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CAN DEAMIDATED GLIADIN PEPTIDE ANTIBODIES BE CONSIDERED MORE SPECIFIC THAN TRADITIONAL MARKERS FOR CELIAC DISEASE- A PILOT STUDY

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Abstract: A prospective study of the clinical diagnosis, duodenal biopsies and serological results in patients with suspected celiac disease was undertaken from Feb 2014 to March 2016.In the study the assay sensitivity and specificity for diagnosing CD were 92.98% (95% CI= 83% to 100%) and 100% (95%CI=96.19% to 100%) for IgG and 55.36% (95% CI=41.47.8% to 68.66%) and 100% (95% CI= 96.23% to 100%)for IgA antibodies to DGP respectively. The sensitivity and specificity of IgA tTG was found to be96.49% (95% CI= 87.892% to 99.57%) and 78.95% (95% CI= 69.38% to 86.64%) respectively. In conclusion the addition of DGP antibody test in combination with tTG antibodies makes the test more acceptable to the patients than undergoing invasive endoscopic procedure.

Key words: Celiac disease,DeamidatedGliadin Peptide,Tissue Trans Glutaminase,Positive Predictive Value,Negative Predictive Value,Upper Limit of Normal.

INTRODUCTION:

The appreciation ofthe importance of gliadin deamidation in the immunopathogenesis of Celiac disease has led to the development of commercially available diagnostic tests based on antibodies to deamidated gliadin peptides.Recently the use of these autoantibodies have shown high specificity and sensitivity in detecting Celiac disease[1]. We could findno publishedreports from the Indian sub-continent regarding the use of DGP as a reliable tool for diagnosis and management of celiac disease. Therefore, we undertook this pilot study to look at its efficacy.

MATERIALS AND METHODS:

A total of 152 patients(both children and adults) were studied.75 subjects acted as controls. IgA antibodies against tTG were determined by ELISA test.Indirect Immunofluorescence tests were employed to detect IgA and IgG class antibodies against deamidated gliadin peptides(GAF3X) and IgA class antibodies against Endomysium.Auto antibodies against endomysium were detected by using tissue sections of primate intestine. For the determination of antibodies against deamidated gliadin a repetitive gliadin-analoguefusion

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peptide(GAF3X)1,2was used consisting of an immunologically dominant deamidated epitope of gliadin with a length of nine aminoacids. To increase the diagnostic competence for its detection,it is linked with an artificial gliadin – homologue octa peptide1,2.Both the ELISA kits and IIFT kits were procured from EUROIMMUN GERMANY. Sensitivity, specificity, positive and negative predictive value (PPV & NPV) are expressed as percentages for ease of interpretation.Their confidence intervals are "exact" Clopper-Pearson confidence interval.

RESULTS:

A total of 152 individuals were evaluated of which 77 had some symptoms and signs related to the upper gastrointestinal tract. The rest 75 individuals were apparently healthy subjects.Among the total 152 cases 75 clinically symptomatic individuals had elevated anti tTG in their serum above the upper limit of normal (ULN) for the kit used. The values ranged from 21.5 to 88050 U/mL. All these 75 cases were further evaluated for the presence of antibodies against IgA DGP, IgG DGP and IgA Endomysium. In 31of the 75 cases with elevated anti tTG (106 U/mL to 88050 U/mL), all three antibodies viz: IgA DGP, IgG DGP and IgA endomysium were present, which indicated a high probability that the individuals were suffering from Celiac disease. Further analysis of these 31 cases shows that in 15 cases the levels of anti IgA tTG that were ≥ 10 X ULN.12 cases had anti tTG≥ 8 < 10 X ULN. The rest 4 cases the anti tTG level was > 1< 8 X ULN as in Table 1. There were 20 cases in which the anti tTG was elevated (range 21.5 U/mL to 65 U/mL) but all the other three serologic markers were negative. These were considered false positive from the diagnostic point. These 20 subjects were advised duodenal biopsy and were convinced to undergo upper gastrointestinal endoscopic examination by the physician. In all these 20 cases the biopsy results returned as normal and was not indicative for Celiac disease. In 152 subjects studied, 75 cases had elevated IgA tTGantibody levels above the upper limit of normal (ULN).2 cases were found to be IgA deficient.None of the 75 cases acting as controls had elevated IgAtTG antibody levels above the ULN. Of the 75 cases with elevated anti IgA tTG, 31 were also positive for IgADGP,IgGDGP antibody levels. In 20 cases only IgGDGP was positive. In 24 cases both IgA / IgGDGP antibodies were not detected.On statistical analysis the sensitivity of IgAtTG, IgADGP and IgGDGPwere found out to be 96.49%,55.36%,and 92.98% respectively.The specificity of IgA tTG,IgADGP and IgGDGP were 78.95%,100%,100% respectively.The sensitivity, specificity, positive predictive value (PPV), negative predictive value are given in Table 2.

Table 1. No of positive DGP and endomysium with different levels of anti tTG.

Anti IgA tTG n =31	≥ 10 ULN	≥ 8 < 10ULN	< 8ULN
Anti IgA DGP, IgG DGP and IgA endomysium	n =15	n =12	n =4

Table 2: Sensetivity, specificity,PPV and NPV of DGP and tTG antibodies.

Serologic markers	Sensitivity	Specificity	PPV	NPV
IgA tTG	96.49%	78.95%	73.33%	97.4%
IgA DGP	53.36%	100%	100%	79.34%
IgG DGP	92.98%	100%	100%	95.96%

DISCUSSION:

Our analysis shows that IgA tTGantibody has greater sensitivity than IgG DGP and IgA-DGP antibody(96.49% vs 53.36% and 92.98%)respectively for the screening and diagnosis of Celiac disorder.However, the sensitivity of IgG DGP is significantly higher as compared to IgA DGP. On the other hand, the specificity of both the DGP antibodies are higher than that of IgA tTG as was shown in published studies[3]. Till date at least partial villous atrophy is required to make a final diagnosis of Celiac Disease.

It is interesting that in eight studies IgG DGP antibodies were compared with IgA DGP antibodies.In seven out of these eight studies the IgG test was sensitive than the IgA test,although it was more specific in seven[4,5,6,7].As sensitivity is more important than specificity in screening,the IgA tTGtest is to be preferred,although the IgG test could be useful to detect 2% ofCeliacdisease patients who have IgA deficiency [7]. Thus it has been suggested that a test for IgG DGP be combined with IgA-tTG antibody.The combination of IgG-DGP with IgA-DGP antibodies has also been studied[4,5,8,9,10] and in general an increased sensitivity is traded off for a long specificity and the resultant specificity / sensitivity were better than IgA tTG[4,5].

CONCLUSION:

The novel anti GAF3X dots representing deamidated gliadin dots detected by IIF test shows a higher specificity to detect Celiac disease associated autoantibodies in patients with Celiac disorder when compared with tests using tTG or Endomysium as substrates alone. The outstanding novelty represented by the high CD specificity of DGP opens new roads for implementation of a different antibody strategy. The newly developed DGP antibodies tests, alone or in combination with tTG antibodies can thus be used as a screening tool to avoid intestinal biopsy.

The essence of diagnosing celiac disease is to start GFD to allay symptoms in patients ingesting gluten. The presence of DGP antibodies is highly predictive of celiac disease particularly in childrenbefore three years of age. This essentially reflects the sensitization of an

individual against gliadin and a great assessment of an immune reaction to gluten. The presence of antibodies to DGP could give a strong indication to the clinician to consider prescribing GFD at least on a trial basis particularly in economically constrained countries.

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