Evaluation of IgA epidermal transglutaminase ELISA in suspected dermatitis herpetiformis patients

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To the Editor:

Dermatitis herpetiformis (DH) is an autoimmune blistering disease occurring in approximately 2.17% to 15% of celiac disease (CD) patients [1,2]. Detection of granular IgA deposits in the dermal-epidermal junction and/or in the papillary dermis using the gold standard test, direct immunofluorescence (DIF), on uninvolved, perilesional skin, serology testing for IgA antibodies to endomysial muscle tissue (EMA), and tissue transglutaminase (TG2 or tTG) antigen testing are all currently used to diagnose patients with DH [3]. Given there are an estimated 3.28 million patients in the U.S. living with undiagnosed CD, with current screening guidelines, up to half a million individuals may also be suffering from undiagnosed DH [4]. Consequently, further defining sensitive and specific serological screening markers that distinguish DH from other similar skin diseases is of paramount importance. Sardy et al. in 2002 [5] identified epidermal transglutaminase (TG3 or eTG) as the main autoantigen of DH. Limited, subsequent studies reported IgA eTG ELISA sensitivity of 52-90% in DH patients [6]. Herein, using serum samples received by Beutner Laboratories between 2013 and 2020, we share our experience on the prevalence and efficacy of IgA eTG ELISA and the potential for its use as an initial screening test in patients with suspected DH. Our study bolsters recent findings [7,8] with data from this additional population and provides clinically relevant information by comparing the sensitivity of IgA eTG ELISA against the sensitivities of both IgA tTG ELISA and EMA. Prior studies have demonstrated heightened sensitivity of IgA eTG ELISA compared with IgA tTG ELISA in confirmed cases [7,8]. Our study evaluates IgA eTG ELISA as a screening test in patients with suspected DH and compares its usefulness with EMA, a serologic test with high diagnostic accuracy [9]. We included EMA in our analysis as EMA is one of the primary serologic tests for diagnosing DH [3]. Our results indicate that IgA eTG ELISA is significantly more sensitive than EMA in detecting DH. Further, our data provide additional evidence to support the use of IgA eTG ELISA as an initial screening test, that when positive, may indicate the need for follow-up testing, including skin biopsy.

Serum samples from 220 patients were retrospectively analyzed for IgA antibodies against eTG, tTG, and EMA. Data on DIF biopsy studies was simultaneously available in 38 patients. We divided these 220 samples into four primary groups: biopsy positive (Group 1), biopsy negative (Group 2), biopsy unknown (Group 3), and control (group 4). Group 4 included 5 patients with other autoimmune bullous diseases (four bullous pemphigoid, one linear IgA bullous dermatosis) and 5 healthy controls. Each sample was tested for IgA EMA antibodies by indirect immunofluorescence assay, and for IgA tTG and eTG antibodies by ELISA. The immunofluorescence assay for EMA was performed as referenced [10]. The Enzyme-Linked Immunosorbent Assay for IgA eTG (Immunodiagnostik AG, Bensheim, Germany) and IgA tTG ELISA (INOVA Diagnostics, San Diego, CA, U.S.A) were performed according to the manufacturer's instructions. A titer of >2.5 for EMA,

Table 1. *Serology results for IgA endomysial muscle tissue (EMA), IgA tissue transglutaminase (tTG) and IgA epidermal transglutaminase (eTG) antibodies in confirmed or suspected dermatitis herpetiformis patients.*

Group	EMA-IgA	IgA-tTG	IgA-eTG
Group 1: Biopsy Positive (n=8)	2/8 (25.0%)	5/8 (62.5%)	7/8 (87.5%)
Group 2: Biopsy Negative (n=35)	0/35 (0.0%)	3/35 (8.6%)	3/35 (8.6%)
Group 3: Biopsy Unknown (n=167)	9/167 (5.4%)	21/167 (12.5%)	28/167 (16.7%)
Group 4: Controls (n=10)	0/10 (0.0%)	0/10 (0.0%)	0/10 (0.0%)

and absorbance value of >20 units/ml for IgA tTG ELISA were considered positive. The following absorbance units were used to interpret IgA eTG ELISA: >22AU/ml: Positive; 16-22AU/ml: Grey range; <16AU/ml: Negative

Table 1 shows serology results for IgA EMA, IgA tTG and IgA eTG antibodies in confirmed or suspected DH patients outlines the serology results for groups 1, 2, 3 and 4. The sensitivities of IgA eTG ELISA, IgA tTG ELISA and EMA were 87.5%, 62.5% and 25% respectively. Based on the one sample proportion test, IgA eTG ELISA was significantly more sensitive than EMA (P=0.05). The sensitivity of IgA eTG ELISA was higher than that of IgA tTG ELISA, however we cannot show statistical significance due to insufficient sample size.

