Research Article

Evaluation of Immunoglobulin's Biochemical and Hematological Parameters in Children with Celiac Disease

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Abstract: Celiac disease (CD) is an autoimmune illness which has affected about 1% of the general population and it has appeared due to gluten intolerance in genetically susceptible people. The current study was designed to determine the level of immunoglobulin IgA and IgG in children with celiac disease and to estimate their impact on biochemical and hematological parameters. The current study was conducted on 120 subjects, including 60 blood samples were collected from diagnosed celiac disease children aged less than 12 years along with 60 healthy control subjects of same age range. The results of the present study have shown a statistically significant difference between celiac disease patients and control group. Value of immunoglobulin IgA in CD patients was 205.98 ± 181.48 U/ml as compared to the control group i.e. 4.18 ± 1.77 U/ml which showed a statistically significant difference. The mean value of immunoglobulin IgG in patients was 77.21 ± 71.35 U/ml as compared to the control group i.e. 6.13 ± 1.33 U/ml which showed a statistically significant difference. The mean value of Serum alkaline Phosphatase in patients was 382.58 ± 114.99 U/L as associated to the control group i.e. 265.75 ± 65.2 U/L which showed a statistically significant change. The mean value of serum phosphorus in CD patients was 4.64 ± 1.01 mg/dl as compared to the control group i.e. 3.85 ± 0.55 mg/dl which showed a statistically significant difference. It has been concluded from the above results that in most of the cases of CD the values of serum IgA, serum IgG, serum alkaline phosphate, serum calcium, serum phosphorus and hemoglobin values can prove important clinical diagnostic markers for celiac disease which would ultimately be helpful in the management of this life-threatening disease.

Keywords: Celiac disease, Immunoglobulin A (IgA), Immunoglobulin G (IgG)

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1. Introduction

Celiac disease (CD) is an autoimmune illness which has affect about 1% of the general population and it has appeared due to gluten intolerance in genetically susceptible people. The clinical presentation is varied and uncommon. Children with CD are reported with diarrhea, fatigue, abdominal pain, nausea, and vomiting symptoms. Autoantibodies against tissue transglutaminase (tTG) during a gluten-containing diet are the most obvious serologic parameter of celiac disease (CD). Diagnosis of this disease has been increased worldwide including Pakistan [1]. The variable clinical description makes diagnosis challenging and celiac disease is generally under diagnosed. Celiac disease is diagnosed using a gluten-free diet and a combination of CD serology and small intestine mucosal histology [2].

The protein arrangement familiarized with the diet is another important element that substantially affects celiac disease (CD) development and clinical presentation in predisposed individuals. Gluten is made up of two components in wheat:

gliadin and glutenin. Glutenin is separated in to high and low molecular weight sub-units, while gliadin proteins are categorized as α/β, γ and ω-gliadins. Protein classes are expressed by vast multigene people (more than 50 genes) that encode comparable protein complexes with similar biochemical activity and are created by a single starting gene being multiplied [3]. Although it is a long-standing tradition to familiarize gluten into a child's diet around six months of age, and the rule is intensely ingrained in numerous developing countries, the best time to make known to gluten into a child's diet has never been systematically evaluated [4].

Monozygotic twins have a significant genetic propensity, with a concordance rate of about 75%. Some groups have a higher occurrence of CD than others. First-degree lineages of patients with histopathology CD, for example, account for 4% to 12% of those with the illness. Second-degree relatives tend to be more common as well. The prevalence of CD in individuals with type 1 diabetes mellitus (IDDM) ranges from 3% to 8%. The presence of CD is found in between 5% and 12% of patients with Down's syndrome [5]. The yeast *Candida albicans* sticky protein (Hwp1) has been characterized as a precursor for mammalian transglutaminase and is identical to two significant CD-related gliadin T-cell epitopes, implying that Candida could be a CD trigger. Candida was recently found as major fungus genera in CD patients after gastric microbiome characterization [6].

