ORIGINAL ARTICLE

Serum Cobalamin Levels and Red Blood Cell Morphology Among Celiac Disease Patients

Samreen Bugti[1], Humera Bugti[2], Yar Muhammad Nizammani[3], Shujaullah[4], Navaid Kazi[5]

Author`s Affiliation

[1,2] Senior lecturer
Bolan Medical College, Quetta
[3]Assistant Professor
[4] Senior lecturer, Isra University, Hyderabad
[5]Professor, Isra University, Hyderabad

Author`s Contribution

[1]Conception, Synthesis and Planning of the research
[,5]Active participation in active methodology
[2,3]Interpretation, analysis and discussion

Article Info

Received: May 12, 2018
Accepted: Sept 24, 2018

Funding Source: Nil
Conflict of Interest: Nil

Address of Correspondence
Dr. Samreen Bugti
samreen.bugti@yahoo.com

Cite this article as: *Bugti S, Bugti H, Nizammani YM, Shujaullah, Kazi N. Serum Cobalamin Levels and Red Blood Cell Morphology Among Celiac Disease Patients. Ann. Pak. Inst. Med. Sci. 2018;14(3): 245-249.*

ABSTRACT

Objective: To evaluate serum cobalamin level and red blood cell morphology among Celiac disease patients.

Methodology: This Case control study was conducted at Department of Physiology, Medicine and Gastroenterology Unit, Isra University Hospital Hyderabad from March 2017 to October 2017. All the participants were divided equally in two groups. Group I. Controls- normal healthy subjects (n=45). Group II. Cases diagnosed cases of Celiac disease (n=45). Red blood cell morphology was assessed under light microscopy at postgraduate Laboratory. Serum cobalamin was determined by competitive EIA technique. All the data was recorded in the proforma.

Results: Total 90 cases were studied out of them 45 were patients of celiac disease and 45 were normal. Mean age of controls cases was 47.53±8.13 years and celiac disease patient's was 46.84±7.69 years. Male were found in majority of both group's cases and controls as 82.2% and 73.3% respectively. Mean of serum cobalamin level was significantly lower among patients as 201.36±74.77 pg/mL in contrast to controls as 247.06±60.34 pg/mL p= 0.002. Hemoglobin and hematocrit levels were also low in cases compared to controls. RBC indices showed macrocytic, microcytic, hyperchromic and megaloblastic changes.

Conclusion: Serum cobalamin was decreased among celiac disease patients and disturbed RBC indices as macrocytic, microcytic, hyperchromic and megaloblastic were common in the Celiac disease patients.

Keywords: Celiac disease, Cobalamin, red blood cell morphology

Introduction

Celiac disease is the lifelong immune mediated systemic disorder.[1] In various diseases the villi and microvilli of the small intestine can be damaged and their function can become impaired leading to incomplete digestion and absorption which is better termed as malabsorption. One such disease is known as celiac disease in which there is failure of absorption and indigestion. According to the world data the prevalence of Celiac disease is 1-3% in Europe and correct prevalence in Pakistan celiac disease is not known.[1,2] Celiac disease was believed to be a frequent disorder among children, but now it is considered to be a common multi-system can occurred at any age due to presence of gluten in diet.[1,3] The celiac disease is found to occur more in females. The female to male ratio is approximately 2: 1 to 3: 1.4.[4] CD is a common cause of various hematologic disorders, the most common of which is anemia.[5] Anemia secondary to malabsorption of iron, folic acid, and/or vitamin B_{12} is a common complication of celiac disease and many patients have anemia at the time of diagnosis.[5] Celiac disease may also be associated with thrombocytosis, thrombocytopenia, leukopenia, venous thromboembolism, hyposplenism and IgA deficiency. Vitamin B_{12} is an essential cofactor and a coenzyme

