Rotavirus and coeliac autoimmunity among adults with non-specific gastrointestinal symptoms

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ABSTRACT

الأهداف: تحديد كيفية تأثير مرض حساسية القمح أو الداء البطني (CD) على الدم وكذلك ماهية فيروس الروتا (RV) باستخدام تقنية التفاعل المبلمر وذلك في البالغين الذين يعانون من مشاكل غير محددة في المعدة والأمعاء.

الطريقة: اختارت الدراسة عشوائياً حوالي 5176 فرداً يعيشون في طهران، إيران، وذلك خلال الفترة من سبتمبر 2006م إلى سبتمبر 2007م. وبالاستفتاء تم التعرف على 670 مصاباً بأعراض في المعدة والأمعاء ومن ثم طُلب منهم المواصلة في الدراسة وذلك بأخذ عينات من البراز والدم. لقد تم اختبار عينات البراز من أجل البحث عن فيروس الروتا بواسطة عملية التضخيم الجيني للجين (VP6) وكذلك الكشف عن الفطريات بواسطة الفحص المجهري وطرق تركيز الفورمالين–ايثر. ويشمل اختبار مرض حساسية القمح الكشف عن الأجسام المضادة (tTG) و (IgA). تم إجراء هذه الدراسة في مركز أبحاث الكبد والمعدة والأمعاء، مستشفى طاليغاني، طهران، إيران.

النتائج: لقد كان جين (VP6) ظاهراً في 150 شخصاً (22.3%) أما الأجسام المضادة (IgA) و(tTG) فكانت موجودة في 22 شخصاً (95% CI 2.3-5.1)، وكانت الأجسام المضادة (IgG) و(tTG) موجودة في 3 أشخاص يعانون من نقص الجسم المضاد (IgA). لقد كان جين (VP6) ظاهراً في 8 مرضى (32%) من أصل 25 مصاباً بمرض حساسية القمح في حين كان ظاهراً في 142 شخصاً (22%) من أصل 645 شخصاً غير مصاب بهذا المرض، وهذا الاختلاف لم يكن كبيراً وذلك اعتماداً على الأرقام الإحصائية (p=0.2).

خاتمة: تُظهر هذه الدراسة بأن التهاب فيروس الروتا يعد شائعاً بين الإيرانيين الذين يعانون من مشاكل غير محددة في المعدة والأمعاء، غير أنها –وبالمقارنة مع الدراسة التي أُجريت على الأطفال– لاتُظهر فرقاً كبيراً بين المصابين الذين يحملون الجسم المضاد (tTG) وهؤلاء الذين لا يحملون هذا الجسم المضاد وذلك اعتمادا على الأرقام الإحصائية.

Objectives: To determine celiac disease (CD) serology and rotavirus (RV) by polymerase reaction (PCR) in adults with non-specific gastrointestinal complaints.

Methods: The study comprised 5176 randomly selected individuals living in Tehran, Iran between September 2006 and September 2007. Six hundred and seventy individuals with GI symptoms were identified with a questionnaire and invited for a further study including stool sampling and blood tests. Stool samples were examined for detection of RV by amplification of specific gene (VP6) and by light microscopy and formalin-ether concentration methods for parasite detection. The subjects also tested for CD including anti-transglutaminase (tTG) antibodies and total immunoglobulin A (IgA). The study was carried out in the Research Center of Gastroentrology and Liver Disease, Taleghani Hospital, Tehran, Iran.

Results: The VP6 gene was detected in 150 (22.3%) individuals. Anti-tissue transglutaminase (tTG-IgA) was positive in 22 individuals (95% CI 2.3-5.1) and IgG-tTG antibody in 3 individuals who were IgA deficient. Amplification of VP6 gene was positive in 8/25 (32%) with positive CD serology and in 142/645 (22%) with negative CD serology. This difference was not statistically significant (p=0.2).

Conclusion: This study shows that RV infection is common among Iranian patients with non-specific gastrointestinal symptoms. However, in contrast to studies in children, this study shows that the prevalence of active RV infection was not statistically significantly different between individuals who were tTG antibody positive and those who were tTG antibody negative.

Saudi Med J 2010; Vol. 31 (8): 891-894

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Received 27th February 2010. Accepted 21st June 2010.

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Celiac disease (CD) is an autoimmune disorder characterized by gluten sensitivity in genetically susceptible individuals.[1] The classic manifestations of weight loss and diarrhea relate to small bowel atrophy, but CD patients may also suffer from extra-intestinal manifestations such as neurological, reproductive, and endocrinological disorders.[2] An increased intestinal permeability permits the absorption of intact gliadin molecules, which may initiate the immune process leading to CD.[3] Intestinal infection and inflammation can also increase intestinal permeability. Few studies have investigated the role of specific infectious agents in the development of CD. The prevalence of rotavirus (RV) infection has been reported to range from 3.3-63.6% in different parts of the world.[4-6] Rotavirus infections is one of the most common causes of acute gastroenteritis worldwide.[7] A prospective study by Stene et al,[8] suggests that RV infection may predispose to CD in genetically susceptible host by either molecular mimicry or by repeated infections in childhood triggering an autoimmune response. The objective of this study was to assess the prevalence of RV and CD serology among Iranian adults with non-specific gastrointestinal (GI) symptoms.

