VALIDATION OF THE RAPID TEST "POINT-OF-CARE TEST" FOR THE DIAGNOSIS OF CELIAC DISEASE COMPARED TO THE STANDARD DIAGNOSTICS

Ayman Emil Eskander[1], Noha Musa[2], Hend El Sherbini[3], Mona Abu-Zekry[4], Marwa Abdalaleem[5*]

Article History: **Received:** 12.12.2022 **Revised:** 29.01.2023 **Accepted:** 15.03.2023

Abstract

Background and aim: The worldwide prevalence of celiac disease (CD) of 1 % results in a global burden which necessitates rapid diagnosis and treatment. The aim of this study was to verify the efficacy of the Point-of-Care Test (POCT); BIOCARD™ Celiac Test for the diagnosis of CD.

Methods: 500 children with symptoms suggestive of CD were included in this study and all of them underwent testing for CD using the BIOCARD™ Celiac Test and those with positive or query test results underwent serological testing for CD by anti-tissue transglutaminase immunoglobulin A (anti-tTG IgA) antibodies and upper gastrointestinal endoscopy along with small intestinal biopsies for histopathological examination for features suggestive of CD to confirm the diagnosis.

Results: The BIOCARD™ Celiac Test results were positive for CD in 16 children (3.2 %), query in 3 (0.6 %) and were negative in 481 children (96.2 %). Out of the 16 positive POCT results, 14 children were confirmed by both biopsy histopathological results and serum anti-tTG IgA antibodies test to have CD, one of them had negative anti-tTG IgA antibodies test but had biopsy-confirmed CD and the last one had positive anti-tTG IgA antibodies test but the biopsy result was not suggestive of CD. The sensitivity of the POCT; BIOCARD™ Celiac Test in detecting CD in our study was 93.75 % when confirmed by histopathological examination of biopsy samples and serum anti-tTG IgA antibodies test as reference standard diagnostic tests.

Conclusion: The POCT; BIOCARD™ Celiac Test was successfully validated as a rapid, simple and cheap biomarker for early detection and diagnosis of CD, and hence it can be applied for CD case-finding in primary care settings.

Keywords: Celiac disease, Point-of-Care Test, Anti-tissue transglutaminase antibodies, Upper gastrointestinal endoscopy, Small intestinal biopsy.

[1,2,4,5*]Department of Pediatrics, Cairo University Children's Hospital, Cairo, Egypt.
[3]Department of Clinical and Chemical Pathology, Faculty of Medicine, Cairo University, Cairo, Egypt.

Email: Marwa Abdalaleem, marwa_aleem@yahoo.com

DOI: 10.31838/ecb/2023.12.s3.193

1. Introduction

Celiac disease (CD) is an immune-mediated enteropathy, induced by intolerance to dietary gluten present in wheat, rye and barley, in susceptible individuals [(1)]. The clinical presentation is extremely variable; patients may have severe gastrointestinal symptoms and mal-absorption, extra-intestinal symptoms or have no symptoms at all [(2)]. Poor growth and anemia tend to be the most common extra-intestinal manifestations in children [(3)]. Owing to the multifaceted clinical presentation, diagnosis remains a challenge and CD is heavily underdiagnosed. There is no single test that can completely exclude or diagnose CD with 100 % certainty; however, the collection of clinical findings, serologic tests, intestinal biopsy and the demonstration of the characteristic histopathological changes in the small bowel biopsy and clinical or histologic response to a gluten free diet (GFD) treatment, all together may diagnose CD [(4)].

CD has emerged as an increasingly recognized public health problem, and is now considered as a global phenomenon affecting both developing and developed nations, being one of the most common autoimmune disorders affecting around 1 % of the population worldwide [(5)]. Given the high prevalence of CD, a delay in diagnosis is expected to increase morbidity, in terms of nutritional deficiencies and, especially, of gluten related autoimmune diseases and mortality. In order to face this non-communicable epidemic, it is necessary to have accurate tests available [(6)].

Aim of the study

The aim of our study was to verify the use of the Point-of-Care Test (POCT); BIOCARD™ Celiac Test for CD diagnosis in children aged 1-18 years with symptoms suggestive of CD (growth disorders and/or persistent diarrhea, vomiting, abdominal discomfort or distension, anemia).

2. Methods

The study, an open-label clinical trial, was conducted at the Pediatric Gastroenterology Unit, Abu El-Reesh Children's Hospital, Cairo University, Cairo, Egypt, over the period from July 2013 till March 2015. The study included 500 children with the following inclusion criteria; children aged 1-18 years, with no specific gender or ethnicity, with symptoms suggestive of CD (growth disorders and/or persistent diarrhea, vomiting, abdominal discomfort or distension, anemia). Those less than one year or above 18 years of age were excluded.