TG2 is the most common transglutaminase in mammals, with significant intra and extracellular manifestation in nearly all tissues. Intracellular TG2 is inactive enzymatically, but for unknown causes, it is transferred extracellularly, where it becomes active enzymatically. TG2 interacts with extracellular matrix proteins, particularly fibronectin protein and is implicated in cell adhesion, extracellular matrix stabilization, tissue repair, receptor signaling, and cellular proliferation (CP), and cellular motility [7]. The average age of diagnosis was similar to what has been reported in the literature. In this study, 60.7 percent of patients had parents who were related by consanguinity. In their celiac cohort, Ouda S et al. found that more than 90% of patients had consanguineous parents. Previous research found consanguinity in 40-96 percent of Pakistani children with celiac disease, leading them to conclude that consanguinity is a substantial risk factor for CD. However, only 9 percent of patients had a favorable family history [8]. Celiac disease has a lot of clinical variabilities, which can make diagnosis confirmation difficult. Thus, the existing study is planned to assess the accuracy of immunoglobulins, biochemical and hematological parameters at par with diagnosis from the commercially available kits in children with Celiac Disease [9].

2. Materials and Methods

The cross-sectional study was conducted on 120 subjects, including 60 blood samples were collected from diagnosed celiac disease children aged less than 12 years along with 60 control subjects. The blood samples were collected from Children's Hospital and University of Child Health Sciences Lahore during the two months period with a prior approval from the Ethical Committee of School of Biochemistry, Minhaj University, Lahore. About 3 ml blood samples were collected from test and control groups using sterile (5ml BD, USA) syringes and transferred in EDTA and gel vials subsequently and then incubated for 30 minutes at 37°C in an incubator. The EDTA vials were shaked gently to mix anticoagulant immediately and gel vials with clotted blood were centrifuged at 4000 rpm for 10 minutes to obtain serum which was collected in separate plastic tubes and used for determination of serum Tissue Transglutaminase Immunoglobulin's IgA, IgG, serum Alkaline Phosphatase (ALP), serum calcium, serum phosphate and hemoglobin. The serum level of Tissue Transglutaminase Immunoglobulin IgA and IgG were determined using ORGENTEC Diagnostika GmbH Carl-Zeiss-StraBe Kit. Whereas, the serum level of Biochemical parameters i.e. Serum Alkaline Phosphatase (ALP),

serum calcium and serum phosphorus were determined by using chemical reagents manufactured by (Beckman Coulter, USA) on fully automated chemistry analyzer AU-480. Similarly, hemoglobin was determined using SYXMEX XP-300.

Statistical Analysis

The data obtained from experimental work analyzed statistically using automated software IBM SPSS (Version, 24.0) for one and two sample t-test respectively, and the p-value were calculated to check the significance level i.e. ($p < 0.05$).

3. Results

The study was done to evaluate the serum level of Tissue Transglutaminase Immunoglobulin's IgA, IgG, Serum Alkaline Phosphatase (ALP), Serum Calcium, Serum Phosphate and Hemoglobin.

The results of the present study have been shown in Table 1 which was statistically significant difference between celiac disease patients and the control group. Value of tTg- IgA in CD patients was 205.98 ± 181.48 U/ml as compared to the control group i.e. 4.18 ± 1.77 U/ml which showed a statistically significant difference. The mean value of tTg-IgG in patients was 77.21 ± 71.35 U/ml as compared to the control group i.e. 6.13 ± 1.33 U/ml which showed a statistically significant difference. The mean value of Serum Alkaline Phosphatase in patients was 382.58 ± 114.99 U/L as compared to control group i.e. 265.75 ± 65.2 U/L which showed a statistically significant change. The mean value of Serum Phosphorus in CD patients was 4.64 ± 1.01 mg/dl as compared to the control group i.e. 3.85 ± 0.55 mg/dl which showed a statistically significant difference. The mean value of Serum calcium in CD patients was 8.69 ± 1.23 mg/dl as compared to the control group i.e. 9.76 ± 0.54 mg/dl which showed a statistically significant difference i.e. ($p < 0.05$). The mean value of Hb in CD patients was 9.18 ± 1.7 g/dl as compared to the control group i.e. 11.46 ± 0.46 g/dl which showed a statistically significant difference.

Table 1. The Studied Parameters with their mean and standard deviation

Parameters	Case/Control	Mean	Std. Deviation
tTg- IgA (U/ml)	Celiac disease	205.98	181.48
	Control	4.18	1.77
tTg- IgG (U/ml)	Celiac disease	77.21	71.35
	Control	6.13	1.33
Serum alkaline Phosphatase (U/L)	Celiac disease	382.58	114.99
	Control	265.75	65.2
Serum Phosphorus (mg/dl)	Celiac disease	4.64	1.01
	Control	3.85	0.55
Serum Calcium (mg/dl)	Celiac disease	8.69	1.23
	Control	9.76	0.54
Hb (g/dl)	Celiac disease	9.18	1.7
	Control	11.46	0.46

*tTg-IgA = Tissue Transglutaminase Immunoglobulin A

**tTg-IgG = Tissue Transglutaminase Immunoglobulin G

***Hb = Hemoglobin

The statistically significant alteration in tTg-IgA, IgG, Serum Alkaline Phosphatase, Serum Phosphorus, Serum calcium and Hemoglobin (Hb) level of the Patients and control group has been shown in Table 2.

