Hematological Findings among Jordanian Children with Celiac Disease at Presentation: A Retrospective Analytical Study

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ABSTRACT

Objective: To describe the hematological findings among children with celiac disease on presentation.

Methods: This is a retrospective review which was conducted in the Pediatric Gastroenterology Department at Queen Rania Al Abdullah Hospital for Children. The records of children with celiac disease were reviewed between January 2006 and December 2012. The age of children included in the study was less or equal to 14 years on diagnosis. Complete blood count, serum ferritin, folate, vitamin B12, prothrombin time, partial prothrombin time, international normalized ratio, and tissue glutaminase antibody (IgA and IgG) were performed for all patients prior to the diagnosis. Upper gastrointestinal endoscopy was performed for all children included in the study and multiple duodenal biopsies samples were obtained during the procedure for routine histological analysis.

Results: A total of 111 children were included in the study; 53 (47.7%) were males and 58 (52.3%) were females. The mean age at diagnosis was 9 years. All children had positive tissue glutaminase antibody IgA, IgG, or both. Eleven children had leukopenia, 13 had lymphopenia, two had neutropenia, while eight had eosinophilia. Thirty four (30.4%) children had anemia. Twenty eight (25.2%) children had serum ferritin less than 7ng/ml, 30 (27%) had serum folate less than 5ng/ml and 9 (8.1%) children had vitamin B12 less than 200 pg/ml. Seventeen (60.7%) children who had serum ferritin less than 7ng/ml had also low serum folate (p=0.001). Four (36.4%) out of eleven children with leukopenia had serum folate below 5ng/dl (p=0.2). Vitamin B12 level in children with absolute lymphocyte count less than 1500/µL was significantly less than that of children with equal or more than 1500/µL (P value 0.02). Twelve (10.8%) children had thrombocytosis. The mean hemoglobin level and serum ferritin were significantly lower in children with thrombocytosis than those with normal platelets (p<0.01).

Conclusion: Celiac disease is associated with a diversity of hematological findings that include leukopenia, lymphopenia, neutropenia, and eosinophilia, as well as anemia, thrombocytopenia and thrombocytosis.

Key words: Anemia, Celiac disease, Leukopenia, Thrombocytosis.

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Introduction

Celiac disease is a common systemic disorder that has genetic, immunologic, and environmental components. Its incidence approaches 1% of the population.[1-5] As the patients with celiac disease have a long duration of symptoms prior to diagnosis, the disease is considered to be under diagnosed.[1,6] The diagnostic intervals between the onset of symptoms and diagnosis of celiac disease are still unacceptably long.[7] Awareness about celiac disease should be increased in the community in order to decrease this interval so that patients can recognize their symptoms and doctors can suspect celiac disease sooner when patients present with suggestive symptoms.

The clinical presentation of celiac disease varies greatly and ranges from asymptomatic to severe malnutrition. Celiac disease may present with classic signs and symptoms of malabsorption syndromes (classic celiac disease), or without these symptoms (atypical celiac disease).[8] The diagnosis of celiac disease required positive serologic markers in addition to presence of characteristic mucosal abnormalities on intestinal biopsy.[9] If the histological examination is negative and the serologic markers are positive with symptoms highly suggestive of celiac disease, the results of biopsies should be reviewed with expert gastrointestinal pathologist before additional biopsies are considered. Because nearly all patients with celiac disease have the HLA-DQ2 or HLA-DQ8 markers, HLA typing may be useful if the biopsy results are equivocal although 30% of the general population has these markers.[10]

Celiac disease is a common cause of various hematologic disorders, the most common of which is anemia, this includes iron, folate, and vitamin B12 deficiency anemias. Additionally, celiac disease may be implicated in the etiology of other abnormalities in blood count, splenic hypofunction, and intestinal lymphoma.[11] Our aim in this retrospective study is to review the hematological abnormalities in children with celiac disease upon diagnosis that were encountered at our institution over a seven-year period.

