original article

THE ANTI-TRANSGLUTAMINASE AUTO-ANTIBODIES IN CHILDREN'S SALIVA WITH A SUSPECT COELIAC DISEASE: CLINICAL STUDY

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SUMMARY

The coeliac disease is an immune-mediated enteropathy triggered by an ingestion of gluten in genetically susceptible individuals. Like some other systemic diseases (Crohn's disease, Sjögren's syndrome) the celiac disease is able to alter the oral ecosystem and the composition of the saliva.

Aim. The aim of this retrospective study has been to examine the incidence of coeliac disease (CD) in paediatric population and to search the presence of anti-transglutaminase auto-antibodies (anti-tTG) in saliva, comparing and quantifying the concentration regard to the serum values of the anti-tTG auto-antibodies, before and after six months from the beginning of the free gluten diet.

Materials and Methods. 105 children (G_0), aged between 5 and 13 years, belonging to the Paediatric Gastroenterology-Endoscopy Unit of PTV Hospital, University of Rome "Tor Vergata", have been examined for a diagnosis of suspected CD.

Results. Of a total of 105 pediatric patients (G_0), only the 16.2% (G_1) has showed to be positive. About the evaluation of the anti-tTG auto-antibodies in the serum, obtained from the second blood sample (T_1), we can observe that 10 (G_2) out of 17 children (G_1) show positivity and for this reason they have been subjected to a sampling of intestinal villi to confirm the diagnosis of CD; in addition the 6.7% has been resulted positive at the first sampling of serum (T_0), but negative to the second one (T_1). The incidence of the CD has been resulted to be equal to 9.5%. About the evaluation of anti-tTG in the G1, we can observe that 58.8% of children are "definitely positive" to the salivary anti-tTG, while 11.8% appear to be weakly positive. About the correspondence of serum and salivary anti-tTG in Group G1, we can observe, that children positive to the anti-tTG in the serum have also the anti-tTG in the salivary fluid (sensibility 100%, specificity 71.4%). The results show that the anti-tTG salivary are present in children with CD, even though they have continued to follow the gluten free diet for 6 months.

Conclusions. The presence of anti-tTG in the saliva may be considered, an additional and useful diagnostic dental marker for an initial, reproducible, non invasive, inexpensive and highly sensitive screening of CD having a predictive and precocious value compared to anti-tTG contained in the serum, as it has been already demonstrated.

Key words: coeliac disease, paediatric patient, saliva.

Introduction

The coeliac disease (CD) is an immune-mediated enteropathy triggered by an ingestion of gluten in genetically susceptible individuals (1, 2). It is one of the most commonly chronic diseases found in Caucasians, with a frequency in the general population between 1:85 and 1:300, both in Europe and in United States (3, 4). Over the last thirty years, the incidence of CD has significantly increased, as a probable consequence of the improvement in the diagnosis of atypical forms, confirming today the prevalence between 0.7% and 2% (5).

Like some other systemic diseases (Crohn's disease, Sjögren's syndrome) the CD is able to alter the oral ecosystem and the composition of the saliva (6, 7). In fact it has been observed that in subjects suffering from CD it's produced a lower amounts of amylase, secretory IgA and IgM compared to those found in the saliva of healthy sub-

jects (8, 9). In addition, some significant differences have been detected between the chemical composition of the saliva in the coeliac subjects and that of healthy subjects, as regards: the buffering capacity, the speed of salivary flow, the concentration of calcium, the Ca/P ratio (6, 10).
Recently it has been observed that in the saliva of coeliac subjects there are anti-transglutaminase (anti-tTG) auto-antibodies, whose blood research, until now, it has been considered the more reliable, simple and less invasive method of the screening protocol of CD (11).
Therefore, the aim of this retrospective study has been to examine the incidence of CD in paediatric population and to research in the group of children positive resulted to the first blood sample, the presence of anti-tTG auto-antibodies even in saliva, comparing and quantifying the concentration compared to the values got by a second blood sample.
In addition, we have evaluated the significant differences in the concentrations of anti-tTG auto-antibodies in the saliva, before and after the beginning of the free gluten diet, both in subjects positive resulted at the second blood sample and to a sampling of intestinal villi, that in children negative resulted at the second blood sample but positive to the first one, and therefore considered potentially coeliac.

