metabolic syndrome and hence cardiovascular disorders[3]. Vitamin B status evaluation of children with coeliac disease reveals that they are more prone to develop deficiencies of vitamin B_1, B_2, B6, folate and vitamin B_{12} as compared to healthy controls, all of which have a role in homocysteine metabolism[4].

Objectives

To study the serum homocysteine levels in children with coeliac disease and to analyse in comparison to a few related markers.

Method

A cross-sectional study was carried out in the Department of Paediatrics from 2015-2017. Sample size was calculated on the basis of a previous study by Valente FX, *et al*[5]. A total of 36 newly diagnosed coeliac patients were enrolled (mean age 7.72 ± 3.26 years; 44.4 % females) from children attending the paediatric gastroenterology clinic, paediatric outpatient department and wards. Thirty six controls, matched by sex and age, were enrolled from among the children coming for routine vaccinations, minor afebrile illnesses and healthy siblings of the cases. After taking informed consent, serum concentrations of homocysteine,

vitamin B_{12} and folic acid were determined after overnight fasting.

Statistical analysis: Comparisons between the two groups were performed by Student's t-test or Mann–Whitney U-test for continuous variables. Pearson's Chi-square test or Fisher's exact test was used for categorical variables. $p<0.05$ was considered significant.

Ethical issues: Ethical clearance was obtained from the Institutional Ethics Committee of PGIMER, Dr RML Hospital, New Delhi (IEC no. T. P (MD/MS) (20/2015) / IEC/PGIMER/ RML 4903). Written informed consent was obtained from the parents of the children participating in the study.

Results

Table 1 shows the levels of serum homocysteine, vitamin B12, folic acid and CRP in cases and controls.

Table 1: ***Levels of serum homocysteine, vitamin B_{12}, folic acid and CRP in cases and controls***

Parameter	Cases	Controls	p-value
Homocysteine (µmol/L)			
Normal n (%)	15 (41.7)	31 (86.1)	<0.05
High n (%)	21 (58.3)	05 (13.9)	
Mean ± Standard deviation (SD)	14.1 ± 1.35	6.78 ± 2.41	<0.05
Vitamin B_{12} (pg/mL)			
Low n (%)	31 (86.1)	01 (02.7)	<0.05
Normal n (%)	05 (13.9)	35 (97.3)	
Mean	127.11± 16.16	381.19 ± 53.51	<0.05
Folic acid (ng/mL)			
Low n (%)	28 (77.8)	03 (08.3)	<0.05
Normal n (%)	08 (22.2)	33 (91.7)	
Mean ± SD	5.96 ± 4.17	18.03 ± 5.46	<0.05
CRP (mg/dL)			
Normal n (%)	10 (27.8)	30 (83.3)	<0.05
High n (%)	26 (72.2)	06 (16.7)	
Mean ± SD	7.3 ± 2.21	1.02 ± 2.46	<0.05

Majority of the cases were less than 5 years old with a mean age of 7.72 ± 3.26 years, with 44.4% females and 55.6% male subjects. The control population was comparable in age and sex distribution. Biochemical assessment (Table 1) of the two groups revealed significantly increased serum levels of homocysteine among the cases with a mean of 14.1 ± 1.35 µmol/L as compared to the controls with a mean of 6.78 ± 2.41 µmol/L ($p<0.05$). Vitamin B_{12} levels were low in cases with a mean of 127.11 ± 16.16 pg/dL compared to the controls with a mean of 381.19 ± 53.51 pg/dL ($p<0.05$) and folic acid levels were low in cases with a mean of 5.96 ± 4.17 ng/dL compared to the controls with a mean of 18.039 ± 5.46 ng/dL ($p<0.05$). C-reactive protein levels were increased among the cases with a mean of 7.3 ± 2.21 mg/dL compared to controls with a mean of 1.02 ± 2.46 mg/dL ($p<0.05$).

Discussion

Although most children with coeliac disease have clinical remission with treatment, histological changes sometimes persist for a longer duration in some patients even with good dietary compliance[6]. Thus, screening these patients for markers of metabolic derangement should be undertaken at diagnosis as well as on follow up. Traditional risk factors for cardiovascular morbidities like presence of metabolic syndrome, inflammatory mediators, etc[7], are seen to be present in childhood persisting into young adulthood[8-11]. Children with chronic inflammatory diseases with numerous associated comorbidities like coeliac disease are expected to display these risk factors.