in multiple biochemical pathways, including the pathways of DNA and methionine synthesis. While the main site of vitamin B_{12} absorption is the distal ileum (where it is absorbed bound to intrinsic factor), a small proportion is also absorbed passively along the entire small bowel.[6] Deficiency of vitamin B_{12} is common in CD and frequently results in anemia. Malabsorption of vitamin B_{12} resulting in anemia has also been described in patients with DH. Macrocytic anemia describes an anemic state characterized by the presence of abnormally large RBCs in the peripheral blood.[7] Macrocytosis can be identified by reviewing peripheral blood smears and/or by automated RBC indices. These important vitamins and cofactors are required for normal maturation of all cells. Marrow erythroblasts are no exception. When either of these two factors is deficient, RBC proliferation and maturation result in large erythroblasts with nuclear/cytoplasmic asynchrony. These abnormalities are caused by a defect in DNA synthesis that interferes with cellular proliferation and maturation.[7,8] The cause of the vitamin B_{12} deficiency in CD is not known but may include 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.[9]

Methodology

This cross-sectional study being carried out at Department of Physiology, Medicine and Gastroenterology Unit, Isra University Hospital Hyderabad. Study design was observational study, from March 2017 to October 2017. Ninety subjects were selected on the basis of purposive sampling; subjects were divided into two groups: Group I. Controls- normal healthy subjects (n=45) and Group 2. Diagnosed cases of Celiac disease (n=45). Sample size was calculated by using the raosoft software, taking proportion of (3% celiac disease)[1] with 95% and 5% margin error. Study was conducted after ethical approval and all the patients were informed that the blood which is drawn was to be used for study purpose only. Vein was engorged by a tourniquet applied above cubital fossa.10 ml of blood sample was collected from ante-cubital vein after application of sterilized alcohol swab. 5 ml was put in EDTA containing blood CP bottle and 5 ml in plain glass tube and was sent to the Hospital laboratory. Complete blood parameters and serum cobalamin levels were noted along with red blood cell indices. Serum cobalamin was categorized as normal >240 (pg/ml), borderline deficiency 170-240 (pg/dl), Deficiency <170 (pg/dl) and severe deficiency <100 (pg/dl). All the cost of tests was done by authors. RBC morphology was studied under light microscopy at Postgraduate Laboratory. All the data was recorded in the proforma. Data was analyzed by SPSS version 20. Categorical data was computed as frequency and percentage. Numerical data was computed as mean and standard deviation. Chi-squire test and t-test were applied, a p-value <0.05 was considered as significant.

Results

In celiac disease patients mean age was 46.84±7.69 years, and in normal cases the mean age was 47.53±8.13 years. Gender distribution is shown in table. No. I

Table I: Gender distribution of study population (n=90)

Gender	Controls	Cases	P-value
Male	33 (73.3%)	37 (82.2%)	0.31
Female	12 (26.6%)	8 (17.7%)	

Mean of serum cobalamin level was higher in control cases as 247.06±60.34 pg/mL in contrast to , celiac disease patients as 201.36±74.77 pg/mL with p-value of 0.002. Mean of hemoglobin level was significantly lower among celiac disease patients as 11.57±1.13 in contrast to normal population as 13.42±1.38 with p-value=0.0001. Mean corpuscular volume of RBCs in cases was higher as 103.26±12.45 as compare controls 96.88±10.01 & p-value=0.009. Mean corpuscular Hb (MCH) of RBCs in controls and cases was noted as 27.84±4.25 and 29.52±3.95 pg/dl respectively with significant difference p-value=0.034. Mean corpuscular Hb concentration (MCHC %) of RBCs in controls and cases was noted as 34.30±2.16 and 35.33±2.37 respectively without significant difference p-value=0.056. Table. No. II

Deficiency and severe deficiency of cobalamin was higher among celiac disease patients as compare to normal population p-value 0.001 as showed in table III.

