Original Article

Detection of CD69 Marker by Flow Cytometry in Iraqi Patients with Coeliac Disease

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Summary:

Background: Coeliac disease is a chronic, immunologically determined form of enteropathy affecting the small intestine in genetically predisposed children and adults. It is precipitated by the ingestion of gluten-containing foods. It is also referred to as celiac sprue, gluten-sensitive enteropathy, or nontropical sprue. CD69 is a lymphoid activation antigen whose rapid expression makes it suitable for the detection of T-cell activation and for subset activation analyses.

Objective: Investigate the clinical value of using CD69 marker in diagnosis of coeliac disease.

Patients and method: A total of 40 patients (21 males & 19 females) with coeliac disease attending the Gastroenterology and Hepatology Teaching Hospital in the Medical City, during the period between February 2013 and June 2013.The control group consisted of 40 apparently healthy individuals who were not complaining of any gastro-intestinal problem. Venous blood samples were taken from each subject (fresh blood tested for detection of CD69 marker by flow cytometry).

Results: The age of coeliac disease patients in this study was ranged between (2-56) years with the mean age of (15.6 ± 13.1) years. Blood level of CD69 positive T-lymphocytes was significantly increased in cases group when compared with controls group.

Conclusion: Detection of CD69 marker is an accurate and rapid method for diagnosis of coeliac disease.

Key words: Coeliac disease, CD69.

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Introduction:

Coeliac disease is characterized by intolerance to gluten ingestion, which is contained in cereals such as barley, rye, hay, and malt, happening in genetically predisposed individuals and presents an inflammatory process that involves the small intestine mucosa, leading to atrophy of intestinal villi, malabsorption and a variety of clinical manifestations. Gluten proteins are relatively resistant to digestive enzymes, resulting in peptide derivatives that may lead to immunogenic response in coeliac disease patients (1). Coeliac disease affects 1% of the population (2). Clinical manifestations of coeliac disease may involve the gastrointestinal tract, as well as skin, liver, nervous system, reproductive system, bones, and endocrine system (3). Diagnosis of coeliac disease can be difficult in patients who begin treatment with a gluten-free diet (GFD) after non-standardized food intolerance tests and in the absence of adequate diagnostic work-up (4). Once this treatment is initiated, diagnosis of coeliac disease requires the reintroduction of gluten into the patient's diet for an appropriate period. Also, diagnosis of coeliac disease can be difficult when the search of serum antibodies and the duodenal biopsy results are not concordant. Genetic markers as the HLA DQ2 – DQ8 genes are of help only in excluding coeliac disease as their presence is common also in non coeliac subjects (5). Cluster of differentiation 69 (CD69) is a homodimeric leukocyte transmembrane
protein that is transiently expressed in vitro upon cell activation and that is detected in vivo on small subsets of T and B cells in peripheral lymphoid tissues from healthy subjects. In addition, CD69 is persistently expressed on leukocyte infiltrates of different chronic inflammatory diseases. After its characterization as an early activation antigen, it was widely proposed that CD69 was involved in the activation and proliferation of different leukocyte subsets. However, in vivo data have indicated that this molecule has a different role in the immune system (6). CD69 contributes to deletion of auto reactive lymphocytes by inducing apoptosis; thus, abnormal expression of this molecule could be involved in the pathogenesis of autoimmune diseases (7).

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Patients and method:

A total of 40 patients (21 males & 19 females) with coeliac disease attending the Gastroenterology and Hepatology Teaching Hospital in the Medical City, during the period between February 2013 and June 2013, and their ages ranged between (2-56) years. The diagnosis was made clinically by specialists. The control group consisted of 40 apparently healthy individuals who were not complaining of any gastro-intestinal problem. Venous blood samples were taken from each subject (fresh blood tested for detection of CD69 marker by flow

cytometry). Monoclonal antibodies detecting human antigen antibody is supplied as 100 µg purified immunoglobulin in 2.0 mL (50µg/mL) of phosphate-buffered saline (PBS).The PE conjugate is supplied as 3.0 µg in 1.0 mL (3.0 µg/mL) of PBS. The PBS contains gelatin and 0.1% sodium azide. Statistical analyses which done using SPSS (Statistical Package for Social Sciences) version 20 computer software.