In Group 3, 39/167 sera were positive for at least one antibody. Of these 39 samples, 9 were positive for EMA, 21 were positive for IgA tTG ELISA, and 28 were positive for IgA eTG ELISA. An additional 7 sera gave readings in the grey range (16-22AU/ml) for IgA eTG ELISA. All 9 sera positive for EMA were positive for either IgA tTG ELISA or IgA eTG ELISA or both (subgroup 3a). Ten sera were positive for both IgA tTG ELISA and IgA eTG ELISA (subgroup 3b). Twelve samples were positive for IgA eTG ELISA alone (subgroup 3c). Five samples in this subgroup gave ratio values in the grey zone. Three sera were positive for IgA tTG ELISA and EMA but were negative for IgA eTG ELISA (2/3 sera gave values in grey range for IgA eTG).

As shown in **Figure 1**, IgA eTG ELISA was elevated in the biopsy confirmed group (Group 1) and in suspected DH patients (Group 3) compared to the biopsy negative group (Group 2) and the control group (Group 4). The mean absorbance units for Groups 1 and 3 were comparable and higher than the means found in the biopsy negative and control groups. For ancillary analysis, we compared the average positive eTG absorbance value in Group 3 with Groups 2 and 4 (DH negative). Using the ANOVA and subsequent Tukey-Kramer post hoc test, we found the mean IgA eTG ELISA value in Group 3 to be significantly higher than the average positive IgA eTG ELISA values in Groups 2 and 4 (P<0.05). This analysis provides statistical evidence that a positive IgA eTG ELISA value is indeed significant and requires further investigation. Additionally, 12 of the 28 positive IgA eTG ELISA samples in Group 3 were positive for IgA eTG ELISA alone. In our study, 38% of biopsy confirmed patients were negative for both EMA and IgA tTG ELISA. Only one patient (13%) in this group was negative for IgA eTG ELISA and the absorbance value for this serum was in the grey zone (15.4). Thus, this preliminary data from our laboratory indicates that IgA eTG ELISA may be a sensitive diagnostic test for DH and serve as an additional

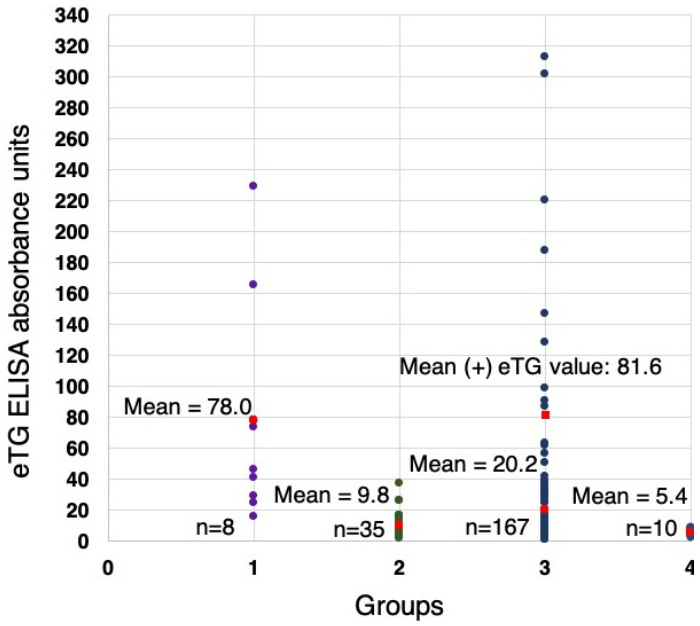

Figure 1. *IgA eTG ELISA absorbance units for Groups 1, 2, 3, and 4.*

primary screening tool in patients with relevant symptoms of DH and a clinical history of gluten-sensitive enteropathy.

Moreover, several publications have recently provided compelling evidence regarding IgA eTG ELISA as a serological marker. Rose et al. [7] found IgA eTG ELISA to be the most sensitive marker for detecting DH in 95% of untreated patients. Jakowski et al. [8] found IgA eTG ELISA to be similarly significant when 20% of DH patients in their study had negative IgA tTG ELISA values and positive IgA eTG ELISA values. These larger studies have reported far greater sensitivity for IgA eTG ELISA compared to IgA tTG ELISA in confirmed cases of DH. Our study supports these findings but differs in that it evaluates the sensitivity if IgA eTG ELISA as an initial screening test compared to the highly accurate EMA in suspected patients with DH [9]. We found IgA eTG ELISA to be significantly more sensitive than EMA for detecting DH specific antibodies in suspected patients.

Although the purpose of this study was to evaluate IgA eTG ELISA as a screening test for DH and not to correlate levels of IgA eTG ELISA with disease presentation, it is important to note that previous studies have found IgA eTG ELISA to be 100% specific in patients with DH (on normal diet), [8]. In our experience, the specificity is 93.3%. Therefore, we can infer that the 28/167 (16.7%) patients in Group 3 with positive IgA eTG ELISA values require additional diagnostic evaluation. To conclude, a positive IgA eTG ELISA may be a strong indication to obtain a biopsy for direct immunofluorescence confirmation of DH. However, further multicenter studies are needed to evaluate the usefulness of IgA eTG ELISA as a screening test for DH in undiagnosed patients and we encourage others to share their experiences by responding to this publication.

Potential conflicts of interest

The authors declare no conflicts of interest.

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