Table II: Mean comparison of Cobalamin level, Hemoglobin, Hematocrit, RBC, MCH and MCHC (n=90)				
Variables		Mean±SD	t-value	p-value
Cobalamin levels	Controls	247.06±60.34 pg/mL	41.3	0.002
	Cases	201.36±74.77 pg/mL		
Hemoglobin levels (g/dl)	Controls	13.42±1.38 g/dl	16.9	0.01
	Cases	11.57±1.13 g/dl		
Hematocrit levels	Controls	43.08±6.13	16.17	0.001
	Cases	36.31±4.05		
Mean corpuscular volume of RBC	Controls	96.88±10.01	14.37	0.009
	Cases	103.26±12.45		
Mean corpuscular haemoglobin (pg/dl)	Controls	27.84±4.25 pg/dl	1.93	0.034
	Cases	29.52±3.95 pg/dl		
Mean corpuscular haemoglobin concentration	Controls	34.30±2.16(%)	12.15	0.056

Table III: Frequency of serum cobalamin among controls and cases(n=90)

Cobalamin	Controls	Cases	P-value
Normal >240 (pg/ml)	31 (68.8%)	18 (40%)	0.0001
Borderline deficiency 170-240 (pg/dl)	7 (15.5%)	8 (17.7%)	
Deficiency <170 (pg/dl)	7 (15.5%)	16 (35.5%)	
Severe deficiency <100 (pg/dl)	0 (0%)	3 (6.6%)	

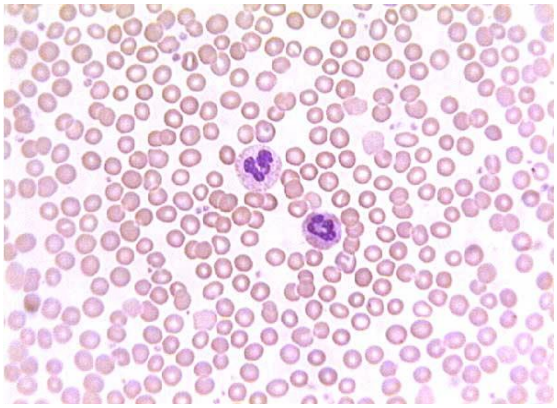

Figure 1. Normal peripheral blood smears showing RBC and white blood cells

RBC indices showed macrocytic, microcytic, hyperchromic and megaloblastic changes as showed in Figures1 to 6.

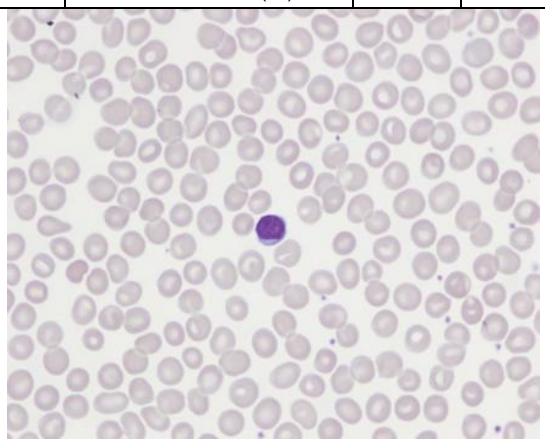

Figure 2. RBC showing macrocytes and oval rbcs; morphology pattern known as Macroovalocytosis

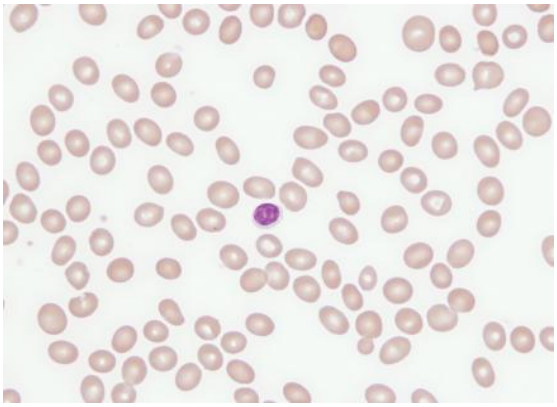

Figure. 3. RBC showing macrocytes and oval rbcs; morphology pattern known as Macroovalocytosis

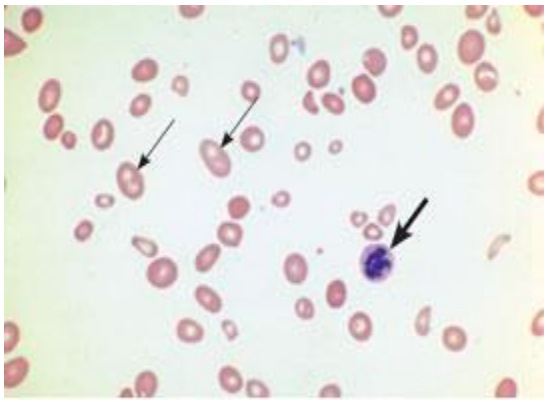

Figure. 4. RBC showing macrocytes, & oval rbcs; morphology pattern known as Macroovalocytosis. Hypersegmented neutrophilsare also visible.

