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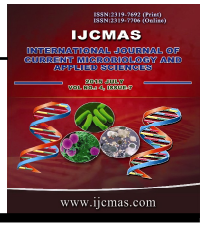

Original Research Article

Detection of Celiac Disease Auto-antibodies in children with Rotavirus infection and their values in the prediction of the disease

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Rotavirus, Celiac Disease, Anti-tTg, Anti-endomusium

A B S T R A C T

Celiac disease (CD) is an auto-immune destruction of the intestinal villi in genetically predisposed individuals provided the presence of environmental triggering factors. Rotavirus infection has implicated as one of these triggers as few studies detected the CD auto-antibodies (Anti-tTg IgA and -IgG, and Anti-Endomysium) in the sera of Rotavirus-infected patients. The objective of the present study was the detection of these auto-antibodies in the sera of children who were infected with Rotavirus, and to assess the value of these auto-antibodies in the prediction of CD in these patients. Sixty Iraqi children (less than five-year old) in whom, the Rotavirus infection has clinically expected and confirmed by Rota ELISA and RT-PCR for Rotavirus VP6 gene were involved in this study. They were serologically assayed for CD auto-antibodies using ELISA. The anti-tTg IgA was detected in 26 (43.3%) of Rotavirus-infected children using RT-PCR, with poor agreement in the prediction of CD (Kappa statistic=0.00). Slight agreements have demonstrated for Anti-tTg IgG (K=0.04) and for Anti-endomysium (k=0.14) as they given positive results in 18 (30%) and in 17 (28.3%) of the same subgroup of patients, respectively. In patients with positive Rota ELISA, the Anti-tTg IgA was the only test to have a slight agreement (K=0.05) in the prediction of CD as it appeared positive in 28 (46.6%) of them. In addition, the duration of Rotavirus illness was at a significant association with the development of detectable levels of the three CD auto-antibodies. In conclusion, these auto-antibodies have no statistical significance value in the prediction of CD in these children. Moreover, for the first time, the duration of Rotavirus illness was found to have a significant impact in the emergence of these auto-antibodies.

Introduction

Celiac Disease (CD) is a chronic auto-immune enteropathy triggered by exposure to Gluten in wheat, barley, and rye in genetically predisposed individuals (Gianfrani *et al*., 2005; Ludvigsson *et al.*, 2013).

With a remarkable differences regarding its' prevalence and geographical distribution, there is an estimation of 1% CD affected persons among general population worldwide, however, an increased prevalence overtime has constantly being

reported (Green and Cellier, 2007; Myleus *et al.*,2009; Mustalahti *at al.,* 2010). The obvious-relevant genetic predisposing factor is thought to be the occurrence of certain Human Leukocyte Antigen, namely; HLA-DQ2 (DQA1**05* and DQB1**02* alleles) and, for less extent, HLA-DQ8 (DQA1**0301* and DQB1**0302* alleles) (Koning *et al.,* 2005). However, not all individuals carry these alleles may develop Celiac disease even on Gluten-containing dietary, so, additional environmental triggers or risk factors have been sought out.

Few studies with different and controversial conclusions were made over these two factors, i.e., genetic and environmental (Mahon *et al.*, 1991; Ivarsson *et al*., 2002; Sandberg-Bennich *et* al., 2002; Forsberg *et* al., 2004). Among other environmental factors, the gastrointestinal Tract (GIT) infections are involved, and the Rotavirus infection has investigated sparsely as thought to play a role in the etiopathogenesis of CD (Dube *et al.,* 2005; Stene *et al*., 2006; Rostami-Nejad *et al.*,2010; Dolcino *et al.*,2013). One possible linkage between GIT infections and development of CD is that, during infection, an increased permeability of the intestinal mucosa may permit the absorption of intact Gliadin molecules and, as consequence, the initiation of auto-immunity in predisposed individuals (Dube *et al.,* 2005). Another perspective is the molecular mimicry between antigens of infectious agent and self-antigens those targets of auto-antibodies in this disease (Dolcino *et al.*,2013). Auto-antibodies against the two main self-antigens in celiac disease course; the tissue TransGlutaminase (tTG) and Endomysium (anti-tTG and anti-endomysium), are often thought to be mediate the intestinal damage. These auto-antibodies are produced before (silent CD) or during the clinical manifestation of CD and their detection in screening trials could aid in the early diagnosis of this disease in relative individuals (Jabri and Sollid, 2009). From the few previous studies on Rotavirus infections and the development of Celiac disease in genetically predisposed persons, an association between these two conditions has pointed out (Stene *et al*., 2006 ; Troncone and Auricchio, 2007; Rostami-Nejad *et al.*,2010; Dolcino *et al.*,2013). But, whether Rotavirus infection, its' duration and its' serological and genetic-based diagnosis may predict or not; the development of CD, was the objective of this study.