Results:

The age of coeliac disease patients in this study was ranged between (2-56) years with the mean age of (15.6 ± 13.1) years. The healthy control group was matched with coeliac disease patients as their age range between (4-45) years with mean age (22.2 ± 12.8) years (Table 1).

Table1: The case-control difference in mean age.

	Study group		
	Controls	Cases(Coeliacdisease)	P*
Age (years)			0.025
Range	(4 - 45)	(2 - 56)	
Mean	22.2	15.6	
SD	12.8	13.1	
SE	2.02	2.07	
N**	40	40	

P* = (Mann-Whitney) for difference in mean
N.**= number

Blood level of CD69 positive T-lymphocytes was significantly increased in cases group that ranged between (1.8 - 25.6) with mean (10.5 ± 5.1) when compared with controls group that ranged between (1 - 9.4) with mean (3.6 ± 2.2) as shown in (table 2, Figure 1).

Table2: The case-control difference in mean CD69 marker tested.

	Study group		
	Controls	Cases (Coeliac disease)	P
CD69 positive T-lymphocytes (%)***			<0.001
Range	(1 - 9.4)	(1.8 - 25.6)	
Mean	3.6	10.5	
SD	2.2	5.1	
SE	0.34	0.81	
N	40	40	

(%)***= percentage

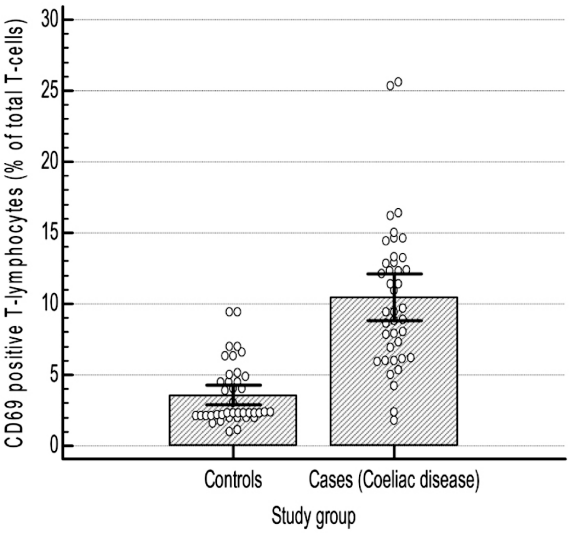

Figure 1: Dot diagram with error bars showing the case-control difference in mean (with its 95% confidence interval) CD69 positive T-lymphocytes (%).

The range and mean value of CD69 marker in blood by selected independent variables (age, gender, duration of disease, GFD, family history) among cases with coeliac disease are shown in table (3).There was a statistically significant effect of age among cases with coeliac disease (CD69 positive T-lymphocytes $p < 0.008$), while there was a statistically non significant effect of (gender, duration of disease, GFD, family history) in CD69 positive T-lymphocytes ($p < 0.64$, $p < 0.6$, $p < 0.07$, $p < 0.16$).

Table3 :The mean CD69 positive T-lymphocytes (%) by selected independent) explanatory (variables among cases with coeliac disease.

	CD69 positive T-lymphocytes (%)					
	Range	Mean	SD	SE	N	P
Age group (years)						0.008
<10	(2.4 - 13.3)	7.8	2.9	0.74	15	
10-19	(1.8 - 25.6)	11	5.7	1.47	15	
20-29	(11.4 - 25.3)	16.6	7.6	4.36	3	
30+	(6 - 16.4)	12.5	3.8	1.42	7	
Gender						0.64[NS*]
Female	(2.4 - 25.3)	10.9	5.2	1.2	19	
Male	(1.8 - 25.6)	10.1	5.1	1.11	21	
Duration of disease (years)-categories						0.6[NS]
<3 months	(1.8 - 16.2)	9.8	4.3	1.15	14	
3-12 months	(2.4 - 25.6)	10.8	5.3	1.37	15	
>1 year	(4.2 - 25.3)	10.9	6.1	1.85	11	
Gluten free diet						0.07[NS]
Negative	(1.8 - 25.3)	9.7	4.7	0.84	31	
Positive	(6 - 25.6)	13.2	5.9	1.97	9	
Family history						0.16[NS]
Negative	(2.4 - 25.6)	10.9	5.2	0.88	35	
Positive	(1.8 - 11.4)	7.5	3.7	1.65	5	