Materials and methods

Between March and October 2011, it has been examined a group of 105 children (11 males and 94 females) (G_0), aged between 5 and 13 years, for diagnosis of suspected CD, belonging to the Paediatric Gastroenterology-Endoscopy Unit of PTV Hospital, University of Rome "Tor Vergata".
Subjects G_0 was carried out blood sampling (T_0) to verify the presence of the anti-tTG auto-antibodies, anti-gliadin deaminated antibodies (AGA) and anti-endomysium antibodies (EMA). Those positive resulted (no.17, including 2 males and 15 females, aged between 5.4 years and 12.10 years, mean age of 9.36 years +/-2 months S.D.) have been included into the G_1.
Subjects of G_1 have been subjected to a second blood sample (T_1) and, in the same session, to a collection of saliva through the procedure called "passive drooling" for evaluating the anti-tTG auto-antibodies.
The *blood samples* have been collected in BD Vacutainer sterile tubes test. Later, at the Department of Molecular Biology of PTV Hospital, University of Rome "Tor Vergata", the tubes test have been centrifuged at 3500 rpm for 5 minutes at ambient temperature; the serum, obtained by centrifugation, has been suspended again 2-3 times to be completely uniformed and it has been divided into two equal parts in Eppendorf tubes test, both numbered. The two test have been sealed and frozen at a temperature of -80°C until assay.
The *fluid saliva samples* have been collected in 50 ml Falcon tubes test, hermetically closed. Subsequently, the samples have been centrifuged at 1000 rpm for 5 minutes at ambient temperature and frozen at a temperature of -80°C.
For each sample of serum and saliva we have examined the anti-tTG auto-antibodies.
The anti-tTG auto-antibodies in the serum have been analyzed by enzyme immunoassay method (ELISA). The values of anti-tTG serum are considered negative between 0 and 5 µg/mL, weak between 5 and 7 µg/mL and positive >7 µg/mL.
The anti-tTG auto-antibodies in the saliva have been analyzed by a Enzyme Linked Immuno Magnetic Electrochemical method (ELIME); they have been examined with the Autolab potentiostat GPSTAT-12 equipped with software GPES (Ecochemie, Ultrecht, The Netherlands).
The test has been validated using an analysis of 15 repetitions of the same negative sample and calculation of:

- Limit Of Detection (LOD) = medium + 3 Standard Deviation;
- Limit Of Quantification (LOQ) = medium + 10 Standard Deviation.

The measured values are 1.5 UA/mL for the LOD and of 3.7 UA/mL for the LOQ.
The values below the LOD are considered as "definitely negative", while the values above the LOQ as "definitely positive"; the intermediate values are considered as "doubtful".

It has been chosen a value so high for the LOQ because there have been problems in the discrimination of values with a low title antibody and therefore we have preferred to classify as positive only those clear samples.
Subsequently, the subjects of G_1, positive resulted for the presence of anti-tTG antibodies in the serum, have been subjected to a gastroscopy with a sampling of intestinal villi.
The patients positive resulted to a gastrointestinal biopsy have been introduced into the system of a gluten-free diet (G_2) (10 children, female, aged between 5.4 and 12.4 years, mean age 9.1 years +/- 2.32 months S.D.). After six months from the beginning of the gluten free diet, the children belonging to G_2 have been subjected to an additional amount of saliva (T_2), to verify the utility of the anti-tTG salivary as a diagnostic marker of CD and index for the follow-up of gluten free therapy.
Moreover, it has been done a second sample of salivary fluid even to children negative resulted to the second blood sample (G_3), but positive to the first one (T_0) and therefore considered potentially coeliac (7 children, including 2 males and 5 females, aged between 6.5 and 12.10 years, mean age 9.8 years +/- 2.25 months S.D).

Results

Out of a total 105 paediatric patients (11 males and 94 females, aged between 5 and 13 years) (G_0) subjected to a first blood sample (T_0) for a suspected diagnosis of CD in order to verify the presence of anti-tTG antibodies, anti-gliadin deaminated antibodies (AGA) and anti-endomysium antibodies (EMA), only 17 have been resulted positive (16.2%) (G_1).
At the time of clinical observation, the G_1 (2 males and 15 females) had an age between 5.4 and 12.10 years, with a mean age of 9.36 years +/- 2 months S.D.
Regarding the evaluation obtained in the second blood sample (T_1) of the anti-tTG auto-antibodies in the serum of the patients belonging to G_1, the results have showed that 10 children (G_2) on 17 have demonstrated positivity to the anti-tTG in the serum (Tab. 1) and therefore they have been subjected to a sample of intestinal villi to confirm the diagnosis of CD.
The incidence of the CD has been proved to be equal to 9.5% (Fig. 1).
The results obtained demonstrate that 7 children out of 17 are positive to the first sample of serum

Table 1 - Values of anti-tTG in the serum and in the saliva in G_1.

