

# Low serological positivity in patients with histology compatible with celiac disease in Perú

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## ABSTRACT

**Objective:** to study the frequency of positive serology for celiac disease (CD) in patients with duodenal biopsies suggestive of this disease.

**Material and methods:** cross sectional study. We included patients with duodenal biopsies histologically compatible with CD and anti gliadin, antiendomysial and IgA antitransglutaminase antibodies. We defined a "case" of CD if there was a positive biopsy and either antiendomysial or antitransglutaminase positive antibodies.

**Results:** thirty one patients were included in our study. Six were antiendomysial positive and 5 antitransglutaminase positive while the anti gliadin was positive in 14 cases. Therefore, out of 31 patients only 10 had a serology compatible with CD and only one had positive both antibodies, antiendomysial and antitransglutaminase.

**Conclusions:** a) we have found that most of the duodenal biopsies compatible with CD are not diagnosed with positive serology; and b) we found a low correlation between serological diagnostic tests.

**Key words:** Celiac disease. Intestinal villous atrophy. Intraepithelial lymphocytes. Perú.

## RESUMEN

**Objetivo:** estudiar la frecuencia de positividad de las pruebas serológicas en pacientes con biopsias compatible con enfermedad celíaca.

**Material y métodos:** estudio transversal. Se incluyeron pacientes con biopsia duodenal histológicamente compatible con en-

fermedad celíaca y determinación de anticuerpos anti gliadina, antiendomysio y antitransglutaminasa IgA. Definimos como caso de enfermedad celíaca a quienes tuvieron biopsia positiva y anticuerpos antiendomysio y/o antitransglutaminasa positivos.

**Resultados:** 31 pacientes fueron incluidos de los cuales 6 fueron antiendomysio positivo, 5 fueron antitransglutaminasa positivo y anti gliadina fue positivo en 14. Por lo tanto de 31 pacientes con cambios histológicos compatibles con enfermedad celíaca sólo 10 tuvieron serología diagnóstica. Sólo uno de los pacientes tuvo positividad tanto para antitransglutaminasa como para antiendomysio.

**Conclusiones:** a) encontramos que la mayoría de biopsias de duodeno con un cuadro histológico sugerente de enfermedad celíaca no se corresponden con serología diagnóstica de esta enfermedad; b) encontramos baja coincidencia en la positividad serológica entre antiendomysio y antitransglutaminasa.

**Palabras clave:** Enfermedad celíaca. Atrfia de vellosidades intestinales. Linfocitos intraepiteliales. Perú.

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## INTRODUCTION

Celiac disease (CD) is usually diagnosed using a combination of histological changes and serological tests in symptomatic and asymptomatic population. Classically, CD was suspected when the patient presented with chronic diarrhea and intestinal malabsorption. More recently, the diagnosis of CD is also considered in patients with dyspepsia and iron deficiency anemia.

Finding villous atrophy and lymphocytic infiltrate in the duodenal epithelium has become the standard for his-

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tological diagnosis of CD. Antitransglutaminase and antiendomysial antibodies have replaced antigliadin antibodies as the main serological tests (1).

Because serological tests are scarce and expensive in our country, histology has become the cornerstone of CD diagnosis in patients with upper gastrointestinal symptoms in Perú.

The aim of our study was to find the frequency of positive serological tests in patients with histological findings suggestive of CD.

## MATERIAL AND METHODS

*Design:* an observational, cross sectional study.

*Patients:* patients were included if they had the following inclusion criteria:

- Adults with gastrointestinal symptoms.
- Duodenal biopsies compatible with CD.

The study was conducted between February 2008 and May 2009. All patients had serological determination for antigliadin, antitransglutaminase and antiendomysial antibodies. Hemoglobin, vitamin B12 and folic acid levels were also assessed. Anemia was defined as hemoglobin below 13 g/dl for men and below 11 g/dl for women. Folic acid deficiency was defined with serum levels below 3 ng/dl and vitamin B12 with levels below 160 pg/dl. In addition, 14 of the 31 patients had a hydrogen breath test to assess the possibility of bacterial overgrowth.

*Exclusion criteria:* patients with gastrointestinal cancer, HIV infection and concomitant intestinal parasites were excluded.

*CD compatible biopsies:* duodenal biopsies were considered compatible with CD if there was an increased lymphocyte count and villous atrophy.

— Lymphocytes in number of 25 or more per 100 epithelial cells in at least 3 different 40x fields (2,3).

— Villous atrophy with a villous/crypt ratio lower than 1/3 in more than 10x field (4).

All duodenal biopsies were obtained from the second portion of the duodenum or beyond. For our present study we only considered patients with villous atrophy, which was classified as mild, moderate and severe according to the MARSH system (5).

*Serological studies:* IgA antitransglutaminase antibodies were assessed using an ELISA commercial kit Inmulisa (IMMCO Diagnosis). Positivity was considered with values above 20 U/ml.

IgA antigliadin antibodies were also determined by an indirect ELISA similar to the above mentioned and with a diagnostic cut off value of 20 U/ml.

Antiendomysial antibodies were determined using monkey's esophagus as substrate and an indirect immunofluorescence (Scimex corporation). Cut off values were dilution of more than 1/5.

All data was processed using the SPSS 15.0 pack. Students t test with a Fishers correction were used for the statistical analysis.

