MATERIALS AND METHODS:
indirect ELISA with available commercial kits (with tTg and htTg).

AIMS: 1) to standardize the indirect ELISA for detection of anti-tTg; 2) to standardize the indirect ELISA for detection of antibodies against the complex tTg/fibronectin; 3) to compare the results of anti-tTg, anti-fibronectin and anti-tTg/fibronectin antibody ELISAS of patients with not treated and treated celiac disease; 4) to compare the results of the in house indirect ELISA with available commercial kits (with tTg and htTg).

STANDARDIZATION OF ANTI-TISSUE TRANSGLUTAMINASE ANTIBODY DETECTION AND ASSESSMENT OF TRANSGLUTAMINASE INTERACTIONS WITH FIBRONECTIN. COMPARISON OF THE RESULTS WITH TWO COMMERCIALY AVAILABLE ESSAYS

INTRODUCTION: anti-endomysial (EMA) and anti-tissue transglutaminase (anti-tTg) are the main circulating autoantibodies in patients with celiac disease. Inasmuch as tissue transglutaminase (tTg) is the target antigen of EMA, specific assays have been developed to establish the serological diagnosis of celiac disease. However, published data are contradictory regarding their superiority over EMA. tTg is a cytoplasm enzyme, but indirect immunofluorescence reaction (IIF) indicates that EMA reacts with components of extracellular matrix, particularly with fibronectin. To date, serological tests have been standardized only with tTg. It is unknown if the addition of fibronectin to tTg substrate will improve the diagnostic accuracy. AIMS: 1) to standardize the indirect ELISA for detection of anti-tTg; 2) to standardize the indirect ELISA for detection of antibodies against the complex tTg/fibronectin; 3) to compare the results of anti-tTg, anti-fibronectin and anti-tTg/fibronectin antibody ELISAS of patients with not treated and treated celiac disease; 4) to compare the results of the in house indirect ELISA with available commercial kits (with tTg and htTg).

MATERIALS AND METHODS: the casuistic was formed by the sera of 173 patients, including 49 with celiac disease without treatment and 124 controls (chronic diarrhea = 30; inflammatory bowel disease, n = 23; autoimmune hepatitis type 1 n = 30; treated celiac disease, n = 23; e healthy individuals, n = 18). EMA was detected by IIF, using sections of human umbilical cord as substrate. Indirect ELISA was carried out for detection of reactivity against tTg (from guinea pig liver), fibronectin (recombinant from human fibroblast) and the complex tTg-fibronectin. The performance of those essays was compared with that of anti-tTg and anti-hTg kits, purchased from Inova Diagnostics, Inc, USA. Statistical analysis was performed using Student t test, Fisher exact test, McNemar test and kappa correlation test when appropriate. RESULTS: Seropositivity to anti-tTg, antifibronectin and anti-tTg/fibronectin was observed in, respectively: 46.9%, 51% e 42.9% in the celiac disease without treatment group; 0%, 13% and 0% in the treated celiac disease group; 39.1%, 65.2% and 56.5% in the inflammatory bowel disease group; 20%, 50% and 20% in the autoimmune hepatitis group; 6.7%, 60% and 26.7% in the chronic diarrhea group. No patient among healthy controls had seropositivity to those aforementioned antibodies. Comparing the anti-tTg and tTg/fibronectin complex seropositivity in the celiac disease without treatment group, it was found that the addition of fibronectin to the tTg substrate did no improved the accuracy of the essay (p = 0.68; relative risk 1.15; 95% confidence interval = 0.7334-1.803). Comparing EMA titers with ELISA positivity, it was found that higher seropositivity to anti-tTg, antifibronectin and anti-tTg/fibronectin complex occurred in sera with EMA titers equal or higher than 1/1280. There was no relation between age and the seropositivity of celiac antibodies. Patients with inflammatory bowel disease had higher rates of seropositivity to anti-tTg (p = 0.029, RR = 6.1 and IC95% = 0.9-41.5) and antifibronectin (p = 0.0097, RR = 6.9 and IC95% 1.0-46.0) when compared with patients with Crohn disease. The concordance coefficients between the in house ELISA and the anti-hTg (commercial kit) was: 46.9% (kappa coefficient kappa 0) in the celiac disease without treatment group; 45.5% (kappa 0) in the treated celiac disease group; 90% (kappa 0.047, p = 0.786; McNemar, p = 1.0) in the chronic diarrhea group; 56.5% (kappa 0.085, p = 0.412; McNemar, p = 0.021) in the inflammatory bowel disease group; 83.3% (kappa 0.359, p = 0.033; McNemar, p = 0.375) in the autoimmune hepatitis group and 100% in the healthy individuals group. The concordance coefficients between the in house ELISA and the anti-tTg (guinea pig, commercial kit) was: 46.9% (kappa coefficient 0) in the celiac disease without treatment group; 54.5% (kappa 0) in the treated celiac disease group; 90% (kappa 0.047, p = 0.786; McNemar, p = 1.0) in the chronic diarrhea group; 52.2% (kappa 0.166, p = 0.235; McNemar, p = 0.033) in the inflammatory bowel disease group; 86.7% (kappa 0.524, p = 0.033; McNemar, p = 0.635) in the autoimmune hepatitis group and 94% (kappa 0) in the healthy individuals group. The concordance coefficients between the two commercial kits varied from 72.7% to 100% in the different groups.

CONCLUSIONS: 1) The in house ELISA was not a good technique for detecting anti-tTg reactivity due to its low rate of positivity in patients with celiac disease without treatment and high rate of positivity in EMA negative control groups; 2) The addition of fibronectin to the substrate tTg did not improve the diagnostic accuracy of the essay in patients with celiac disease without treatment; 3) There was no difference in anti-tTg seropositivity rates between children and adults with celiac disease without treatment; 4) There was a correlation between the EMA titers and seropositivity to anti-tTg, detected by the in house ELISA; 5) The seropositivity of all autoantibodies by ELISA was higher in patients with ulcerative colitis when compared with Crohn disease; 6) Commercial kits were adequate for the identification of positive cases (with celiac disease without treatment) and negative cases among controls; 7) The seronegativity to anti-tTg antibodies by commercial kit was not systematically followed by the negativation of EMA in patients with celiac disease. Those findings suggest that anti-tTg ELISA are suitable for therapeutic monitoring of celiac disease; 8) The standardization of any ELISA for detection of celiac antibodies should include the comparison with both available commercial kits and AAE; 9) According to the results of the current study, the detection of anti-tTg antibodies, using an in house ELISA with guinea pig liver tTg as substrate, could not be incorporated in the routine work-up for the serodiagnosis of celiac disease in our hospital.

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SUMMARY OF THESIS*

ABRANTES-LEOMS, Clarice Pires - Pesquisa do anticorpo antitransglutaminase tissular avaliando as interações da transglutaminase com a fibronectina e comparação com os resultados de dois ensaios comerciais. São Paulo, 2005. (Dissertação de Mestrado - Faculdade de Medicina da Universidade de São Paulo).