Methods

This review was conducted at the Pediatric Gastroenterology Department of Queen Rania Hospital for Children which is a tertiary teaching hospital for children in Amman, Jordan. The medical records of children with celiac disease were reviewed between January 2006 and December 2012. The age of children included in the study was less or equal to 14 years on diagnosis. Children included in the study presented to the Pediatric Gastrointestinal Clinic because of failure to thrive (4.5%), short stature (13.5%), chronic diarrhea (18.9%), abdominal distention 7.2%), constipation (10.8%), first degree relative with celiac disease (14.4%), unexplained iron deficiency anemia (11.7%), type I diabetes mellitus (17.1%), and Down syndrome (1.8%).

Diagnosis of celiac disease was based on the clinical presentation, positive serology, and histological changes suggestive of celiac disease. The new diagnostic criteria for celiac disease from the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) published in 2012 may be based on a combination of symptoms, high antibody levels specific for celiac disease, and HLA testing (HLA-DQ2 and/or HLA-DQ8), thus omitting the duodenal biopsy. The diagnosis is confirmed by an antibody decline and preferably a clinical response to a gluten free diet. Gluten challenge and repetitive biopsies will be necessary only in selected patients in whom diagnostic uncertainty remains.

Complete blood count, serum ferritin, folate, vitamin B12, prothrombin time, partial prothrombin time, international normalized ratio, and tissue glutaminase antibody (IgA and IgG) were performed for all patients prior to the diagnosis. Serum ferritin below 7ng/ml, serum folate below 5ng/ml and vitamin B12 below 200pg/ml were considered low.

Upper gastrointestinal endoscopy was performed for all children included in the study with a Pentax endoscope (model PMK, Endoscope pentax EG 2770 K, EG 2490 K). Multiple duodenal biopsy samples were obtained during the procedure for routine histological analysis. Celiac disease March scoring was used to assess the severity of damage in the small intestine.[12] The spectrum of histological changes according to March classification is as follows; March I: normal villous architecture with

Table I: Descriptive data for main blood indices of the whole group

		Mean	95% CI	
White blood count (/µL)		7593	7167-8020	
	Males	6951	6441-7462	P-Value
	Females	8180	7534-8820	0.003
ANC (/µL)[ı]		3624	3383-3864	
ALC (/µL)[£]		3127	2864-3389	
AEC (/µL)[¥]		212	162-262	
Hemoglobin (g/dl)		11.8	11.49-12.12	
	Males	11.82	11.35-12.3	P-Value
	Females	11.78	11.35-12.2	0.89
Platelets ($\times 10^3$)[π]		327.4	307.5-347.2	
S. ferritin (ng/ml)[π]		25.97	19.95-31.99	
S. folate(ng/ml)[π]		8.02	7.17-8.88	
Vitamin B12(pg/ml)		388.36	355.45-421.27	

ı: Absolute neutrophil count; £: Absolute lymphocyte count; ¥: Absolute eosinophil count; π: Serum.

Table II: Hematological findings among the study group

	Normal	**High**	**Low**
White blood count (/µL)	100 (90.1%)	0	11(9.9%)
ANC (/µL)[ı]	109 (98.2%)	0	2(1.8%)
ALC (/µL)[£]	98 (88.3%)	0	13(11.7%)
AEC (/µL)[¥]	103 (92.8%)	8(7.2%)	0
Hemoglobin (g/dl)	77 (69.4%)	0	34(30.6%)
Platelets ($\times 10^3$)			0
S. ferritin(ng/ml)[π]	83 (74.8%)		28(25.2%)
S. folate(ng/ml)[π]	81 (73%)		30(27%)
Vitamin B12(pg/ml)	102(91.9%)		9(8.1%)

ı: Absolute neutrophil count; £: Absolute lymphocyte count; ¥: Absolute esinophil count; π: Serum.

intraepithelial lymphocytosis abnormalities, Marsh II: intraepithelial lymphocytosis is accompanied by crypt hypertrophy, Marsh III: partial, subtotal, and total villous atrophy. Data is presented as counts and percentages. Chi-square test was used to study the relation between variables. P-Value was considered significant at 0.05. Confidence intervals (CI) were calculated when appropriate.