*Case definition:* we considered a "case" of CD any patient with villous atrophy and positive antiendomysial or antitransglutaminase antibodies.

## RESULTS

Our study was conducted between March 2008 and April 2009 and 31 patients with the above inclusion criteria were included.

According to our case definition, 10 (32.3%) were considered as CD. Positive results for each serological test are shown in table I. Only one patient had positivity for both antitransglutaminase and antiendomysial antibodies.

Table II shows the clinical characteristics of CD patients and those with non celiac enteropathy. We found no differences between both groups.

Table III shows the positivity of each serological test according to the degree of villous atrophy. We found increasing positivity for antiendomysial as well as for antigliadin, but not for the antitransglutaminase antibodies, with increasing villous atrophy.

**Table I. Positivity of serological findings in patients with duodenal biopsy suggestive of CD**

	n = 31	%
AGA	14	45,16
EMA	6	19,2
TTG	5	16,1

**Table II. Clinical characteristics of cd patients and those with non celiac enteropathy**

	Celiac disease	Non celiac enteropathy	p
Age in years (range)	62.9 ± 14.1 (29-80)	50.6 ± 21.3 (22-85)	0.069
Sex			
Female	8 (80%)	14 (66.6%)	
Male	2 (20%)	7 (33.3%)	0.67
Dyspepsia	10 (100%)	18 de 21 (85.7%)	0.53
Diarrhea	8 (80%)	19 de 21 (90.4%)	0.57
Anemia	5 de 9 (55.5%)	6 de 19 (31.5%)	0.40
Folic acid deficit	1 de 8 (12.5%)	5 de 17 (29.4%)	0.62
Vit B12 deficit	4 de 10 (40%)	6 de 20 (30%)	0.69

Fisher test.

**Table III. Serology positivity according to the degree of villous atrophy**

	Mild (MARSH I)	Moderate (MARSH II)	Severe (MARSH III)
AGA (%)	1 (20)	9 (47.4)	4 (57.1)
EMA (%)	0 (0)	3 (15.8)	3 (42.9)
TTG	0 (0)	5 (26.3)	0 (0)
Total	5	19	7

A hydrogen breath test was performed in 14 patients, and only one had a positive result. This single patient had also positive results for antiendomysial antibodies and so considered a case of CD.

## DISCUSSION

Celiac disease is a clinical entity rarely diagnosed in Perú. A search in MEDLINE, LILACS and Scielo data bases revealed only one paper published in Perú regarding CD (6). One of the reasons for this is that serological diagnostic tools are not commonly available; therefore when CD is sought we usually rely on histological findings for its diagnosis.

We aimed to study how frequently a suggestive duodenal biopsy really reflects a CD diagnosis with serological confirmation.

In our study only 10 out of 31 (32.3%) patients with duodenal histology compatible with CD were serologically confirmed. Our results differ from those of Ludwinson (7) and Greco (8) both of whom found a high correlation between villous atrophy and increased number of lymphocytes on one hand and serological markers on the other (95 and 100% respectively). These differences may be explained by the fact that in developing countries there are many other enteropathies of probable infectious origin that may give a similar histological pattern. Our results confirm that histological findings are unspecific and should not prompt an immediate diagnosis of CD.

IgA deficiency could explain low rates of serological positivity in our patients, but it is important to remember that this condition is extremely rare, in fact so rare that it is not a routine to rule out IgA deficiency routinely.

It is also important to remember that there are serology negative CD cases described, but this situation is usually occurring in patients with minimal histological changes, something we have tried to avoid by including only those patients with MARSH III histological compromise (10).

Antiendomysial antibodies are the gold standard for the diagnosis of CD (9,11), but the American Gastroenterological Association has recommended that antitransglutaminase should be used as first line test in primary care (9). Our findings show a poor coincidence between both antibodies. Only one patient had positivity of both

serological tests. This lack of coincidence has been previously shown by others and there are several explanations for this. One is the different techniques used by several laboratories. Another possibility is that there could be other proteins (different than tissue transglutaminase) acting as substrate for the antiendomysial antibodies (10,12). A third possible explanation is that the cutoff level used may be different. The literature shows values ranging from 2 to 20 U/ml (3).

A female predominance in CD has been described (13). Our findings show a F/M ratio of 4. Age at presentation has also been of interest. CD can be present at any age. Our study included only adults and among them we found patients in the 3rd and the 9th decade of life. Our age average was above 60 years, stressing the importance to consider this diagnosis in all age groups.

There has been described an association between degree of villous atrophy and serological positivity, especially in pediatric population (14-16). Our results have shown this relation for antiendomysial antibodies but not so for antitransglutaminase. We acknowledge that our series is a small one and this point requires clarification with bigger numbers.

We have found a high percentage of patients with an enteropathy with characteristics of CD but serologically negative. There are several clinical situations that could explain these histological findings, tropical sprue, diet protein intolerance and bacterial overgrowth among them (5,17). In 9 patients (with negative CD serology) a breath test was done to search for bacterial overgrowth and none of them tested positive.

We must acknowledge that our study population is small and our findings need confirmation with other series, nevertheless we can conclude the following: a) the frequency of positive serology in patients with duodenal biopsies compatible with CD is low; and b) we have found a discrepancy in the positivity of antiendomysial and antitransglutaminase antibodies, and so we believe that in our country the diagnosis of CD should not rely on a sole serological test.

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