Patients	Anti-tTG in the serum (μg/mL)	Coeliac Disease	Anti-tTG in the saliva (UA/mL) I sample	Anti-tTG presence in the saliva
1	42,16	+	8,9	+
2	0,53	-	0,9	-
3	>100	+	8,9	+
4	2,5	-	0,6	-
5	>100	+	17,0	+
6	0,22	-	3,9	+
7	0,2	-	0,7	-
8	67,1	+	3,8	+
9	0,25	-	0,9	-
10	12,4	+	2,8	weakly positive
11	53,3	+	4,6	+
12	37,1	+	7,8	+
13	0,35	-	0,6	-
14	>100	+	2,0	weakly positive
15	0,27	-	4,0	+
16	86,4	+	11,7	+
17	22,9	+	6,9	+

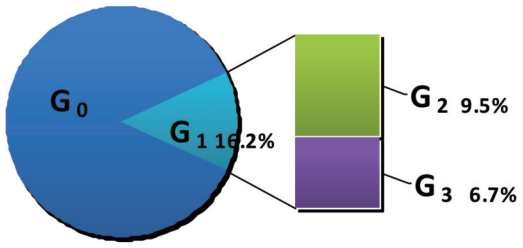

Figure 1
Incidence of coeliac disease.

(T_0) but negative to the second one (T_1) (6.7%). For this reason these subjects haven't been subjected to the sample of intestinal villi and they haven't been even classified as coeliac.

Since they have showed positivity to the anti-tTG, even just once, we can consider them at the beginning as potential coeliac, then as patients in whom the disease could occur in a second time of their lives.

Regarding the evaluation of the anti-tTG in the saliva (T_1) of patients belonging to G_1, the obtained results show that 10 children out of 17 are "definitely positive" to the salivary anti-tTG (58.8%), 2 children are weakly positive (11.8%) and the reaming 5 "definitely negative" (29.4%) (Tab. 1).

As regards the correspondence of the serum and salivary anti-tTG in the G_1, the results obtained have shown that children positive to the anti-tTG in the serum have anti-tTG even in the salivary fluid (Tab. 2).

The sensitivity of the study is guaranteed 100% and when we talk about "sensitivity" we mean the measure of the frequency of a positive test in the presence of the disease in question and it is expressed as the percentage ratio between true positives and total (true positives + false negatives).

The specificity is equal to 71.4%; even here when we talk about "specificity" we mean the measure of the frequency of the negative of a test in the absence of the disease studied and it is expressed as the percentage ratio between total and true negatives (true negatives + false positives).

We're talking about the 71.4% since there are 2 false positive; in fact 2 girls, belonging to the Group G_3, are negative to the anti-tTG of the serum, but positive to the anti-tTG salivary. In addition, the patients are characterized by the positive genetic polymorphism, by a family history of CD and by symptoms referable to the disease; the 2 girls are healthy carriers of CD, since they have the disease genes in the DNA and the anti-tTG in the saliva.

The monitoring of the follow-up through the salivary anti-tTG has predicted that the children, belonging to the G_1, have been subjected to a further salivary fluid collection (T_2), after 6 months from the first sample of salivary fluid (Tab. 3).

The results obtained show the correspondence between the first and the second sampling of saliva in the same children (Tab. 4).

Looking at the results we can notice that in children with CD the anti-tTG salivary are always present, despite of the inclusion in the program of the gluten-free diet.

The check of the anti-tTG in the serum is expected after 6 months from the start of the diet, when it will be occurred the remission or not of the disease and the presence of the markers in the blood/saliva.

Table 2 - Correspondence of the serum and salivary anti-tTG in G_1.