Results

A total of 111 children were included in the study; 53 (47.7%) were males and 58 (52.3%) were females. The mean age at diagnosis was nine years. Forty eight (43.2%) children presented with gastrointestinal symptoms including chronic diarrhea, abdominal distention and constipation. All children had positive tissue glutaminase antibody IgA, IgG or both.

Table I shows the mean and 95% confidence interval for blood indices in our patients. The mean white blood cells, neutrophil, lymphocyte and eosinophil count were 7593, 3624, 3127, 212 /µL respectively. Eleven (9.9%) children had leukopenia, 13 (11.7%) had lymphopenia, two (1.8%) had neutropenia, and eight (7.2%) had eosinophilia (Table II). March III histological changes were found in one hundred four (93.7%) patients while March II in seven (6.3%) patients.

Thirty four (30.4%) children had hemoglobin level below the third percentile for their age. There were no significant difference between hemoglobin level or white blood count between males and females (p=0.08, p=0.31, respectively).

Twenty eight (25.2%) children had serum ferritin less than 7ng/ml, 30 (27%) had serum folate less than 5ng/ml and nine (8.1%) children had vitamin B12 less than 200pg/ml. Seventeen (60.7%) children who had serum ferritin less than 7ng/ml had also low serum folate (p value 0.001). Of nine children who had low vitamin B12, three (33.3%) had iron deficiency, two (22.2%) had folate deficiency, three (33.3%) had isolated vitamin B 12 deficiency and only one (11.1%) had combined deficiency in iron, folate and vitamin B12. Serum folate had a positive correlation with serum ferritin (p=0.02) and vitamin B12 (p=0.005).

Four (36.4%) out of 11 children with leukopenia had serum folate below 5ng/dl. In addition, there was no difference between the mean white blood cell count in children with normal serum folate and those with low serum folate (p=0.8). Vitamin B12 level in children with absolute lymphocyte count less than 1500/µL was significantly less than that of children with equal or more than 1500/µL (p=0.02).

Twelve (10.8%) children had thrombocytosis. The means of hemoglobin level and serum ferritin are significantly lower in children with thrombocytosis than those with normal platelets (p<0.01). The mean lymphocyte count in children with thrombocytosis was significantly higher than those with normal platelets (p<0.01). Prothrombin time and partial prothrombin time were normal in all studied children.

Discussion

Celiac disease is a multisystem disorder that has gastrointestinal and extra intestinal manifestation. Hematologic abnormalities that may be associated with celiac disease include leukopenia, anemia, thrombocytosis or thrombocytopenia.

Leukopenia has been reported in some children with celiac disease.[13] The possible etiology implicated for this finding in cases of celiac disease is deficiencies of both folate and copper.[14-16] In this study, 36.4% of children with leukopenia had low serum folate, but there is no difference in the means of white blood cell count in patients with normal or those with low serum folate. In addition we found lower levels of vitamin B12 in association with lymphopenia. Serum copper was not performed for our patients at time of diagnosis to determine the combined effect of copper and folate on total white blood cells. Interestingly all children had normal total white blood cell count on follow up after initiation of gluten free diet.

In patients with untreated celiac disease, peripheral reduction of both total and T-lymphocytes was confirmed.[17] In addition, an increased susceptibility of peripheral blood lymphocytes from untreated celiac disease patients to undergo Fas-mediated apoptosis.[18] This will act as protective mechanism to limit the expansion of unwanted T-cells and responsible for both lymphopenia and immunogenic exposure of phospholipids with subsequent production of auto antibodies. In this study, thirteen children (11.7%) had lymphopenia.

Data regarding peripheral blood eosinophilia in children with celiac disease is limited. A recent study showed that peripheral blood eosinophilia was linked with several genes including celiac disease locus that contains SH2B3 (also known as LNK).[19] We had eight children with absolute eosinophils above 500, two of them had type I diabetes mellitus, two had first degree relative with celiac disease, and one had first degree relative with celiac disease and type I diabetes mellitus. Other causes of eosinophilia were excluded. These findings may be explained by celiac disease locus genes abnormalities on DR3-DQ2 which is shared by celiac disease and type I diabetes mellitus. Homozygosity for DR3-DQ2 in a population with type I diabetes carries a 33% risk for the presence of tissue transglutaminase auto antibodies.[20]

Anemia may be encountered frequently in patients with celiac disease and it may be the only presenting feature or abnormality identified.[21,22,26] The prevalence of anemia in newly diagnosed children with celiac disease varies and has been reported in 12% to 69%.[21-25] In our study the prevalence was 30.6%.