Table 2. t-test for Equality of Means (Two Independent sample t-tests)

Parameters	Mean value of Celiac patients	Mean value of Control persons	p-value
tTg –IgA (U/ml)	205.98	4.18	<0.05
tTg-IgG (U/ml)	77.21	6.13	<0.05
Serum alkaline Phosphatase (U/L)	382.58	265.75	<0.05
Serum Phosphorus (mg/dl)	4.64	3.85	<0.05
Serum Calcium (mg/dl)	8.69	9.76	<0.05
Hb (g/dl)	9.18	11.46	<0.05

The significant change in the mean of tTg-IgA of celiac disease patients i.e. 10 the significant difference in the mean of tTg-IgG of celiac disease patients i.e. 8 the significant variance in the mean of Serum Phosphorus of celiac disease patients i.e. 175 the probable difference in the mean of Serum Phosphorus of celiac disease patients i.e. 3.5 the significant difference in the mean of Serum calcium of celiac disease patients i.e. 9.8 the significant alteration in the mean of Hb of celiac disease patients i.e. 14.5 were shown in Table 3.

Table 3. One Sample t-test

Parameters	t	df	p-value	Mean Difference	95% C. I of the Difference	
					Lower	Upper
Test Value = 10						
tTg-IgA	2.782	59	0.007	468.596	131.52	805.67
Test Value = 8						
tTg-IgG	7.57	59	0.000	71.28	52.43	90.13
Test Value = 175						
Serum alkaline Phosphatase	13.983	59	0.000	207.583	177.87	237.28
Test Value = 3.5						
Serum Phosphorus	8.792	59	0.000	1.142	8.882	1.40
Test Value = 9.8						
Serum Calcium	-7.024	59	0.000	-1.111	-1.438	-0.795
Test Value = 14.5						
Hb	-24.314	59	0.000	-5.324	-5.762	-4.886

*df = degrees of freedom

**C.I = confidence intervals

Figure 1 illustrates that 35% of cases have Serum alkaline Phosphatase level less than 350 U/L and 65% of cases Serum Alkaline Phosphatase level is greater than 350 U/L while, 97% of the control group have Serum Alkaline Phosphatase level less than 350 U/L and 3% of control group have Serum Alkaline Phosphatase level greater than 350U/L.

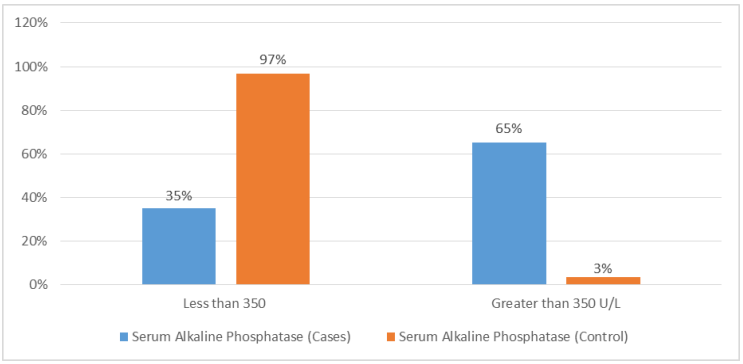

Figure 1. Comparison of Serum Alkaline Phosphatase between Control and Celiac Disease Patients

Figure 2 was showing that 48% of cases had Serum Phosphorous levels less than 4.5 mg/dl and 52% of cases had Serum Phosphorous level is greater than 4.5 mg/dl while, 97% of the control group has Phosphorous levels less than 4.5 mg/dl and 3% of control group had Phosphorus level greater than 4.5 mg/dl.