Patients	Anti-tTG in the serum	Anti-tTG in the saliva
1	+	+
2	-	-
3	+	+
4	-	-
5	+	+
6	-	+
7	-	-
8	+	+
9	-	-
10	+	weakly positive
11	+	+
12	+	+
13	-	-
14	+	weakly positive
15	-	+
16	+	+
17	+	+

Table 3 - Values of anti-tTG in the saliva in G_1 after 6 months.

Patients	Anti-tTG in the saliva (UA/ml) II sample	Anti-tTG presence in the saliva
1	2,3	weakly positive
2	1,8	-
3	5,6	+
4	2,4	-
5	33,6	+
6	5,4	+
7	1,4	-
8	4,9	+
9	1,3	-
10	4,1	+
11	3,9	+
12	11,8	+
13	1,3	-
14	4,4	+
15	5,6	+
16	5,8	+
17	8,4	+

Discussion

The CD, seen as a systemic immunological disorder, can have therefore numerous influences on the content of the saliva (8, 12). Lenander-Lumikari has shown that individuals with CD secrete lower amounts of IgA, IgG and amylase; in his study, he hasn't found significant differences between coeliac and healthy subjects, as also it has been said more recently by Mina (8, 13). Some studies, over the last decade, have also demonstrated the presence of anti-tTG auto-antibodies in the saliva of coeliac children (14-16).

This study has took into consideration the anti-tTG auto-antibodies in the saliva of 17 children with suspected CD, in order to attest the usefulness of the salivary anti-tTG as diagnostic and predictors markers of the CD.

The obtained results demonstrate the simultaneous presence of the anti-tTG both in the serum and in the saliva of paediatric patients with suspected CD. In particular, in all children that have shown only later such pathology (100%), the saliva, as indeed also the serum, contained the anti-tTG even before the clinical onset of the CD (Tab. 2). This is also linked to the data reported recently by Bonamico who, on a sample of 32 coeliac children (11 males and 21 females, aged between 5.8 and 8.7, mean age 7.4 years) shows the presence of the anti-tTG in the saliva. In particular, over the whole sample, 31 children have showed correspondence with the anti-tTG both in the serum and the salivary fluid, thus con-

Table 4 - Correspondence of anti-tTG in the saliva between the first and the second sample.

Patients	Anti-tTG in the saliva (UA/mL) I sample	Anti-tTG in the saliva I sample	Anti-tTG in the saliva (UA/ml) II sample	Anti-tTG in the saliva II sample
1	8,9	+	2.3	weakly positive
2	0,9	-	1.8	-
3	8,9	+	5.6	+
4	0,6	-	2.4	-
5	17,0	+	33.6	+
6	3,9	+	5.4	+
7	0,7	-	1.4	-
8	3,8	+	4.9	+
9	0,9	-	1.3	-
10	2,8	weakly positive	4.1	+
11	4,6	+	3,9	+
12	7,8	+	11,8	+
13	0,6	-	1,3	-
14	2,0	weakly positive	4,4	+
15	4,0	+	5,6	+
16	11,7	+	5,8	+
17	6,9	+	8,4	+

firming the hypothesis that the saliva can be a valuable help for diagnostic screening of such pathology (11). However, a previous study, conducted by Baldas, sayed that the saliva, because of its low sensitivity, couldn't be considered as a source of the typical auto-antibodies of the CD and for this reason such biological fluid couldn't be used for diagnostic purposes for the earlier interception of the disease (17).

Before the discover of the salivary anti-tTG, several studies have been carried out in order to seek the specific salivary antibodies for the diagnosis of the CD; in particular anti-gliadin deaminated antibodies (AGA) and anti-endomysium antibodies (EMA) have been evaluated and analyzed (18, 25).

As regards as the AGA, they are resulted to be in possess, in general, of a low sensitivity and a limited specificity and therefore less reliable for diagnostic purposes. However the EMA have been specifically examined in two studies, both dated: in the first one, led by Lahteenoja in 1999, it is stated that the salivary EMA aren't useful for the diagnosis or for the follow-up of the CD; in the second one, led by Di Leo, it is declared that the salivary EMA have a high specificity (100%) but low sensitivity (46.5%) and therefore, they should be considered also unreliable for the diagnosis of CD (23, 24).

This is the reason why in the present study it has been used the ELIME's method; it represents a simple, reproducible, non-invasive test that has, in addition, an excellent sensitivity for the screening of CD, like the RIA method (Radio Immuno Assay) used in the study of 2011 of Bonamico regarding paediatric coeliac patients (11).