Iron deficiency anemia is a common finding in children with celiac disease which may reach up to 47% of cases with subclinical celiac disease.[21] Iron deficiency anemia is characterized by microcytic, hypochromic anemia, low serum iron levels, elevated total iron-binding capacity, and low ferritin levels.[27] In our study, we depended on blood film interpretation by expert hematopathologist and on serum ferritin level to diagnose patients with iron deficiency anemia. The prevalence of iron deficiency anemia in this study was 25.2%. Iron deficiency anemia in patients with celiac disease may result from defect in absorption of iron and occult blood loss in the gastrointestinal tract depending on the degree of villous atrophy.[28-30]

Folic acid deficiency is another common finding in patients with newly diagnosed celiac disease and in those with celiac disease detected by screening.[31-33] Serum folate level is dependent mainly on folate intake and is frequently increased in patients with vitamin B12 deficiency.[34-35] In this study, thirty (27%) children had low serum folate. A correlation

between serum folate and vitamin B12 levels was also seen in this cohort of patients ($p< 0.01$). Our data showed that 8.1% of children had vitamin B12 deficiency. Previous studies suggested that 8% to 41% of previously untreated subjects with celiac disease were deficient in vitamin B12.[36-37] Vitamin B12 deficiency in celiac disease may be due to decreased gastric acid, bacterial overgrowth, autoimmune gastritis, decreased efficiency of mixing with transfer factors in the intestine, or perhaps subtle dysfunction of the distal small intestine.[38-39]

Thrombocytosis was reported in up to 60% of patients with celiac disease.[39-41] It is more common than thrombocytopenia.[13,43-47] About 10.8% of our patients had thrombocytosis. In addition there was a positive correlation between lymphocyte count and platelet count which might need further studies. Thrombocytosis may be secondary to inflammatory mediators, iron-deficiency anemia or functional hyposplenia.[48] This finding may resolve after institution of a gluten free diet.[40,43]

Conclusion

Anemia, due to iron deficiency, folate deficiency, vitamin B12 deficiency or combined remains the most common hematological finding in children with celiac disease. Other hematological findings including leukopenia, neutropenia, lymphopenia, eosinophilia and thrombocytosis were found. Celiac disease should be considered in the differential diagnosis of any unexplained hematological finding in children.

