



RED BLOOD INDICES, PLATELET COUNT AND TRANSFERRIN LEVELS IN CELIAC PATIENTS WITH AND WITHOUT TYPE I DIABETES MELLITUS IN HYDERABAD, SINDH, PAKISTAN

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ABSTRACT

OBJECTIVE: To analyze the hematological manifestations among celiac disease (CD) and CD with type-I diabetes mellitus (T1DM) children to aid in differential diagnosis and management of disease.

METHODS: This study was conducted at Institute of Biotechnology and Genetic Engineering, University of Sindh, Jamshoro, Pakistan. CD pediatric patients were diagnosed through serological or intestinal biopsy. The individuals were divided into 5 groups (35 each); Control (A), diagnosed cases of CD (B), individuals affected with celiac-like indications (C), T1DM patients with celiac-like symptoms (D) and T1DM patients (E).

RESULTS: Mean age of 175 participants was 9.5 ± 1.2 years. Mean hemoglobin level (mg/dl) was 9.1 ± 1.1 , 9.03 ± 1.4 , 8.64 ± 1.2 and 11.7 ± 1.3 in group-B, group-C, group-D and group-E respectively as compared to 13.02 ± 1.5 in Group-A ($p < 0.001$). Mean corpuscular volume (MCV) [fl] was 64.1 ± 5.4 , 68.5 ± 7.8 , 57.6 ± 6.0 and 69.4 ± 4.4 in group-B, group-C, group-D and group-E respectively as compared to 84.4 ± 7.3 in Group-A ($p < 0.001$). Mean corpuscular hemoglobin (MCH) [group-B: 24.1pg, group-C: 25.5pg, group-D: 24.6pg] and mean corpuscular hemoglobin concentration (MCHC) [group-B: 29.3 g/dl, group-C: 277.9 g/dl, group-D: 31.5 g/dl] were decreased among all celiac affected cases. For group-E the hemoglobin, MCV and MCHC were in the normal ranges. Platelet Count (cells/ul) was $689 \pm 178 \times 10^3$, $702 \pm 141 \times 10^3$, $518 \pm 124 \times 10^3$ and $529 \pm 101 \times 10^3$ in group-B, group-C, group-D & group-E respectively as compared to $326 \pm 98 \times 10^3$ in group A ($p < 0.01$). Mean Transferrin levels (mg/dl) were 411.5 ± 24.8 ; 387.7 ± 22.4 ; 406.4 ± 21.4 & 368.8 ± 22.0 in group-B, group-C, group-D and group-E and 271.1 ± 39.1 in group-A ($p < 0.01$).

CONCLUSION: Hematological anomalies; like anemia and thrombocytosis are frequently present in mismanaged and late diagnosed CD children.

KEY WORDS: Celiac disease (MeSH); Anemia, Iron-Deficiency (MeSH); Erythrocyte Indices (MeSH); Blood Platelets (MeSH); Thrombocytosis (MeSH); Diabetes Mellitus, Type I (MeSH).

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INTRODUCTION

Globally, about 1% of adult population is affected with Celiac disease (CD). It is also known as gluten-sensitive enteropathy, or non-tropical sprue.¹ It is genetic autoimmune disorder in which mucosal membrane of small intestine becomes hypersensitive to prolamins and gliatins; the polypeptides

found in wheat, rye and barley. Due to which the enterocyte lining in intestine is damaged, consequently leading to digestive problems and poor absorption of nutrients.² CD can be asymptomatic or symptomatic with severe malnutrition giving rise to many complications; like anemia, ulcers, diarrhea, fatigue, late puberty and osteoporosis.³ It is common food related disorder in western

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populations.⁴ It seems to be a common disorder among Pakistanis and it affects children to adults. However, there is no data regarding its prevalence.⁵ The high probability of occurrence of CD (~515%) is found in first and second-degree relatives of the affected individuals. Besides this, other vulnerable individuals are those suffering with other autoimmune disorders, e.g., thyroiditis, type I diabetes mellitus (T1DM), and Down syndrome.⁶ Hematologic misbalance (84%) has been described in CD patients, in considerable frequency, ever since decades ago.⁷ The main obstacle is the misdiagnosis of CD, which paves the way to chronic and unresponsive iron-deficiency anemia. Hematologic evaluation can be a helpful adjunct and prognostic marker for patients with CD, since it is difficult to make a diagnosis accurately due to its varied clinical presentations. Hematologic features of CD are well described in the guidelines of the British Society of Gastroenterology, American College of Gastroenterology, and European Society for Pediatric Gastroenterology, Hepatology, and nutrition.^{8,9} Although the guidelines cover a wide range of complications, yet numerous other deficiencies have been documented. Such as; insufficient folate causing megaloblastic anemia, iron-deficiency anemia (IDA), vitamin B₁₂ deficiency and coagulopathy product of vitamin K deficiency.¹⁰ The most common clinical symptom is IDA, which could aid as an extra-intestinal diagnostic tool for CD screening. About 3.2% of patients