CRP is an important marker of inflammation with a strong linear relation with the incidence of cardiovascular events[12]. Vitamin B status evaluation of children with coeliac disease reveals that they are more prone to develop deficiencies of vitamin B_1, B_2, B_6, folate and vitamin B_{12} as compared to healthy controls[4,13]. This is true even in patients following a strict gluten free diet with biopsy proven remission[13]. A study done by Hallert C, *et al*[14] revealed that more than half of the children with coeliac disease with biopsy proven remission had signs indicative of poor vitamin status. Gluten free diet replacing wheat is often produced with refined flours without any fortification. Thus, it lacks many vitamins including folic acid and vitamin B_{12}[15-17]. Results have shown that coeliac patients have up to five times increased risk of developing folate deficiency than healthy individuals with up to 20-40% of

coeliac patients demonstrating low folate and vitamin B_{12} levels[18].

In our study, 72.2% of the cases had increased CRP whereas only 16.7% of the controls had increased CRP. In cases, 86.1% and 77.8% had decreased levels of vitamin B_{12} and folic acid respectively while among controls, 2.7% had decreased vitamin B_{12} levels and 8.3% had decreased levels of folic acid ($p<0.05$). Vitamin B_2, B_6, B_{12} and folic acid all have a role in homocysteine metabolism[19-21], an intermediate in methionine synthesis. These are substrates and essential factors for enzymes in its metabolism and their inadequate intake happens to be the most common cause of higher concentration of homocysteine in children with coeliac disease [22-24]. Occurrence of genetic abnormalities and mutations leading to hyper-homocysteinaemia have been found to be no different than in the general population[23-25]. American Heart Association recommends screening of patients with malnutrition and malabsorption syndromes for hyper-homocysteinaemia[26]. In untreated patients hyper-homocysteinaemia has been linked to osteoporosis and cardiovascular disease[27-31]. Homocysteine may be involved in pro-thrombotic mechanisms and has toxic effects on the vascular endothelium. Homocysteine levels have been found to be increased in patients with coeliac disease even at diagnosis and has been seen to decrease with gluten free diet[5,32]

In our study, serum homocysteine was increased in 21 (58.3%) cases and 5 (13.9%) controls and the difference was statistically significant ($p<0.05$). Among the 21 patients with increased homocysteine levels, 19 (90.5%) had decreased vitamin B12 levels, 10 (71.4%) had decreased folic acid levels and 15 (71.4%) had increased CRP levels. We found a negative correlation of statistical significance between vitamin B12 levels, folic acid levels and homocysteine levels. We also found a statistically significant positive correlation between CRP and homocysteine levels. Hence long term vitamin deficiency commonly seen in children having coeliac disease should be frequently monitored and treated with dietary supplementation. Further, associated serum homocysteine levels need to be monitored and followed up accordingly.

We could not carry out follow up assessment and comparison after treatment with gluten-free diet and vitamin supplements. This is a limitation

Conclusions

There were statistically significantly decreased levels of vitamin B_{12} and folic acid in this cohort of children with coeliac disease as compared to controls. There were also statistically significantly increased levels of CRP and serum homocysteine levels along with correlation between vitamin B_{12} levels, folic acid levels, CRP, and homocysteine levels.

References

1. Braski D, Troncone R, Fasano A. Coeliac disease (Gluten-Sensitive Enteropathy). In: Kleigman. R.M, editor. Nelson Textbook of Pediatrics. 20 ed. Canada: Elsevier; 2015.p. 1835-38.

2. Nicholson JF, Pesce MA. Reference ranges for laboratory tests and procedures. In: Behrman R. E, Kleigman. R.M, Jenson H.B, editors. Nelson Textbook of Pediatrics. 17 ed. India: Saunders; 2004. p. 1835-38.

3. Tortora R, Capone P, De Stefano G, Imper atore N, Gerbino N, Donetto S *et al.* Metabolic syndrome in patients with coeliac disease on a gluten-free diet. *Alimentary Pharmacology and Therapeutics* 2015; **41** (suppl 4): 352-9. https://doi.org/10.1111/apt.13062 PMid: 25581084

4. De Marchi S, Chiarioni G, Prior M, Young adults with celiac disease may be at increased risk of early atherosclerosis; *Alimentary Pharmacology and Therapeutics* 2013; **38**:162-9. https://doi.org/10.1111/apt.12360 PMid: 23730933

5. Valente FX, Campos Tdo N, Moraes LF, Hermsdorff HH, Cardoso Lde M, Pinheiro-Sant'Ana HM *et al.* B vitamins related to homocysteine metabolism in adult coeliac disease patients: a cross-sectional study. *Nutrition Journal* 2015; **14**:110. https://doi.org/10.1186/s12937-015-0099-8 PMid: 26487487 PMCid: PMC4617727