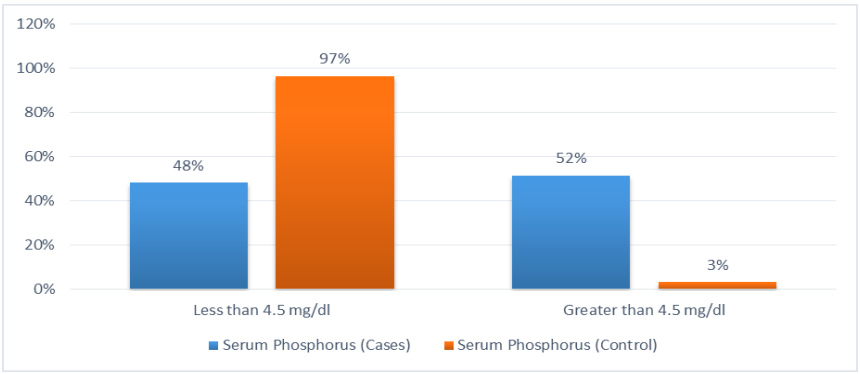

Figure 2. Comparison of Serum Phosphorous between Control and Celiac Disease Patients

Figure 3 was showing that 50% of cases had Serum calcium levels less than 8.8 mg/dl and 50% of cases had Serum calcium levels greater than 8.8 mg/dl similarly, 7% of control group had calcium levels less than 8.8 mg/dl, and 93% of control group had calcium level greater than 8.8 mg/dl.

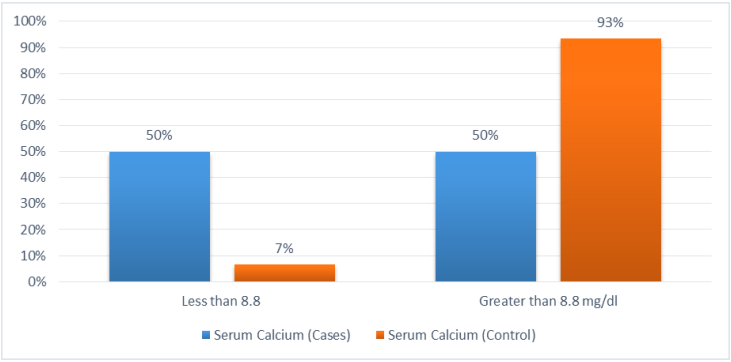

Figure 3. Comparison between Serum Calcium Levels between Control and Celiac Disease Patients

Figure 4 was showing that 74% of cases had hemoglobin (Hb) level was < 10.5 g/dl and 26% cases had (Hb) level greater than 10.5 g/dl while, 0% of the control group had hemoglobin (Hb) level is less than 10.5 g/dl and 100% of Control group had hemoglobin (Hb) level greater than 10.5 g/dl.

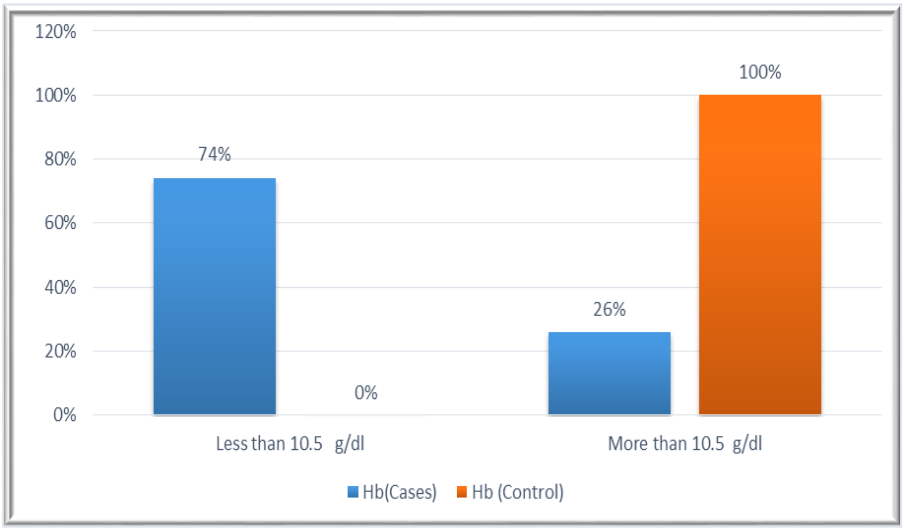

Figure 4. Comparison of Hemoglobin (Hb) between Control and Celiac Disease patients

Figure 5 showed that 5% cases had Immunoglobulin (IgA) level less than 8 U/ml and 95% cases had Immunoglobulin (IgA) level greater than 8 U/ml similarly, 100% of healthy children had Immunoglobulin (IgA) level less than 8 U/ml and 0% of healthy children had Immunoglobulin (IgA) level greater than 8 U/ml.