NS*= Not significant

Validity parameters of CD69 marker differentiating cases with coeliac disease from healthy controls was determined according to sensitivity, specificity, accuracy, Matthew's correlation coefficient, positive predictive value (PPV) and negative predictive value (NPV). The cut off value associated with highest (perfect) sensitivity (100%) ≥ 1.8 for CD69 positive T-lymphocytes.The cut off value associated with highest accuracy (86.3%) ≥ 5.2 for CD69 positive T-lymphocytes. This cut off value qualifies as the optimum cut off value, being able to classify a tested individual into patient infected with coeliac disease or not. Testing positive at this optimum cut-off value will establish a possible diagnosis of coeliac disease with 83.7% for CD69 positive T-lymphocytes confidence in a clinical setting where the primary diagnosis of coeliac disease has equal odds probability (50% pretest probability), and with 97.9% for CD69 positive T-lymphocytes, confidence in a clinical setting where the primary diagnosis (based on case history and examination) of coeliac disease has a high probability (90% pretest probability). The highest specificity cut-off value is (100%) ≥ 9.6 for CD69 positive T-lymphocytes. Testing positive at this cut-off value will establish diagnosis of coeliac disease.

Table 4: Validity parameters for CD69 positive T-lymphocytes when used as test to diagnose cases with coeliac disease differentiating them from healthy controls.

				Matthew's correlation coefficient	PPV at pretest probability =		NPV at pretest probability = 10%
Positive if ≥ cut-off value	Sensitivity	Specificity	Accuracy		50%	90%	
CD69 positive T-lymphocytes (%)							
1.8 the highest sensitivity	100.0	10.0	55.0	0.229	52.6	90.9	100.0
4.4	92.5	67.5	80.0	0.62	74.0	96.2	98.8
4.7	92.5	75.0	83.8	0.686	78.7	97.1	98.9
5.0	92.5	77.5	85.0	0.708	80.4	97.4	98.9
5.2 optimum cut-off	90.0	82.5	86.3	0.727	83.7	97.9	98.7
8.7	60.0	95.0	77.5	0.587	92.3	99.1	95.5
8.9	57.5	95.0	76.3	0.566	92.0	99.0	95.3
9.2	55.0	95.0	75.0	0.546	91.7	99.0	95.0
9.6 the highest specificity	50.0	100.0	75.0	0.577	100.0	100.0	94.7

Discussion:

In the current study, the demographic distribution of data showed that coeliac disease was more prevalent in younger age which is in agreement with results published by many other studies (8, 9, 10). The current study showed that the mean CD69 positive T-lymphocytes were significantly higher among coeliac disease patients in comparison with cells from controls. This finding was in agreement with many studies (11, 12, 13, 4). In current study, we found significant changes in the percentage of CD69 positive T-lymphocytes ($p<0.008$) among coeliac disease patients for only one of independent variable which is age. This is in agreement with study in USA (14) and with study in Finland (15).Our study showed that the effect of the other independent variables (gender, duration of disease, GFD, family history) on CD69 positive T-lymphocytes was not significant among coeliac disease patients. These findings were in disagreement with many studies conducted that ageing has different effects in males and females with males having a shorter life-span than females (14).

Conclusion:

Detection of CD69 marker is an accurate and rapid method for diagnosis of coeliac disease.

Author Contributions:

Karrar A. MH Al-Sakini / data collection, acquisition of data analysis, drafting of manuscript and interpretation of data.

Hayfaa S. Al-hadithi / study conception, interpretation of data and critical revision

Bassim A. Asker / interpretation of data and critical revision

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