References

1. **West J, Logan RF, Hill PG, *et al.*** Seroprevalence, correlates, and characteristics of undetected celiac disease in England. *Gut* 2003; 52: 960-965.
2. **Maki M, Mustalahti K, Kokkonen J, *et al.*** Prevalence of celiac disease among children in Finland. *N Engl J Med* 2003; 348: 2517-2524.
3. **Tatar G, Elsurer R, Simsek H, *et al.*** Screening of tissue transglutaminase antibody in healthy blood donors for celiac disease screening in the Turkish population. *Dig Dis Sci* 2004; 49: 1479-1484.
4. **Mustalahti K, Catassi C, Reunanen A, *et al.*** The prevalence of celiac disease in Europe: Results of a centralized, international mass screening project. *Annals of Medicine* 2010; 42 (8): 587-595.
5. **Bingley PJ, Williams AJ, Norcross AJ, *et al.*** Undiagnosed celiac disease at age seven: population based prospective birth cohort study. *BMJ* 2004; 328: 322- 323.
6. **Green PHR, Stavropoulos SN, Panagi SG, *et al.*** Characteristics of adult celiac disease in the USA: results of a national survey. *Am J Gastroenterol* 2001; 96: 126-131.
7. Lan**kisch PG, Martinez SA, Petersen F, *et al.*** Diagnostic intervals for recognizing celiac disease. *Z Gastroenterol* 1996; 34: 473-477.
8. **Rampertab S, Pooran N, Brar P, *et al.*** Trends in the Presentation of Celiac Disease. *The American Journal of Medicine* 2006; 119 (4): 355.e9-355.e14.
9. **Sbarbati A, Valletta E, Bertini M, *et al.*** Gluten sensitivity and 'normal' histology: is the intestinal mucosa really normal? *Dig Liver Dis* 2003; 35: 768-73.
10. **Dewar D, Pereira SP, Ciclitira PJ.** The pathogenesis of coeliac disease. *Int J Biochem Cell Biol* 2004; 36:17-24.
11. **Brousse N, Meijer JW.** Malignant complications of coeliac disease. *Best Pract Res Clin Gastroenterol* 2005; 19: 401-412.
12. **Marsh MN.** Gluten, major histocompatibility complex, and the small intestine. A molecular and immunobiologic approach to the spectrum of gluten sensitivity ('celiac sprue'). *Gastroenterology* 1992; 102: 330-354.
13. **Fisgin T, Yarali N, Duru F, *et al.*** Hematologic manifestation of childhood celiac disease. *Acta Haematol* 2004; 111: 211-214.
14. **Halfdanarson TR, Kumar N, Hogan WJ, *et al.*** Copper Deficiency in Celiac Disease. *Journal of Clinical Gastroenterology* 2009; 43 (2) : 162-164.
15. **Jameson S, Hellsing K, Magnusson S.** Copper malabsorption in coeliac disease. *Sci Total Environ* 1985; 42: 29-36.
16. **Pittschieler K.** Neutropenia, granulocytic hypersegmentation and coeliac disease. *Acta Paediatr* 1995; 84: 705-706.
17. **Di Sabatino A, Bertrandi E, Casadei Maldini M, *et al.*** Phenotyping of peripheral blood lymphocytes in adult coeliac disease. *Immunology* 1998; 95: 572-576.
18. **Di Sabatino A, D'aloa S, Millimaggi D, *et al.*** Apoptosis and peripheral blood lymphocyte depletion in celiac disease. *Immunology* 2001; 103: 435-440.
19. **Gudbjartsson DF, Bjornsdottir US, Halapi E, *et al.*** Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction. *Nat Genet* 2009; 41: 342-347.
20. **Bao F, Yu L, Babu S, *et al.*** One third of HLA DQ2 homozygous patients with type 1 diabetes