TABLE I: HEMATOLOGICAL MANIFESTATIONS AMONG DIFFERENT GROUPS (N=175)

Blood Parameters	Groups	Mean±S.D	F-Value	P-Value
Hemoglobin levels (mg/dl)	A	13.02±1.5	20.88	0.0001
	B	9.1±1.1		
	C	9.03±1.4		
	D	8.64±1.2		
	E	11.7±1.3		
Mean corpuscular volume [MCV] (fl)	A	84.4±7.3	24.23	0.0001
	B	64.1±5.4		
	C	68.5±7.8		
	D	57.6±6.0		
	E	69.4±4.4		
Mean Corpuscular Hemoglobin [MCH] (pg)	A	32.4±1.3	45.23	0.0001
	B	24.1±1.4		
	C	25.5±1.8		
	D	24.6±0.8		
	E	26.4±0.7		
Mean Corpuscular Hemoglobin Concentration [MCHC] (g/dl)	A	34.1±0.6	64.78	0.0001
	B	29.3±0.9		
	C	27.9±1.1		
	D	31.5±0.8		
	E	30.2±0.3		
Platelet Count (cells/ul)	A	326±98x10 ³	87.96	0.0001
	B	689±178x10 ³		
	C	702±141x10 ³		
	D	518±124x10 ³		
	E	529±101x10 ³		

Group A: Control, Group B: Diagnosed cases of Celiac Disease, Group C: Patients with Celiac-like symptoms, Group D: Type 1 Diabetes patients with Celiac-like symptoms, Group E: Type 1 Diabetes patients

with IDA have biopsy-proven CD verified by recent meta-analysis and systematic review.¹¹ Hemorrhagic cases can be the representing characteristic of CD, including cases with extreme malabsorption and coagulation deficits.¹² The probable rationale of hemorrhagic diathesis is vitamin K deficiency, while other analysis have also theorized mimicry between tissue transglutaminase and factor XIII. Different studies showed different frequencies of hematological manifestations of CD.¹³

In this study we evaluated the pattern of hematological manifestations among different pediatric Celiac groups i.e.; diagnosed CD vs. suspected CD vs CD-T1DM cases. The analyzed parameters were hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelet count and transferrin levels disturbed in poorly and late diagnosed CD cases. The purpose of the study was to know the prevalence of varied clinical features of CD in children from Sindh. This research may help in differential diagnosis and proper management of

celiac disease.

METHODS

Study setting

This cross-sectional study was organized after approval of ethical research committee of Institute of Biotechnology and Genetic Engineering, Board of Advance Studies and Research, University of Sindh, Jamshoro, Pakistan. Study duration was January to December 2018. A brief oral and written consent was taken from all the parents/guardians of the subjects in this study.

In total 175 pediatric patients (age 3-15 years) diagnosed as the cases of CD through serological or intestinal biopsy, patients having symptoms but undiagnosed for CD and diagnosed cases of T1DM with CD were included in the study. All samples were collected from Liaquat University of Medical and Health Sciences Hospital (LUMHS), Isra University Hospital and Asian Institute of Medical Sciences (AIMS) Hospital, Hyderabad, Pakistan. The experiments were performed in Molecular Biotechnology laboratory at the Institute of

Biotechnology and Genetic Engineering, University of Sindh, Jamshoro and at Isra university laboratory, Hyderabad, Pakistan. The individuals were equally divided into 5 groups (n=35 each group); Control (Group A), Diagnosed cases of Celiac Disease (Group B), Patients with Celiac-like symptoms (Group C), T1DM patients with Celiac-like symptoms (Group D) and T1DM patients (Group E).

Blood analysis

Five (5) ml of venous blood sample was taken from each patient. The blood collected in EDTA tube was then sent for blood complete picture (CP) report. For all patients the blood CP was carried out. For complete blood count, automated analyzer (Hitachi Roche, Japan) was used.