Patients and methods

Patients

Three hundred and forty-five children aged less than five-year old were investigated for antibiotic-nonreponding diarrheal cases, in whom, rotaviral infection was clinically suspected by specialist pediatricians in the teaching central children hospital/Baghdad/ Iraq during Jun. 2013-Aug. 2014. Age, gender, and duration of the illnesses were registered in a pre-designed questionnaire.

Stool exam; ELISA, and RT-PCR

A general stool exam was done to exclude other infective agents. All patients children were subjected to stool RT-PCR for VP6 Rota (Bioneer, south Korea) and for stool ELISA for ROTA antigen, viral RNA extraction and its' reversible amplification were carried-out according to manufacturer instructions.

Celiac disease auto-antibodies

Based on the positive results for one of/ or both Rotavirus infection tests above, sixty patients were further assayed for serum Anti-tTg and anti-enomysial auto-antibodies

(AESKULISA/Germany) using ELISA. The latter two tests are routinely used for the detection and diagnosis of CD in clinically suspected individuals, the positive/negative results of these two tests were calculated as depicted in the instructions of the manufacturer.

Statistical analysis

The associations and concordances of various results obtained were analyzed using KAPPA statistics to assess their degree of agreement in predicting the development of celiac disease in children with Rotavirus infection (Viera and Garrett, 2005).

Results and Discussion

The general results

The sixty children patients included in this study were at a mean age of 3.10±1.18 years (range=1-5 years). Duration of illness varied from 1 up to 8 days with a mean of 3.43±1.77 days. The Rotavirus ELISA was positive in 70% of patients, and the virus has detected in the stool of 66.6% of them by RT-PCR.

Celiac disease serology

The Anti-tTG IgA was positive in 65% of the patients while Anti-tTG IgG was positive in 43.33% of them. The positive rate of Anti-Endomysium auto-antibodies was 36.67% as shown in table 1.

Concordance and association of different tests

Tables 2 and 3 show the concordance and association of different tests' results in our patients, there were high frequencies of double positives for two tests in the same patient, for example, 43.3% (not presented in table 1) of patients were positive for Anti-tTG of IgA class as well as for PCR ROTA VP6 with poor agreement to predict celiac disease using this test in this group of patients. Another high proportion of 46.6% (not presented in table 2) of patients were positive for Anti-tTG of IgA class as well as for Rotavirus ELISA, with slight agreement in the same issue.

Slight agreements have also observed between positive PCR for Rotavirus and each of Anti-tTG of IgG class and Anti-Endomysium auto-antibodies. However, there was no significant association between any test for Rotavirus and tests of CD.

The impact of Rotavirus illness duration

Surprisingly, table 4 shows that the duration of Rotavirus illness has a significant impact on the positive/negative rate of each of serological tests used for prediction of celiac disease. In other words, as longer as the duration of Rotavirus illness is, as the positive rate of each of CD serological tests increased.

The aim of the present study was to evaluate whether the coincidence of Anti-tTG and Anti-endomysium auto-antibodies in children with Rotavirus infection have an agreement in the prediction of Celiac disease in these patients or not. Celiac disease, as an auto-immune disease is triggered by environmental factors in genetically predisposed individuals (Sollid, 2002). Among other environmental factors, GIT infections are thought to play a role in the etiopathogenesis of CD with lacking of obvious, direct linkage as many other factors may interfere like intestinal microbiota, dietary habits, infections, hypersensitivity and the genetic factors as principle (Rubio-Pzo *et al.*, 2012).