Ethical approval: The study was approved by the Ethical Committee of the Faculty of Medicine, Cairo University, Cairo, Egypt on 17/06/2013. Informed consents were obtained from the parents or legal guardians of the children prior to inclusion in the study after explaining the nature of the study to them.

Initial assessment: Demographic data and medical history were taken from the participating children regarding their personal details (name, age, sex and residence), symptoms suggestive of CD (diarrhea, abdominal discomfort, abdominal distension, constipation, vomiting, nausea, faltering growth or failure to gain weight), history of weight loss, nutritional history (breast or artificial feeding and timing of introduction of wheat) and family history of CD. Also, full physical examination was done and anthropometric parameters including weight, height and body mass index (BMI) were taken for all the included children.

All the included children were tested for CD using the Point-of-Care Test (POCT); BIOCARD™ Celiac Test. For the children with positive or query POCT results, further testing by serum anti-tTG IgA antibodies and upper gastrointestinal endoscopy and histopathological examination of the small intestinal biopsies were performed, as reference standard diagnostic tests.

BIOCARD™ Celiac Test (Ani Biotech, Vantaa, Finland):

It is an immunochromatographic test for the qualitative detection of anti-tTG IgA antibodies from a fingertip capillary whole blood sample. The test requires only 1 drop (10 microliter) of blood from the fingertip, but also intravenous whole blood samples may be used. If the sample contains anti-tTG IgA antibodies, these will bind with the gold-labeled antibodies, with the tTG derived from the red blood cells in the sample and with the stationary reagents in the test membrane forming a visible red test line. The test also contains an integrated detection system for total IgA. Red line in the control window shows that the sample contains enough IgA antibodies for CD diagnosis. The test can be carried out and evaluated in about 5 minutes, but no longer than 15 minutes; positive results can be seen already after 1-2 minutes.

Interpretation of the test results (Figure 1):

The test result is positive if a red control line appears in the control field (C) and a light to dark red line forms in the test field (T) which indicates that there are anti-tTG antibodies in the blood sample, with a high probability of an existing CD. The test result is negative if a red control line appears in the control field (C) and no red line forms in the test field (T), which indicates that there are no anti-tTG antibodies in the tested blood or the antibody level is below the cut-off of the test (5 U/ml). In this case, CD can virtually be ruled

out. However if gastrointestinal complaints are present, further medical investigation is necessary. The test result is query if there is no line in the test field or in the control field or the line intensity in the control field is faint, which indicate that there are either no IgA antibodies or that the titer of the IgA antibodies is low in the sample (since the test line measures IgA antibodies against tTG). In this case there is no possibility to detect CD with the BIOCARD™ Celiac Test and separate testing of the IgG antibodies against tTG (anti-tTG IgG) should be done, as there is higher prevalence of selective IgA deficiency in patients with CD [(7)].

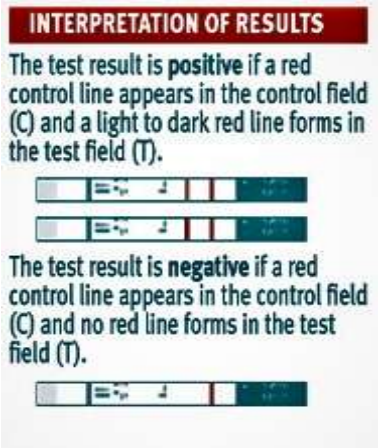

Figure 1: Interpretation of the test results

Measuring serum anti-tissue transglutaminase IgA antibodies:
An enzyme immunoassay test (IPR-Immuno Pharmacology Research, Catania, Italy) indicated for the detection of antibodies of class A anti-human transglutaminase present in the serum sample was used. The presence of anti-tTG IgA antibodies in the sample means a positive result and should be considered indicative of CD.

Upper gastrointestinal endoscopy and histopathological examination of the small intestinal biopsy:
The duodenum and upper part of the small intestine were examined by upper endoscopy using fiber-optic video-scope Olympus Type GIF XP 260 (Olympus Medical Systems, Hamburg, Germany) and multiple biopsies were obtained from the duodenum and the bulb using punch biopsy forceps. Microscopic (histopathologic) description of the small intestinal villous lesion characteristic of CD was classified according to the modified Marsh-Oberhuber classification, where Marsh type 2 or 3 at histology were considered the gold standard of CD diagnosis [(8)].