Methods. This cross-sectional study was performed in Tehran, Iran. It was designed to investigate the association between RV and CD. Five thousand and one hundred seventy-six adults were identified randomly from the population of Tehran, Iran between September 2006 and September 2007. Randomization was achieved on the basis of the list of postal codes.[9-12] Random samples of postal codes and their related address were drawn from the databank registry of the Tehran central post office, approximately 1000 households selected and all members (5176 persons) surveyed in each corresponding address. Interviewers asked subjects questions with yes or no answers regarding 8 GI symptoms including abdominal pain, constipation, diarrhea, bloating, dyspepsia, nausea and vomiting, weight loss and heartburn. Subjects who reported at least one of these 8 GI symptoms were selected for further participation in this study. Altogether 670 (mean age 40, range 14-83 years) with GI symptoms comprised the actual study group; 427 (63.7%) were women (mean age 42 years). Those with similar symptoms, but an established diagnosis, such as inflammatory bowel disease, pancreatitis or underlying malignancy were excluded from the study. Blood and stool samples were collected from each suitable subject: the samples were transferred to the National Research Department of Food Borne Diseases (NRDFD) located in the Research Center of Gastroenterology and Liver Diseases, Shaheed Beheshti University, MC, Tehran, Iran. The sera were assayed within 24 hours after collection or stored at -80°C until analysis. Determinations of IgA anti-tTG antibody were carried out using a commercially available kit (Aeskulisa tTG, Wendelsheim, Germany) and an enzyme-linked immunosorbent assay (ELISA) method and total serum IgA values were evaluated by an immunoturbidometric assay (Pars Azmoon, Tehran, Iran). Immunoglobulin A class human anti-tissue transglutaminase (tTG) antibody and total serum IgA values for CD were measured as described previously.[13] Stool sample were kept in closed containers, refrigerated at 4˚C, rapidly sent to NRDFD and stored at -20˚C until processed.

The study was carried out and approved by the Institutional Ethics Committees of Research Center for Gastroenterology and Liver Disease, Shaheed Beheshti University, M.C., Tehran, Iran and all participants signed a written informed consent.

Viral ribonucleic acid (RNA)-extraction. For stool samples preparation, the cured fecal samples (0.5 ml) were diluted with 150 µl of phosphate-buffered saline, vortex for 10 sec and incubated at room temperature with gentle mixing for 15 minutes. For RNA extraction QIAamp viral RNA mini kit (Qiagen, Westburg, Leusden, The Netherlands) was used according to the manufacturer's instruction.

Rotavirus VP6-specific polymerase chain reaction (PCR). A reverse transcriptase-PCR (RT-PCR) was performed to amplify a 382 bp of segment 6 RNA. This region was chosen to determine the group of a specific VP6 protein of RV as previously described by Gomara et al.[15]

Parasites detection. Specimens preserved in sodium acetate, glacial acetic acid and formalin (SAF) were sent to NRDFD where the stools were examined using light microscopy and the formalin–ether concentration method for detection of protozoa and of geohelminth eggs.[16] In addition, a modified acid-fast staining was used to identify *Cryptosporidium parvum*.

Descriptive statistics was performed to analyze the results. Fisher exact test was carried out using SAS software. *P* value of 0.05 was considered statistically significant.

Results. Of the 670 symptomatic GI patients who participated in this screening, we found an organic etiology in 290 cases; 380 reported self-limiting symptoms of short duration. Immunoglobulin A tTG

Disclosure. This study was financially supported by the Research Center of Gastroenterology and Liver disease (RCGLD), Shaheed Beheshti University, MC, Tehran, Iran.