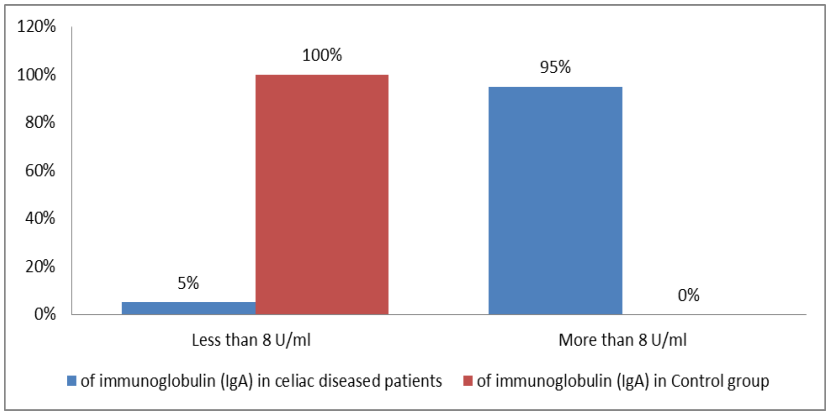

Figure 5. Comparison of tTG immunoglobulin (IgA) of Control and Celiac Disease Patients

Figure 6 was showing that 12% of cases had immunoglobulin (IgG) level less than 10 U/ml and 88% cases had immunoglobulin (IgG) level greater than 10 U/ml while, 0% of control children have immunoglobulin (IgG) levels less than 10 U/ml and 100% of healthy children had immunoglobulin (IgG) level greater than 10 U/ml.

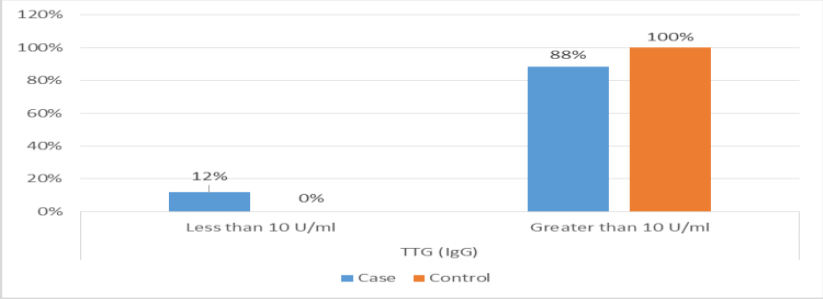

Figure 6. Comparison of tTg Immunoglobulin (IgG) between Control and Celiac Disease Subjects

4. Discussion

CD is dangerous autoimmune disease that distresses more than 0.5 % of the world's population, according to estimates. Gluten (Amino Acids present in wheat, rye and barley) causes celiac disease sufferers to have a strong immune response that assaults the small intestine and destroys the villi lining the small intestine. Celiac disease is a genetic disorder that spread from generation to generation. People who have a first-degree virtual with celiac disease have a one-in-ten probability of emerging the disease [10].

Celiac disease (CD) caused by gluten containing foods or medications and can occur at any age. CD symptoms often affect the intestinal absorption but they can affect other sections or organ of the body as well. Children and adults often had distinct sets of Symptoms. Paeds population with celiac disease may experience fatigue and irritability. They may also be smaller than average and experience delayed puberty [11]. Weight loss, vomiting, abdominal bloating, stomach discomfort, chronic diarrhea or constipation and pale, greasy, foul-smelling faeces are other typical symptoms. According to the 2017 statistics census, Pakistan has around 207.8 million inhabitants (2007), because CD is expected to affect more than 1% of the population. Over 2 million celiac disease patients are estimated in Pakistan [12]. However, in terms of epidemiology and occurrence, there is limited information obtainable for celiac disease in Pakistan despite the availability of extremely profound and specific serological tests such as IgA-tissue transglutaminase antibodies. Up till now, only 34 studies have been conducted on various aspects of celiac disease so far. However, neither study has described the biochemical and Immunoglobulin contents of celiac disease patients. As a result, the present study aims to assess immunoglobulins and biochemical parameters in celiac disease children. To diagnose a patient, a physical examination and a medical history are required. Blood tests can identify raised levels of anti-endomysium and anti-tissue transglutaminase (tTGA) antibodies in celiac disease patients. CBC, LFTs, cholesterol test, alkaline phosphatase level test and serum albumin test are all common blood tests [13].