Data management and analysis
The collected data was revised, coded, tabulated and introduced to a personal computer (PC) using Statistical package for Social Science (SPSS 15.0.1 for windows; SPSS Inc, Chicago, IL, 2001). Data was presented and suitable analysis was done according to the type of data obtained for each parameter.

3. Results

The mean age of the study participants was 3.7±3.1 years and ranged between 1 to 18 years with a median of 2.5 year. The mean duration of breast feeding and the mean time of wheat introduction was 11.7±4.3 months and 7.5±3 months, respectively. The mean BMI was 15.5±3. Males represented 51.2 % of the participants; where the male: female ratio was almost 1:1. About 44 %, 66.7 %, and 68 % had normal (average) weight for age, height for age and weight for length, respectively. The most common manifestations of CD among the participants were failure to gain weight (88 %) followed by diarrhea and abdominal discomfort (74.8 % each), vomiting (57.2 %), abdominal distension (55.2 %), faltering growth (20 %), while the least common manifestations were dystrophy and dermatitis (8.8 % and 8.4 %, respectively).

Table 1: The prevalence of the signs and symptoms suggestive of celiac disease among the participating children

Signs and symptoms	Number of patients	Percentage (%)
Failure to gain weight	440	88

Diarrhea	374	74.8
Abdominal discomfort	374	74.8
Vomiting	286	57.2
Abdominal distension	276	55.2
Nausea	144	28.8
Faltering growth	100	20
Pallor	70	14
Constipation	64	12.8
Dystrophy	44	8.8
Dermatitis	42	8.4

Among the total number of the 500 participating children in this study, the POCT rendered positive results in 16 of them (3.2 %), query results in 3 (0.6 %) and was negative in 481 children (96.2 %)(Figure 2).

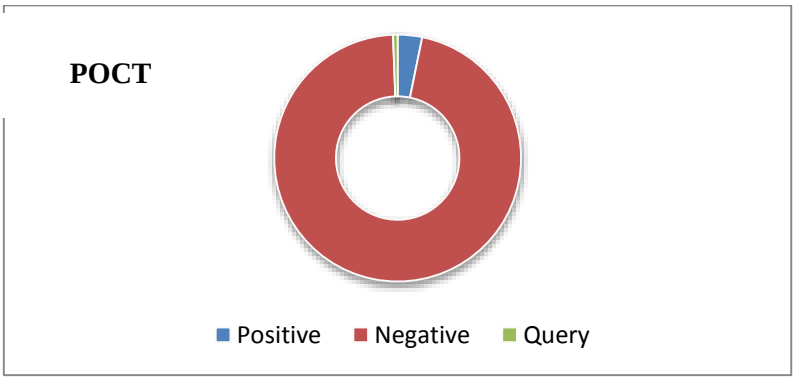

Figure 2: The results of the POCT among all of the participating children

Out of the 16 children with positive POCT results, 14 of them were diagnosed to have CD as confirmed by both serum anti-tTG IgA antibodies test results and histopathological results of the biopsy samples; one child had negative anti-tTG IgA antibodies test but had biopsy histopathological results suggestive of CD and the last one had positive serum anti-tTG IgA antibodies test but the biopsy result was not histopathologically suggestive of CD. The 3 cases with query POCT results showed also negative serum anti-tTG IgA antibodies test and only one of them had CD as confirmed by biopsy histopathological assessment (Table 2).

Table 2: The relation between POCT results and the results of the serum anti-tTG IgA antibodies test and biopsy histopathological assessment results

			serum anti-tTG IgA antibodies test results		**Biopsy histopathological findings suggestive of CD**	
			Positive	**Negative**	**Yes**	**No**
		Number	**Number**	**Number**	**Number**	**Number**
POCT	**Positive**	**16**	**15**	**1**	**15**	**1**
	Query	**3**	**0**	**3**	**1**	**2**

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the POCT when compared to the biopsy-confirmed cases as a reference were 93.75 %, 99.79 %, 93.75 % and 99.79 %, respectively with an overall 99.5 % diagnostic accuracy. The sensitivity,

specificity, PPV and NPV of the POCT when compared to the serum anti-tTG IgA antibodies test confirmed cases as a reference, were 93.75 %, 75 %, 93.75 % and 100 %, respectively with an overall 94.7 % diagnostic accuracy.