Looking at the data obtained, regarding the correspondence of the anti-tTG salivary between the first (T_1) and the second sampling (T_2) of salivary fluid in the G_1, it is clear that:

- the anti-tTG, after 6 months from the beginning of the exclusion diet, are always present in 10 coeliac children (G_2) and in the 2 girls healthy carriers of CD (Tab. 4);
- 4 children out of 12 positive resulted before to the anti-tTG salivary (T_1), show a decrease in the concentration of anti-tTG (33.3%), while 8 out of 12 show an increase of the anti-tTG (66.7%).

The results consider a period of time of the exclusion diet equivalent to 6 months, demonstrating that the concentration of the anti-tTG salivary can't be used as an early parameter of monitoring for the follow-up of the patients after only 6 months from the beginning of the gluten-free diet. Surely a wider range, namely up to 9 months or more, could be more significant, as it's affirmed by Bonamico in his study, in which it is demonstrated that the salivary anti-tTG can be used for the follow-up of the disease, because after 9 months or more from the beginning of the exclusion diet, 50% of paediatric patients with CD has low levels of both anti-tTG salivary than serum (16). In this study, it has been further corroborated the hypothesis that the presence of salivary anti-tTG after the beginning of the exclusion diet, can reveal a poor adherence (16). In fact, after one year of the gluten-free diet, the negativity of the EMA and anti-tTG is indicative of a strict adherence to it, while the positivity of the AGA, EMA and anti-tTG is a symptom of poor adhesion (26).

Conclusions

The results obtained from this study have shown how it could be possible to make a simple, reproducible, non invasive, inexpensive and highly sensitive screening of the CD using the saliva of paediatric patient with suspected CD. Therefore, it's possible to say that the saliva and the presence of anti-tTG auto-antibodies may be considered, like other signs and clinical symptoms, intra and extra oral; also like the results produced by the diagnostic gastroenterology protocol and in addition like a useful diagnostic dental markers, used for a first screening of the disease, having, as it has been demonstrated, a predictive and early value than anti-tTG which are in the serum.