express celiac disease associated transglutaminase autoantibodies. *J Autoimmun* 1999; 13: 143-148.
21. **Unsworth DJ, Lock FJ, Harvey RF.** Iron deficiency anaemia in premenopausal women. *Lancet* 1999; 353: 1100.
22. **Bottaro G, Cataldo F, Rotolo N, *et al.*** The clinical pattern of subclinical/silent celiac disease: an analysis on 1026 consecutive cases. *Am J Gastroenterol* 1999; 94: 691-696.
23. **Harper JW, Holleran SF, Ramakrishnan R, *et al.*** Anemia in celiac disease is multifactorial in etiology. *Am J Hematol* 2007; 82: 996-1000.
24. **Hin H, Bird G, Fisher P, *et al.*** Coeliac disease in primary care: case finding study. *BMJ* 1999; 318: 164-167.
25. **Lo W, Sano K, Lebwohl B, *et al.*** Changing presentation of adult celiac disease. *Dig Dis Sci* 2003; 48: 395-398.
26. **Hoffbrand AV.** Anaemia in adult coeliac disease. *Clin Gastroenterol* 1974; 3: 71-89.
27. **Cook JD.** Diagnosis and management of iron deficiency anemia. *Best Pract Res Clin Haematol* 2005; 18: 319-332.
28. **de Vizia B, Poggi V, Conenna R, *et al.*** Iron absorption and iron deficiency in infants and children with gastrointestinal diseases. *J Pediatr Gastroenterol Nutr* 1992; 14: 21-26.
29. **Kosnai I, Kuitunen P, Siimes MA.** Iron deficiency in children with coeliac disease on treatment with gluten-free diet: role of intestinal blood loss. *Arch Dis Child* 1979; 54: 375-378.
30. **Fine KD.** The prevalence of occult gastrointestinal bleeding in celiac sprue. *N Engl J Med* 1996; 334: 1163-1167.
31. **Howard MR, Turnbull AJ, Morley P, *et al.*** A prospective study of the prevalence of undiagnosed coeliac disease in laboratory defined iron and folate deficiency. *J Clin Pathol* 2002; 55: 754-757.
32. **Kemppainen TA, Kosma VM, Janatuinen EK, *et al.*** Nutritional status of newly diagnosed celiac disease patients before and after the institution of a celiac disease diet: association with the grade of mucosal villous atrophy. *Am J Clin Nutr* 1998; 67: 482-487.
33. **Haapalahti M, Kulmala P, Karttunen TJ, *et al.*** Nutritional status in adolescents and young adults with screen-detected celiac disease. *J Pediatr Gastroenterol Nutr* 2005; 40: 566-570.
34. **Lenhardt A, Plebani A, Marchetti F, *et al.*** Role of human-tissue transglutaminase IgG and antigliadin IgG antibodies in the diagnosis of coeliac disease in patients with selective immunoglobulin A deficiency. *Dig Liver Dis* 2004; 36: 730-734.
35. **Carmel R.** Megaloblastic anemia's: disorders of impaired DNA synthesis. In: Greer JP, Foerster J, Lukens JN, Rodgers GM, Paraskevas F, Glader B, eds. Wintrobe's Clinical Hematology. Vol 1. Philadelphia, PA: Lippincott Williams & Wilkins; 2004: 1367-1395.
36. **Dahele A, Ghosh S.** Vitamin B12 deficiency in untreated celiac disease. *Am J Gastroenterol* 2001; 96: 745-750. 65.
37. **Dickey W.** Low serum vitamin B12 is common in celiac disease and is not due to autoimmune gastritis. *Eur J Gastroenterol Hepatol* 2002; 14: 425-427.
38. **Gillberg R, Kastrup W, Mobacken H, *et al.*** Gastric morphology and function in dermatitis herpetiformis and in celiac disease. *Scand J Gastroenterol* 1985; 20: 133-140.
39. **Dickey W, Hughes DF.** Histology of the terminal ileum in celiac disease. *Scand J Gastroenterol* 2004; 39:665-667.
40. **Nelson EW, Ertan A, Brooks FP, *et al.*** Thrombocytosis in patients with celiac sprue. *Gastroenterology* 1976; 70: 1042-1044.
41. **Patwari AK, Anand VK, Kapur G, *et al.*** Clinical and nutritional profile of children with celiac disease. *Indian Pediatr* 2003; 40: 337-342.
42. **Carroccio A, Giannitrapani L, Di Prima L, *et al.*** Extreme thrombocytosis as a sign of celiac disease in the elderly case report. *Eur J Gastroenterol Hepatol* 2002; 14: 897-900.
43. **Croese J, Harris O, Bain B.** Coeliac disease: haematological features, and delay in diagnosis. *Med J Aust* 1979; 2: 335-338.
44. **Mulder CJ, Pena AS, Jansen J, *et al.*** Celiac disease and geographic (serpiginous) choroidopathy with occurrence of thrombocytopenic purpura. *Arch Intern Med* 1983; 143: 842.
45. **Eliakim R, Heyman S, Kornberg A.** Celiac disease and keratoconjunctivitis: occurrence with thrombocytopenic purpura. *Arch Intern Med* 1982; 142: 1037.
46. **Stenhammar L, Ljunggren CG.** Thrombocytopenic purpura and celiac disease. *Acta Paediatr Scand* 1988; 77: 764-766.
47. **Kahn O, Fiel MI, Janowitz HD.** Celiac sprue, idiopathic thrombocytopenic purpura, and hepatic granulomatous disease: an autoimmune linkage? *J Clin Gastroenterol* 1996; 23: 214-216.
48. **Schafer AI.** Thrombocytosis. *N Engl J Med* 2004; 350: 1211-1219.