The prevalence of CD in our study group; when confirmed by the POCT results, the serum anti-tTG IgA antibodies test results and the biopsy histopathological findings suggestive of CD; was 3 %. It was found that the most common classical signs and symptoms of CD presenting in the diagnosed cases were failure to gain weight (93.3 %), followed by diarrhea (86.8 %), abdominal discomfort (75 %), abdominal distension and vomiting (60 % each).

4. Discussion

The sensitivity of the POCT; BIOCARD™ Celiac Test (AniBiotech®, Vantaa, Finland), in detecting CD in our study was 93.75 % when confirmed by histopathological examination of biopsy samples and serum anti-tTG IgA antibodies test as reference standard diagnostic tests. The sensitivity, specificity, PPV and NPV of the POCT when compared to the biopsy-confirmed cases as a reference were 93.75 %, 99.79 %, 93.75 % and 99.79 %, respectively with an overall 99.5 % diagnostic accuracy, and when compared to the serum anti-tTG IgA antibodies test confirmed cases as a reference, they were 93.75 %, 75 %, 93.75 % and 100 %, respectively with an overall 94.7 % diagnostic accuracy. Point-of-Care tests (POCTs), such as BIOCARD™ Celiac Test, have been developed and their high diagnostic accuracy for CD diagnosis has also been previously validated in many studies, in order to screen the general population and speed up CD diagnosis. A study similar to ours by Singh et al., [9] successfully validated the BIOCARD™ Celiac Test for CD diagnosis, in north India, in children aged 2-18 years with symptoms suggestive of CD. The test showed 83.6 % sensitivity and 90 % specificity compared to reference tests (positive serology plus histology suggestive of CD). Also two previously published studies which were performed in two centers, from countries participating in the MEDICEL network project (Mediterranean Network for the Management of Food Induced Diseases), obtained the highest PPV (100 %) by using the same POCT; one of them in Greece by Karagiozoglou-Lampoudi et al., [10] and the other one in Tunisia by Ben Hariz et al., [11]. The efficacy of a POCT based on deamidated gliadin peptides (DGP) for the detection of CD in pediatric patients was also evaluated and rendered similar results. One was a study by Polanco et al., [12] which showed 95.8 % sensitivity, 98.1 % specificity, 97.9 % PPV and 96.2 % NPV value with a 100 % diagnostic accuracy and another study by Esteve et al., [13] with a sensitivity of POCT in detecting CD of 100 %, a specificity of 93 %, PPV of 14 % and NPV of 100 %. In a systematic review by Singh et al., [14]; the pooled sensitivity and specificity for tTG-IgA-based POCTs were 90.5 % and 94.8 %, respectively and the pooled sensitivity and specificity of all POCTs (based on tTG or DGP or tTG + anti-gliadin antibodies) for diagnosing CD were 94.0 % and 94.4 %, respectively. The prevalence of CD in our study was 3 % which is higher than the prevalence in the general population studies. This could be the result of the higher prevalence rate of CD in those at-risk, like subjects included in our study with symptoms suggestive of CD (growth disorders and/or persistent diarrhea, vomiting, abdominal discomfort or distension, anemia), than in the general population screening studies. A study by Abu-Zekry et al., [15] showed the prevalence of CD to be 0.5 % among the not-at risk (general population) and 4.7-6.4 % in the at-risk (type 1 diabetes mellitus, diarrhea) Egyptian children, based on serological and biopsy-confirmed cases. In a systematic review, the prevalence of CD reported worldwide was 1.4 % based on serologic test results and 0.7 % based on biopsy results. This prevalence was found to vary with sex, age and location [16].

5. Conclusion

Detection of anti-tTG IgA antibodies in the serum using commercial enzyme-linked immunosorbent assays (ELISA) for testing for CD represents a valuable tool for diagnosis. However, it is time-consuming, requires a serum sample, and is too expensive for countries with poor resources and needs equipped laboratories that are not readily available in developing countries where patients are not able to reach referral centers or centralized laboratories located far away. Introducing a simple blood drop based POCT may represent the only way to diagnose CD in these countries, as it is a low-cost, quick with immediate results, widely accessible, easy-to-perform test, that does not require a laboratory or experienced staff and has comparable sensitivity and specificity to the standard diagnostics methods. BIOCARD™ Celiac Test may be also used for monitoring the effects of GFD, where the anti-tTG IgA antibodies titers diminish with the institution of GFD, often within weeks, and may become undetectable after 6 months.

Conflicts of interest: The authors have no conflict of interest to declare.