References

1. Lionetti E, Catassi C. New clues in celiac disease epidemiology, pathogenesis, clinical manifestations, and

treatment. Int Rev Immunol 2011 Aug; 30(4):219-31.
2. Condò R, Costacurta M, Maturo P, Docimo R. The dental age in the child with Coeliac disease. European Journal of Paediatric Dentistry 2011; (12)3:184-188.
3. Carroccio A, Campisi G, Iacono G, Iacono OL, Maresi E, DI Prima L, Compilato D, Barbaria F, Arini A, DI Liberto C, Pirrone G, Craxì A, DI Marco V. Oral mucosa of coeliac disease patients produces antiendomysial and antitransglutaminase antibodies: the diagnostic usefulness of an in vitro culture system. Aliment Pharmacol Ther 2007 Jun 15; 25(12):1471-7.
4. Costacurta M, Condò R, Sicuro L, Perugia C, Docimo R. Cervical vertebral maturation and dental age in coeliac patiets. Oral & Implantology 2011; IV(3-4):23-29.
5. Lamireau T, Clouzeau H. Epidemiology of celiac disease. Pathol Biol (Paris). 2011 May 24.
6. Mina SS, Azcurra AI, Dorronsoro S, Brunotto MN. Alterations of the oral ecosystem in children with celiac disease. Acta Odontol Latinoam 2008; 21(2):121-6.
7. Toscano V, Conti FG, Anastasi E, et al. Importance of gluten in the induction of endocrine autoantibodies and organ dysfunction in adolescent celiac patients. Am J Gastroenterol 2000; 95:1742-8.
8. Lenander-Lumikari M, Ihalin R, Lähteenoja H. Changes in whole saliva in patients with coeliac disease. Arch Oral Biol 2000 May; 45(5):347-54.
9. Samaşca G, Iancu M, Farcău D, Butnariu A, Pop T, Pîrvan A, Andreica M, Miu N, Cristea V, Dejica D. IgA anti-tissue transglutaminase antibodies, first line in the diagnosis of celiac disease. Clin Lab 2011; 57(9-10):695-701.
10. Acar S, Yetkıner AA, Ersın N, Oncag O, Aydogdu S, Arıkan C. Oral Findings and Salivary Parameters in Children with Celiac Disease: A Preliminary Study. Med Princ Pract 2011 Oct 21.
11. Bonamico M, Nenna R, Montuori M, Luparia RP, Turchetti A, Mennini M, Lucantoni F, Masotti D, Magliocca FM, Culasso F, Tiberti C. First salivary screening of celiac disease by detection of anti-transglutaminase autoantibody radioimmunoassay in 5000 Italian primary schoolchildren. J Pediatr Gastroenterol Nutr 2011 Jan; 52(1):17-20.
12. Kaufman E, Lamster IB. The diagnostic applications of saliva a Review. Crit Rev Oral Biol Med 2002; 13:197-212.
13. Mina SS, Riga C, Azcurra AI, Brunotto M. Oral ecosystem alterations in celiac children: A follow-up study. Arch Oral Biol 2011 Sep 13.
14. Bonamico M, Ferri M, Nenna R, Verrienti A, Di Mario U, Tiberti C. Tissue transglutaminase autoantibody detection in human saliva: a powerful method for celiac disease screening. Institute of Paediatrics and Department of Clinical Science, University of Rome La Sapienza, Italy. J Pediatr 2004 May; 144(5):632-6.
15. Ocmant A, Mascart F. Effective detection of celiac disease using salivary anti-transglutaminase. Am J Med 2007 Oct; 120(10):e15; author reply e17.
16. Bonamico M, Nenna R, Luparia RP, Perricone C, Montuori M, Lucantoni F, Castronovo A, Mura S, Turchetti A, Strappini P, Tiberti C. Radioimmunological detection of anti-transglutaminase autoantibodies in human saliva: a useful test to monitor coeliac disease follow-up. Aliment Pharmacol Ther 2008 Aug 1; 28(3):364-70.
17. Baldas V, Tommasini A, Santon D, Not T, Gerarduzzi T, Clarich G, Sblattero D, Marzari R, Florian F, Martellossi S, Ventura A. Testing for anti-human transglutaminase antibodies in saliva is not useful for diagnosis of celiac disease. Clin Chem 2004 Jan; 50(1):216-9.
18. al-Bayaty HF, Aldred MJ, Walker DM, et al. Salivary and serum antibodies to gliadin in the diagnosis of celiac disease. J Oral Pathol Med 1989; 18:578-81.
19. Kelly CP, Feighery CF, Gallagher RB, Gibney MJ, Weir DG. Mucosal and systemic IgA anti-gliadin antibody in celiac disease. Contrasting patterns of response in serum, saliva, and intestinal secretions. Dig Dis Sci 1991 Jun; 36(6):743-51.
20. O' Mahony S, Arranz E, Barton JR, Ferguson A. Dissociation between systemic and mucosal humoral immune responses in coeliac disease. Gut 1991; 32:29-35.
21. Hakeem V, Fifield R, al-Bayaty HF, et al. Salivary IgA antigliadin antibody as a marker for coeliac disease. Arch Dis Child 1992; 67:724-7.
22. Rujner J, Socha J, Barra E, Gregorek H, Madaliński K, Woźniewicz B, Giera B. Serum and salivary antigliadin antibodies and serum IgA anti-endomysium antibodies as a screening test for coeliac disease. Acta Paediatr 1996 Jul; 85(7):814-7.
23. Di Leo M, Weisz G, Ansaldi Balocco N. Serum and salivary antiendomysium antibodies in the screening of coeliac disease. Panminerva Med 1999 Mar; 41(1):68-71.
24. Lähteenoja H, Toivanen A, Räihä I, Syrjänen S, Viander M. Salivary antigliadin and antiendomysium antibodies in coeliac disease. Scand J Immunol 1999 Nov; 50(5):528-35.
25. Marinello D, Rapa A, Osello R, et al. Celiac disease screening: exploring the iceberg with salivary antigliadin antibodies. J Pediatr Gastroenterol Nutr 2001; 32:227-8.
26. Mohaidle A, Mella JM, Pereyra L, Luna P, Fischer C, Cimmino DG, Pedreira SC, Boerr LA. Role of antibodies in celiac disease after one year of treatment to predict the adherence to gluten-free diet. Acta Gastroenterol Latinoam 2011 Mar; 41(1):23-8.

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