In the current study, there is a substantial alteration in the mean of tTG (IgA) of celiac disease patients from 10 there is a significant difference in mean of tTG (IgG) of celiac disease patients from 8. CD-related antibodies are classified as IgA or IgG, but only those classified as IgA are highly sensitive and specific for CD Volta et al. (2010).Because of the large percentage of false positives, the use of IgG markers (save for DGP) is frequently deceptive, and their use should be confined to individuals with IgA deficiency Villalta, (2010). 7% cases have tTG (IgG) level less than 10 U/ml and 60% cases have tTG (IgG) level greater than 10 U/ml, 53% of healthy children have tTG (IgG) level less than 10 U/ml and 0% of healthy children have tTG (IgG) level greater than 10 U/ml. Antibodies titers were performed by [14].

CD related antibodies are classified as IgA or IgG, but only those classified as IgA are highly sensitive and specific for CD because of the large percentage of false positives, the use of IgG markers (save for DGP) is frequently deceptive, and their use should be confined to individuals with IgA deficiency [15]. Nearly 7% cases had TTG (IgG) level less than 10 U/ml and 60% cases have TTG (IgG) level greater than 10 U/ml, 53% of healthy children had TTG (IgG) level less than 10 U/ml and 0% of healthy children have TTG (IgG) level greater than 10 U/ml. Antibodies titers was performed by [16]. On a gluten free diet, juvenile patients 18 years or younger, straight likened fundamental data from 16 antibody testing. Patients with a low level of TGA-IgA but no other symptoms of celiac disease were labelled as having no CD.

An immunoglobulin test determines the amount of different kinds of antibodies in the blood. Antibodies are produced by the immune system to defend the body from invading microorganisms and allergens. To combat these diseases, the body produces various antibodies, or immunoglobulins. The antibody for chickenpox, for example, is not the same as the antibody for mononucleosis. Sometimes the body will wrongly produce antibodies against itself, mistaking healthy organs and tissues for invading intruders. This is referred to as an autoimmune illness [17].

In the current study, there was a momentous alteration amid the hemoglobin (Hb) level of the patients and the control group. The changes in Hb contents might be due to malabsorption of multiple micronutrients required for normal hematopoiesis. A similar conducted by Bergamaschi et al (36) on CD patients around low hemoglobin (Hb) in 34%, with higher prevalence in women (21% in men vs 41% in women, $P = 0.021$).

Moreover, current analysis of blood phosphorus level 48% cases Phosphorus level is less than 4.5 mg/dl and in 52% cases Phosphorus level is greater than 4.5 mg/dl and Calcium level less than 8.8 mg/dl and 50% cases have Calcium level superior than 8.8 mg/dl in Celiac disease patients. The first clinical manifestations of celiac disease vary depending on the severity and activity of the illness, but patients often appear with lactose intolerance, steatorrhea, and weight loss [18].

Ca^{+} malabsorption has been linked to vit-D deficiency, intraluminal calcium binding to unabsorbed fatty acids, and decreased transport by the sick intestinal mucosa and it can result in hypocalcemia, secondary hyperparathyroidism, osteopenia and elevated alkaline phosphatase levels [19].

Another study by Doganci and Bozkurt (2004) reported a total of 45 CD children with chronic diarrhea, anemia, and short stature were assessed. There was no statistically significant difference in hemoglobin levels between children with CD who presented with anemia. The authors concluded that generalized malabsorption may not impact children, particularly newborns with chronic diarrhea with CD. Anemia is observed in CD patients, whether or not they are the predominant presenting symptoms [20].

The result shows that people suffering from Celiac disease condition are enormous who elude correct diagnosis. But certain routine and comparatively cheaper parameters like serum phosphorous, calcium have shown significant results validation disease in pediatric patients. Hence, serological testing can help provide substantial proof that CD is present. A further larger cohort is needed to confirm and validate the results of the current finding.

5. Conclusions

On the basis of current study findings, it has been concluded that serum alkaline phosphatase, serum calcium, serum phosphorus and hemoglobin (Hb) can prove important clinical diagnostic markers for celiac disease which would ultimately be helpful in the management of this life-threatening disease.

Conflict of interest

All authors do not have any conflict of interest.

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