6. References

Lindfors K, Ciacci C, Kurppa K, Lundin KEA, Makharia GK, Mearin ML, Murray JA, Verdu

EF, Kaukinen K. Coeliac disease. Nat Rev Dis Primers. 2019 Jan 10; 5(1):3.

Bai JC, Verdú EF. Evolving Paradigms in the Diagnosis of Adult Patients with Celiac Disease. Am J Gastroenterol. 2019 Jun; 114(6):854-857.

Nurminen S, Kivela L, Huhtala H, Kaukinen K, Kurppa K. Extraintestinal manifestations were common in children with coeliac disease and were more prevalent in patients with more severe clinical and histological presentation. Acta Paediatr. 2019 Apr; 108(4):681-687.

Ensari A, Marsh MN. Diagnosing celiac disease: A critical overview. Turk J Gastroenterol. 2019 May; 30(5):389-397.

Mogul D, Nakamura Y, Seo J, Blauvelt B, Bridges JF. The unknown burden and cost of celiac disease in the U.S. Expert Rev Pharmacoecon Outcomes Res. 2017 Apr; 17(2):181-188.

Costa S, Astarita L, Ben-Hariz M, Currò G, Dolinsek J, Kansu A, Magazzu' G, Marvaso S, Micetic-Turku D, Pellegrino S, Primavera G, Rossi P, Smarrazzo A, Tucci F, Arcidiaco C, References 139 Greco L. A Point-of-Care test for facing the burden of undiagnosed celiac disease in the Mediterranean area: a pragmatic design study. BMC Gastroenterol. 2014 Dec 18; 14:219.

Valletta E, Fornaro M, Pecori S, Zanoni G. Selective immunoglobulin A deficiency and celiac disease: let's give serology a chance. J Investig Allergol Clin Immunol. 2011; 21(3):242-4.

Marsh MN, Rostami K. What is a normal intestinal mucosa? Gastroenterology. 2016; 151:784-8.

Singh P, Wadhwa N, Chaturvedi MK, Bhatia V, Saini S, Tandon N, Makharia GK, Maki M, Not T, Phillips A, Bhatnagar S. Validation of Point-of-Care testing for coeliac disease in children in a tertiary hospital in north India. Arch Dis Child. 2014 Nov; 99(11):1004-8.

Karagiozoglou-Lampoudi T, Zellos A, Vlahavas G, Kafritsa Y, Roma E, Papadopoulou A, Fotoulaki M, Karyda S, Xinias I, Savvidou A. Screening for coeliac disease in preschool Greek children: the feasibility study of a community-based project. Acta Paediatr. 2013 Jul; 102(7):749-54.

Ben Hariz M, Laadhar L, Kallel-Sellami M, Siala N, Bouraoui S, Bouziri S, Borgi A, Karouia F, Maherzi A, Makni S. Celiac disease in Tunisian children: a second screening study using a "new generation" rapid test. Immunol Invest. 2013; 42(4):356-68.

Polanco I, Koester Weber T, Martínez-Ojinaga E, Molina M, Sarria J. Efficacy of a Point-of-Care test based on deamidated gliadin peptides for the detection of celiac disease in pediatric patients. Rev Esp Enferm Dig. 2017 Nov; 109(11):743-748.

Esteve M, Rosinach M, Llordés M, Calpe J, Montserrat G, Pujals M, Cela A, Carrasco A, Ibarra M1, Ruiz-Ramirez P, Tristán E, Arau B, Ferrer C, Mariné M, Ribes J, Fernández-Bañares F. Case-finding in primary care for coeliac disease: Accuracy and cost-effectiveness of a rapid Point-of-Care test. United European Gastroenterol J. 2018 Jul; 6(6):855-865.

Singh P, Arora A, Strand TA, Leffler DA, Mäki M, Kelly CP, Ahuja V, Makharia GK. Diagnostic Accuracy of Point-of-Care Tests for Diagnosing Celiac Disease: A Systematic Review and MetaAnalysis. J Clin Gastroenterol. 2019 Aug;53(7):535-542

Abu-Zekry M, Kryszak D, Diab M, Catassi C, Fasano A. Prevalence of celiac disease in Egyptian children disputes the east-west agriculture-dependent spread of the disease. J Pediatr Gastroenterol Nutr. 2008 Aug; 47(2):136-40.

Singh P, Arora A, Strand TA, Leffler DA, Catassi C, Green PH, Kelly CP, Ahuja V, Makharia GK. Global Prevalence of Celiac Disease: Systematic Review and Meta-analysis. Clin Gastroenterol Hepatol. 2018 Jun; 16(6):823-836.