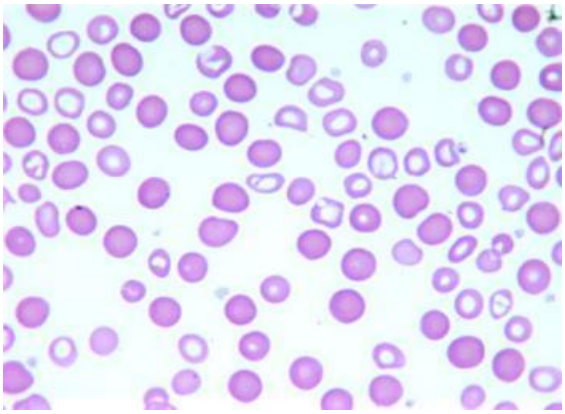

Figure. 5. RBC showing different cell population. Some cells have normal chromic appearance, whilst other have prominent Central pallor; the morphology picture reveals "Dimorphic anaemia"

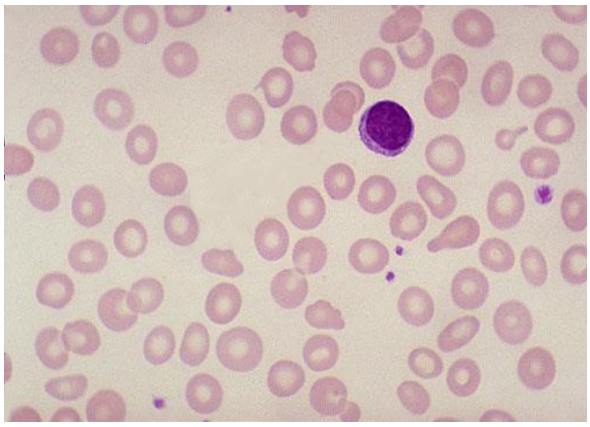

Figure. 6. RBC with central pallor; a morphology pattern suggestive of iron deficiency anaemia. (Microcytic Hypochromic anaemia)

Discussion

Celiac disease which is a common cause of malabsorption occurs in genetically predisposed individuals. In this study mean age of cases was 46.84±7.69 years and mean age of controls was 47.53±8.13 years without significant difference p-value 0.68. Abbas Z et al[2] reported that mean age of the patients was 29.9 ± 12.7 years. Asghar A et al[10] reported that mean age of the cases was 34.12 ±11.23 years. Volta U et al[11] found median age was 36 years. Mean age of these studies is lower in contrast to this study, and this difference may be because of different selected age ranges. In this series male were in majority among both groups, similarly Abbas Z et al[2] reported male were in majority 41 (53.2%), while Asghar A et al[9] inconsistently reported that females in majority.

In this study mean of serum cobalamin level was significantly lower among celiac disease patients as 201.36±74.77 pg/mL in contrast to normal 247.06±60.34 pg/mL with p-value 0.002. Studies stated that causes of cobalamin deficiency in celiac disease is not known, but several reduced gastric acid, over growth of bacteria, autoimmune gastritis, reduced efficiency of mixing with the transfer factor in intestine, or indirect distal cases of CD were with deficiency of Vitamin B12.[11-13] Dahele A et al[11] demonstrated that out of total celiac disease patients 41% patients had vitamin B12 deficiency.