Table.1 The general results of the PCR and ELISA tests applied in clinically suspected of having Rotaviral infection patients

	Positive		Negative	
Test	**No.**	**%**	**No.**	**%**
Anti-tTG IgA	39	65.00	21	35.00
Anti-tTG IgG	26	43.33	34	56.67
Anti-Endomysium	22	36.67	38	63.33
Rotavirus ELISA	42	70.00	18	30.00
PCR of Rotavirus VP6	40	66.67	20	33.33

Table.2 Concordance and agreement degree between Rotavirus infections detected by RT-PCR and celiac disease serological markers

Test for Celiac disease		**PCR of Rotavirus VP6**			**P-value**	**p_o**	**p_e**	**K**	Interpretation
		-ve	**+ve**	Total					
Anti-tTG IgA	**-ve**	**7**	14	21	1.000	0.55	0.55	0.00	Poor agreement
	+ve	13	**26**	39					
Anti-tTG IgG	**-ve**	**12**	22	34	0.713	0.50	0.48	0.04	Slight agreement
	+ve	8	**18**	26					
Anti-Endomysium	**-ve**	**15**	23	38	0.185	0.53	0.46	0.14	Slight agreement
	+ve	5	**17**	22					
	Total	20	40	60					

p_o=Observed agreement, **p_e**=expected agreement, **K**=Kappa statistic

Table.3 Concordance and agreement degree between Rotavirus infections detected by ELISA and celiac disease serological markers

Test for Celiac disease		**Rotavirus ELISA**			**P-value**	**po**	**pe**	**K**	Interpretation
		-ve	**+ve**	**Total**					
Anti-tTG IgA	**-ve**	**7**	14	21	0.679	0.58	0.56	0.05	Slight agreement
	+ve	11	**28**	39					
Anti-tTG IgG	**-ve**	**9**	25	34	0.495	0.43	0.47	-0.08	Less than chance agreement
	+ve	9	**17**	26					
Anti-Endomysium	**-ve**	**11**	27	38	0.815	0.43	0.45	-0.02	Less than chance agreement
	+ve	7	**15**	22					
	Total	20	40	60					

p_o=Observed agreement, **p_e**=expected agreement, **K**=Kappa statistic

Table.4 Association between duration of Rota virus infection diagnosed by PCR and/or ELISA and celiac disease serology

Test	Mean duration/days in Rota positive cases	Mean duration/days in Rota negative cases	P-value
Anti-tTG IgA	3.90±1.85	2.57±1.25	0.005
Anti-tTG IgG	4.04±2.07	2.97±1.36	0.019
Anti-Endomysium	2.76±1.28	4.59±1.92	<0.001

In one study, having three or more infection, regardless of the type of infection during the first six months of life was associated with a significantly increased risk for later Celiac Disease (Myleus *et al.,* 2012). In this scenes, the Rotavirus infections have draw a special attention by few authors. The presence of CD auto-antibodies in the sera of patients with Rotavirus infections have been reported in children (Stene *et al*., 2006; Myleus *et al.,* 2012; Dolcino *et al.*,2013) as well as in adults (Rostami-Nejad *et al.*,2010). The first indication of increased risk for CD as function of high frequency of Rotavirus infections has been prospectively reported in genetically predisposed children, i.e. children who carry either HLA haplotype (DR3-DQ2) or (DR4-DQ8) (Stene *et al*., 2006).

At the same time, Zanoni *et al* (2006) have revealed as subset of anti-tTG IgA antibodies recognized the Rotaviral protein VP-7, suggesting a mechanism of molecular mimicry that may trigger the CD etiopathogenesis. In the present work, despite the high frequencies of anti-tTG IgA and IgG as well as anti-endomysium auto-antibodies in children with Rotavirus infection, these tests have failed to get a strong agreement to predict CD in these patients, as the Kappa statistic revealed. In one study in Iran, it was found that in adult patients with active Rotavirus infection, there was no significant difference between the number of individual who had had positive anti-tTG and those with negative for the test (Rostami-Nejad *et al.*,2010).

A recent possible explanation for such controversy is that recent data have demonstrated the presence of anti-Rotavirus VP7 antibodies in 81% of children patients with type 1 Diabetes Mellitus (T1DM) and with CD in the same time, moreover, a fraction (27%) of T1DM patients without CD were at detectable anti-Rotavirus VP7, too (Dolcino *et al.*,2013). These complicated and confusing findings confirm the overlapping features of auto-immune diseases; meanwhile, they may support the non-significant concordance of findings in our study.

Finally and exclusively, we may conclude that, in addition to the frequencies of Rotavirus infections, the duration of the infection have a significant association with emergence of CD auto-antibodies, whether it is because of the long-time shedding of the self-antigen mimicked viral antigens, it is a matter of further studies.

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