antibody serology was positive in 22 subjects (3.3% of samples, 17 females). Three patients with IgA deficiency had positive for IgG tTG serology. The prevalence of tTG antibody in the screened samples was 3.7% when including IgA-deficients with positive tTGG. One hundred and twenty-six stool samples (19%) were positive for pathogens, 73 were positive for non-pathogenic parasites and 544 were negative. Among the pathogen parasites identified, *Giardia labmlia* was the most common intestinal pathogen (41/670) followed by *Blastocystis hominis* (30/670), *Iodomoeba butchelii* (13/670), *Entamoeba histolytica/Entamoeba dispar complex* (11/670), and *Cryptosporidium parvoum* (3/670). Among the non-pathogen parasites *Entamoeba coli* was the most prevalent species (39/670) followed by *Endolimax nana* (34/670). Altogether 150 (22.3%) out of 670 subjects had positive RT-PCR testing for the RV VP6 gene in their stool samples (76 female and 74 male). Eight (32%) of 25 positive CD serology cases were also infected by RV; the proportion of patients with positive RV infection was not significantly different when patients with positive CD serology were compared with patients with negative CD serology. The most frequent GI symptom in 150 cases positive for RV was heartburn (47/150, 31%). For patients with positive CD serology, the most common symptom was abdominal pain (n=10, 40%). **Table 1** shows the clinical signs and symptoms, and associated conditions in RV-infected patients and positive CD serology patients. The proportion of patients with positive CD serology with symptoms of weight loss (4/17 versus 44/670, p=0.007) and abdominal pain (10/17 versus 185/670, p=0.005) was increased compared to CD serology negative patients. The proportion of RV positive patients with bloating (32/150) was increased compared to RV negative patients (200/670) (p<0.05). There was no other significant difference in the symptoms when groups were compared according to CD serology and RV infection results.

Discussion. Rotavirus is an important cause of diarrhea in children. An asymptomatic carriage state has also been described. This is the first report of RV infection in Iranian adults and our results are similar to those obtained in other comparable studies. Unlike previous studies that reported the prevalence of RV infection in subjects with diarrhea, this study reports the prevalence of RV infection among adults with other GI symptoms, such as heartburn. Approximately 22.3% of our subjects were positive to RV, compared to the prevalence of 20-40% of RV reported elsewhere such as Brazil, Tunisian and India.[16-18] Repeated infections are also common, detectable by an increase in the level of RV specific antibodies and RT-PCR.[20,21] From birth, individuals can be infected by RV and upon reaching the age of 3 years, the most individuals in a community have been in contact with the virus and have developed antibodies.[6] In one study from Tehran, from total of 1250 stool samples that were collected from children under 5 years old, RV was detected in 32.3% of subjects.[19] Comparing the results of our study with the above study in children in Tehran, RV seems to be more prevalent in children. This may partly explain why RV found to be associated with CD in children. Spread by fecal-oral transmission, RV infection in adults typically manifests with nausea, malaise, headache, abdominal pain, diarrhea, and fever. Infection can also be symptomless.[22] The most common symptoms for patients with evidence of RV in the current survey were heartburn and abdominal pain, suggesting atypical and possibly frequent asymptomatic carriage. There was, however, a significant correlation between RV and bloating. Bloating is a non-specific symptom, but it is possible RV infection may be the causative factor for some of these individuals. Symptoms of weight loss

Table 1- Clinical signs and symptoms, and associated conditions in RV-infected patients and positive CD serology patients (8 patients whom diagnosed with both CD and RV excluded from the analysis).

Symptom, sign, associated condition or, test	Rotavirus- positive (N=142)	Positive celiac disease serology (N=17)	*P* value
Diarrhea	4 (2.8)	1 (5.9)	0.43
Abdominal pain	36 (25.3)	10 (58.8)	<0.001
Nausea & vomiting	3 (2.1)	8 (47.0)	0.07
Constipation	27 (19.0)	6 (35.2)	0.12
Weigh loss	7 (4.9)	4 (23.5)	0.02
Heart burn	46 (39.4)	9 (52.9)	0.10
Bloating	32 (22.5)	8 (47.0)	0.01
Dyspepsia	3 (2.1)	0	

and abdominal pain were more prevalent proportion of patients with positive CD serology and evidence of RV infection than in patients with negative CD serology and evidence of RV infection when compared. In this study 3.7% of our subjects were positive CD serology. We have no histological confirmation for CD in these subjects, but tTG antibodies are highly specific for the condition.[22] Within the limitations of the present study (lack of performing small bowel biopsy), one can infer that the prevalence of undiagnosed CD autoimmunity among adults with non-specific GI symptoms is 2-3 times higher than the general population in Iran.[23] In contrast to the study in children this study did not find a significant correlation between CD and RV infection. Nevertheless, the infected cases with RV scored higher on what in patients with positive tTG with 32% versus 22% in those with negative CD serology (p=0.2). One of the reason for the lack of this association might be the lower prevalence of RV infection in adult with non-specific symptoms compared to children with specific symptoms such as diarrhea.[8] We acknowledge the limitation of our cross sectional study in assessing the association between CD and RV. Further longitudinal studies in a larger population in adults with specific symptoms would be required to identify the possible triggering role of RV in CD.

Acknowledgment. *The authors would like to thank Dr. MA Pourhoseingholi for his kind cooperation in statistical analysis and Dr. H. Dabiri for his sincere cooperation in the laboratory.*

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