In this study mean of haemoglobin level was significantly lower among celiac disease patients as 11.57±1.13 g/dL in contrast to normal population p-value=0.0001. Asghar A et al[10] found mean haemoglobin level 8.71±1.03 g/dL. Shahzad et al[14] also found mean haemoglobin level 8.81±1.23 g/dL. In a study reported that iron deficiency is the most commonly recognized cause of anaemia in patients with coeliac disease, followed by folate and vitamin B12 deficiencies, which are also common at the time of diagnosis. Macrocytic anaemia is unusual due to B12 or folate deficiency.[15] In this study RBC indices showed macrocytic, microcytic and hyperchromic. In the literature generally studies stated that megaloblastic anemia is the different type of anemia categorized by macrocytic RBCs and typical morphological alteration in RBC precursors.[15]

Vitamin B12 deficiency disturbs the rapidly proliferating cells of the bone marrow and in the resulting ineffective erythropoiesis cause large immature red blood cells formation known as megaloblasts.[16] No proper studies found regarding association of red cell morphology and cobalamin deficiency among celiac disease patients.

Conclusion

The recent study showed that decreased serum cobalamin and fluctuated RBC indices in the patients of Celiac disease. Macrocytic, hyperchromic changes in RBC morphology were noticed frequently. Addressing the role of malabsorption and degree of derangement of cobalamin in celiac disease are important and necessary in future studies. Thus, more studies are recommended to be conducted to establish the relationship of serum cobalamin in Celiac disease and its effect on red blood cell morphology.

References

1. Mantegazza C, Zuccotti G, Dilillo D, Koglmeier J. Celiac Disease in Children: A. International Journal of Digestive Diseases. 2015;1(1):9.
2. Abbas Z, Raza S, Yakoob J, Abid S, Hamid S, Shah H, Jafri W. Varied presentation of celiac disease in Pakistani adults. Journal of the College of Physicians and Surgeons Pakistan. 2013;23(7):522.
3. Gujral N, Freeman HJ, Thomson AB. Celiac disease: prevalence, diagnosis, pathogenesis and treatment. World journal of gastroenterology: WJG. 2012 Nov 14;18(42):6036.
4. Babar MI, Ahmad I, Rao MS, Iqbal R, Asghar S, Saleem M. Celiac disease and celiac crisis in children. J Coll Physicians Surg Pak 2011; 21 (8): 487-90.
5. Fernández-Bañares F, Monzón H, Forné M. A short review of malabsorption and anemia. World journal of gastroenterology: WJG. 2009 Oct 7;15(37):4644.
6. Kruzliak P. Hematologic manifestations of celiac disease. In Celiac Disease-From Pathophysiology to Advanced Therapies 2012. InTech.
7. Aslinia F, Mazza JJ, Yale SH. Megaloblastic anemia and other causes of macrocytosis. Clinical medicine & research. 2006 Sep 1;4(3):236-41.
8. George RM. Role of reticulocyte count in the differential diagnosis of macrocytic anemia (Doctoral dissertation).2010;1-48
9. Dickey W, Hughes DF. Histology of the terminal ileum in coeliac disease. Scand J Gastroenterol. 2004;39:665–667.
10. Asghar A, Zafar MH, Munir S. Frequency of Celiac Disease in Patients Presenting with Nutritional Anemia. APMC 2018;12(2):151-3
11. Volta U, Caio G, Giancola F, Rhoden KJ, Ruggeri E, Boschetti E, Stanghellini V, De Giorgio R. Features and progression of potential celiac disease in adults. Clinical Gastroenterology and Hepatology. 2016 May 1;14(5):686-93
12. Kumar VS, Parmeshwarappa SB, Vadde AR. Celiac disease manifesting as isolated cobalamin deficiency megaloblastic anemia: Case series and review. Journal of the Scientific Society. 2014 Sep 1;41(3):211.
13. Weiss G, Goodnough LT. Anemia of chronic disease. N Engl J Med 2005;352:1011-23
14. Shahzad A, Sahto AA, Samina. Frequency of celiac disease; patients presenting with iron deficiency anemia at tertiary care hospital. Professional Med J. 2016;23(7):812-6.
15. Harper JW, Holleran SF, Ramakrishnan R, Bhagat G, Green PH. Anemia in celiac disease is multifactorial in etiology. Am J Hematol 2007;82(11):996-1000
16. Chandra J. Megaloblastic anemia: back in focus. The Indian Journal of Pediatrics. 2010 Jul 1;77(7):795-9.