Transferrin test

Total serum transferrin concentration was determined by using Human transferrin receptor kit (by Bioassay Technology Laboratory) and analyzed on ELISA reader (Diasource 2000, Belgium). This kit follows the sandwich model ELISA.

All the data was collected via detailed questionnaire; to know the history, demographic details clinical features and gluten-free diet status of each patient. Data was analyzed by using SPSS version 2.0.

RESULTS

The mean age of the study participants (n=175) was 9.5±1.2 years. There were 112 (64%) male and 63 (36%) females in this survey. It was observed that CD is diagnosed later and is poorly treated. The only treatment for CD is strict gluten absence from diet. Almost 80% patients and families were unaware of the concept of gluten free diet, so many of the cases had severe malnutrition. The group A was the diagnosed cases of Celiac either by serological or intestinal biopsy tests. For the celiac-suspected groups including T1DM group, the serological testing (anti-tTG) was carried out.

Red blood cell indices

The most common and in few cases (15%) the only symptom observed was

TABLE II: TRANSFERRIN LEVELS AMONG DIFFERENT GROUPS (N= 175)

	Groups	Mean ± S.D	F-Value	P-Value
Transferrin levels (mg/dl)	A	271.1 ± 39.1	46.98	0.0001
	B	411.5 ± 24.8		
	C	387.7 ± 22.4		
	D	406.4 ± 21.4		
	E	368.8 ± 22.0		

Group A: Control, Group B: Diagnosed cases of Celiac Disease, Group C: Patients with Celiac-like symptoms, Group D: Type 1 Diabetes patients with Celiac-like symptoms, Group E: Type 1 Diabetes patients

anemia. In the cohort studied for Celiac disease the mean hemoglobin levels in Group B, C and D were 9.1, 9.03 and 8.64 mg /dl respectively. Whereas the normal hemoglobin levels should be in the range of 12 to 16 mg/dl, as observed in-group A and E. Table I shows comparison of RBC indices among different celiac groups. The mean of MCV and MCH were significantly lower in all patients groups as compared to control group (p=0.0001). MCHC level was significantly decreased in groups B, C and D; while it was found 34.1 ± 0.6 g/dl in control group and 30.2 ± 0.3 g/dl in-group E.

Platelet count

In many of the cases, secondary thrombocytosis was observed. The mean platelet level was significantly higher $689 \pm 178 \times 10^3$ cells/ μ L in-group B, in contrast to control group (p=0.0001). Platelet Count was also raised in other groups (Table I). There were six cases with severe thrombocytosis having levels above $\sim 1200 \times 10^3$ cells/ μ L.

Transferrin

For healthy individuals the range of transferrin should be 170-370 mg/dl, which was observed in the group A. For group B the highest transferrin level (411.5 ± 24.8 mg/dl) was observed (Table II). Because majority of patients had severe anemia and were not on strict gluten free diet, the symptoms of celiac were persistent with less recovery. Also for group C the ranges were observed to be on higher end. In participants, suffering from both diabetes and celiac (group D) the transferrin was observed to be 406.4 mg/dl.

DISCUSSION

Several research over the years have widened the understanding about clinical features of CD¹⁴. But from typical to atypical Celiac the varied symptoms

pose challenges in proper diagnosis of CD. In this cross-sectional study out of 175 children; males were 64% and 36% were females. Studies have observed that CD is more prevalent in females than males; like in other autoimmune disorders.¹⁵ Another study conducted by Green PH, et al.¹⁶ on 1612 patients from all United States of America analyzed that the females are more affected (2.9:1).

The celiac children cohort had typical symptoms of disease such as chronic diarrhea, abdominal pain, and fatigue and weight loss. The blood complete picture revealed imbalance in RBC indices and platelet count. The mean hemoglobin level was observed lower among celiac disease patients as compared to healthy controls (p=0.0001). In comparison to our results, study conducted by Chen CM, et al.¹⁷ reported that occurrence of iron deficiency (ID) (3.7% infants) and IDA (2.7% infants) under 6 months of age was observed, also it further amplified to 20.4% and 6.6% respectively, in children above six months of age. The healthy group had a higher total iron than ID and IDA groups. Sanseviero MT, et al.¹⁸ also stated in their findings that 156 patients comprising of (19.8%) females and (10.2%) males had anemia, they also showed symptoms of IDA (21.62%), few had thalassemia characteristics without ferric deficiency and the remaining affected with other forms of anemia. About (22.20%) of the patients had decreased ferritin levels and with border line hemoglobin values.