6. Lee SK, Lo W, Memeo L, Rotterdam H, Green PH. Duodenal histology in patients with coeliac disease after treatment with a gluten-free diet. *Gastrointestinal Endoscopy* 2003; **57**(suppl 2): 187-91. https://doi.org/10.1067/mge.2003.54 PMid: 12556782

7. Berenson GS, Srinivasan SR, Bao W, Newman WP, Tracy RE, Wattigney WA.

Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. *New England Journal of Medicine* 1998; **338**(suppl 23): 1650-6. https://doi.org/10.1056/NEJM199806043382302 PMid: 9614255

8. Webber LS, Voors AW, Srinivasan SR, Frerichs RR, Berenson GS. Occurrence in children of multiple risk factors for coronary artery disease: the Bogalusa heart study. *Preventive Medicine* 1979; **8**(suppl 3): 407-18. https://doi.org/10.1016/00917435(79)90018-5

9. Khoury P, Morrison JA, Kelly K, Mellies M, Horvitz R, Glueck CJ. Clustering and interrelationships of coronary heart disease risk factors in schoolchildren, ages 6-19. *American Journal of Epidemiology* 1980; **112**(suppl 4):524-38. https://doi.org/10.1093/oxfordjournals.aje.a113022 PMid: 7424901

10. Smoak CG, Burke GL, Webber LS, Harsha DW, Srinivasan SR, Berenson GS. Relation of obesity to clustering of cardiovascular disease risk factors in children and young adults. The Bogalusa Heart Study. *American Journal of Epidemiology* 1987; **125**(suppl 3): 364-72. https://doi.org/10.1093/oxfordjournals.aje.a114543 PMid: 3544817

11. Bao W, Srinivasan SR, Wattigney WA, Berenson GS. Persistence of multiple cardiovascular risk clustering related to syndrome X from childhood to young adulthood. The Bogalusa Heart Study. *Archives of Internal Medicine* 1994; **154**(suppl 16): 1842-7. https://doi.org/10.1001/archinte.154.16.1842 PMid: 8053753

12. Ridker PM, Rifai N, Rose L, Buring JE, Cook NR. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *New England Journal of Medicine* 2002; **347** (suppl 20): 1557-65. https://doi.org/10.1056/NEJMoa021993 PMid: 12432042

13. Luciani A, Villella VR, Vasaturo A, Giardino I, Pettoello-Mantovani M, Guido S *et al.* Lysosomal accumulation of gliadin p31-43 peptide induces oxidative stress and tissue transglutaminase-mediated PPARgamma downregulation in intestinal epithelial cells and coeliac mucosa. *Gut* 2010; **59**(suppl 3): 311-9. https://doi.org/10.1136/gut.2009.183608 PMid: 19951908

14. 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**: 1333–9. https://doi.org/10.1046/j.13652036.2002.01283.x PMid: 12144584

15. Thompson T. Thiamin, riboflavin, and niacin contents of the gluten-free diet: is there cause for concern? *Journal of the American Dietetic Association* 1999; **99**(suppl 7): 858-62. https://doi.org/10.1016/S00028223(99)00205-9

16. Thompson T. Folate, iron, and dietary fibre contents of the gluten-free diet. *Journal of the American Dietetic Association* 2000; **100**(suppl 11): 1389-96. https://doi.org/10.1016/S00028223(00)00386-2

17. Bardella MT, Fredella C, Prampolini L, Molteni N, Giunta AM, Bianchi PA. Body composition and dietary intakes in adult coeliac disease patients consuming a strict gluten-free diet. *American Journal of Clinical Nutrition* 2000; **72**(suppl 4): 937-9. https://doi.org/10.1093/ajcn/72.4.937 PMid: 11010934

18. Mariani P, Viti MG, Montuori M, La Vecchia A, Cipolletta E, Calvani L *et al.* The gluten-free diet: a nutritional risk factor for adolescents with coeliac disease? *Journal of Pediatric Gastroenterology and Nutrition* 1998; **27(**suppl 5): 519-23.

https://doi.org/10.1097/00005176199811000-00004
PMid: 9822315

19. Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomised trials. Homocysteine Lowering Trialists' Collaboration. *British Medical Journal* 1998; **316**(suppl 7135): 894-8.
https://doi.org/10.1136/bmj.316.7135.894
PMCid: PMC28491

20. McKinley MC, McNulty H, McPartlin J, Strain JJ, Pentieva K, Ward M *et al.* Low-dose vitamin B-6 effectively lowers fasting plasma homocysteine in healthy elderly persons who are folate and riboflavin replete. *American Journal of Clinical Nutrition* 2001; **73**(suppl 4): 759-64.
https://doi.org/10.1093/ajcn/73.4.759
PMid: 11273851

21. McNulty H, Dowey le RC, Strain JJ, Dunne A, Ward M, Molloy AM *et al.* Riboflavin lowers homocysteine in individuals homozygous for the MTHFR 677C->T polymorphism. *Circulation* 2006; **113**(suppl 1): 74-80.
https://doi.org/10.1161/CIRCULATIONAHA.105.580332
PMid: 16380544

22. Dickey W, Ward M, Whittle CR, Kelly MT, Pentieva K, Horigan G *et al.* Homocysteine and related B-vitamin status in coeliac disease: Effects of gluten exclusion and histological recovery. *Scandinavian Journal of Gastroenterology* 2008; **43**(suppl 6): 682-8.
https://doi.org/10.1080/00365520701881118
PMid: 18569985

23. Hadithi M, Mulder CJ, Stam F, Azizi J, Crusius JB, Peña AS *et al.* Effect of B vitamin supplementation on plasma homocysteine levels in coeliac disease. *World Journal of Gastroenterology* 2009; **15**(suppl 8): 955-60.
https://doi.org/10.3748/wjg.15.955
PMid: 19248194 PMCid: PMC2653396

24. McNulty H, Scott JM. Intake and status of folate and related B-vitamins: Considerations and challenges in achieving optimal status. *British Journal of Nutrition* 2008; **99**(suppl 3): S48-54.
https://doi.org/10.1017/S0007114508006855
PMid: 18598588

25. Hozyasz KK, Mostowska A, Szaflarska-Poplawska A, Lianeri M, Jagodzinski PP. Polymorphic variants of genes involved in homocysteine metabolism in coeliac disease. *Molecular Biology Reports* 2012; **39**(suppl 3): 3123-30.
https://doi.org/10.1007/s11033-011-1077-7
PMid: 21688148 PMCid: PMC3271217

26. Malinow MR, Bostom AG, Krauss RM. Homocysteine, diet, and cardiovascular diseases: a statement for healthcare professionals from the Nutrition Committee, American Heart Association. *Circulation* 1999; **99**(suppl 1): 178-82.
https://doi.org/10.1161/01.CIR.99.1.178
PMid: 9884399

27. West J, Logan RF, Card TR, Smith C, Hubbard R. Risk of vascular disease in adults with diagnosed coeliac disease: a population-based study. *Alimentary Pharmacology and Therapeutics* 2004; **20**:73-9.
https://doi.org/10.1111/j.13652036.2004.02008.x
PMid: 15225173

28. van Meurs JB, Dhonukshe Rutten RA, Pluijm SM, van der Klift M, de Jonge R, Lindemans J *et al.* Homocysteine levels and the risk of osteoporotic fracture. *New England Journal of Medicine* 2004; **350**(suppl 20): 2033-41.
https://doi.org/10.1056/NEJMoa032546
PMid: 15141041

29. Gefel D, Doncheva M, Ben-Valid E, el Wahab-Daraushe A, Lugassy G, Sela BA. Recurrent stroke in a young patient with coeliac disease and hyper-homocysteinaemia. *Israel Medical Association Journal* 2002; **4**(suppl 3): 222-3.

30. Lim PO, Tzemos N, Farquharson CA, An derson JE, Deegan P, MacWalter RS *et al.* Reversible hypertension following coeliac disease treatment: the role of moderate hyper-homocysteinaemia and vascular endothelial dysfunction. *Journal of*

Human Hypertension 2002; **16**(suppl 6): 411-5.
https://doi.org/10.1038/sj.jhh.1001404
PMid: 12037696

31. McLean RR, Jacques PF, Selhub J, Tucker KL, Samelson EJ, Broe KE *et al.* Homocysteine as a predictive factor for hip fracture in older persons. *New England Journal of Medicine* 2004; **350**(suppl 20): 2042-9.
https://doi.org/10.1056/NEJMoa032739
PMid: 15141042

32. Saibeni S, Lecchi A, Meucci G, Cattaneo M, Tagliabue L, Rondonotti E *et al*. Prevalence of hyper-homocysteinaemia in adult gluten-sensitive enteropathy at diagnosis: role of B12, folate, and genetics. *Clinical Gastroenterology and Hepatology*. 2005; **3**(suppl 6): 574-80.
https://doi.org/10.1016/S15423565(05)00022-4