Among all the groups studied the MCV, MCH and MCHC were significantly lower with p-value of 0.0001. The main reason behind this is the poor or no absorption of iron from the damaged intestinal walls. Radlovic N, et al.¹⁹ had reported that in chronic and late diagnosed cases 52% of the patients were anemic, out of these (72 %) were

microcytic (MCV < 70 fl) and remaining were normocytic (MCV 70-87 fl). The authors also reported that they observed iron deficiency along with hypo-ferritinemia in majority of celiac patients.

In this survey the platelet levels were unusually high indicating secondary thrombocytosis with the highest levels observed in all three celiac groups (B, C & D). There were six cases observed in group B having severe thrombocytosis with levels as high as 1200×10^3 cells/ μ L. Similarly in group C, D and E platelet count with mean of 702×10^3 cells/ μ L, 518×10^3 cells/ μ L, and 529×10^3 cells/ μ L, respectively was observed. There are many reports that coincide with our data showing elevation in platelet count. Halfdanarson et al. TR, et al.²⁰ has described that in CD patients anemia may follow thrombocytosis. Another study by Dan stated that in majority of Celiac affected children the average platelet count is $\sim 700 \times 10^3$ cells/ μ L.²¹ However, studies show relationship between anemia and thrombocytosis, but the clear mechanisms and cause is still under debate.²² In anemic CD children, the erythropoietin is up-regulated. It is interesting to point out that the amino acid sequence of protein erythropoietin is quite similar to that of thrombopoietin and it might be one explanation for the secondary thrombocytosis occurrence.²³ For healthy individuals the range of transferrin should be 170-370 mg/dl, which was observed in the group A. For group B the highest transferrin level was observed. Because majority of patients had severe anemia and were not on strict gluten free diet due to which symptoms of celiac were persistent with less recovery. Also, for group C the ranges were observed to be on higher end. In participants suffering from both diabetes and celiac: group D the transferrin was observed to be 406.4 mg/dl.

The poorly managed CD cases in all groups had elevated levels of transferrin (Table II). When iron is absent, the transferrin protein levels are raised. Transferrin is an iron-binding glycoprotein that maintains the levels of iron in bio-fluids. When transferrin levels are higher, there is reciprocal decrease in transferrin iron saturation and corresponding increase in TIBC.^{24,25}

The CD children in concurrence with

T1DM were severely malnourished with life threatening anemia for some cases in our study. After autoimmune thyroiditis, diabetes mellitus type I is the second common autoimmune disorder in comorbidity to CD. Due to mutual sharing of genetic loci in CD and T1DM the chances of acquiring celiac disease are increased than the normal population.²⁶ One Pakistani study by Rasheed, et al. from Multan tertiary hospital has reported a list of many genetic disorders in concurrence with CD; among those T1DM (8.3%) being most prominent.²⁷ Moreover, most of these cases were observed to be late diagnosis. In addition, association of T1DM, epilepsy and arthritis with CD in Turkish and Dutch cohorts has been published.²⁸ The CD- T1DM group D in this survey showed, the mean hemoglobin, MCV, MCH and MCHC levels significantly decrease, while platelet count was at higher end. T1DM patients suffer from cardiac, kidney and pancreatic stress which leads to hematologic anomalies and when in conjunction with CD the anemic profile intensifies.²⁹

CONCLUSION

This study emphasizes the need for better awareness about varied clinical symptoms of Celiac, which causes delays in diagnosis. The hematological abnormalities were frequently present in CD children studied. Markers of iron-deficiency (RBC indices) and changes in platelet count should be considered to aid in management and diagnosis of celiac disease at clinical settings. Children having other autoimmune disorders like T1DM must also be tested for CD, if they are suffering from any of the celiac related symptoms.

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AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

KS: Conception and study design, acquisition of data, drafting the manuscript, critical review, approval of final version to be published

MR: Acquisition of data, drafting the manuscript, approval of final version to be published

AB: Analysis and interpretation of data, drafting the manuscript, approval of final version to be published

AAU: Acquisition, analysis and interpretation of data, critical review, approval of final version to be published

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

CONFLICT OF INTEREST

Authors declared no conflict of interest

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DATA SHARING STATEMENT

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