5.5 Detection of gluten immunogenic peptides (GIP) in stools as a method of monitoring the gluten-free diet in children

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Introduction

Methods of evaluating the compliance to the gluten-free diet (GFD) include clinical, serological and histological tests, but currently a specific, non-invasive and standardised method is lacking. A recent study has shown that a significant part of α-gliadin 33-mer (33Eps) is resistant to gastrointestinal (GI) digestion [1]. The G12 and A1 monoclonal antibodies (mAbs) against the main immunogenic epitope of the α-gliadin 33-mer, already proven to successfully detect toxic peptides in food samples [2-3], have recently been tested to quantify immunogenic peptides in faeces. A G12 competitive ELISA test has been shown to easily quantify traces of gluten in faeces and furthermore a recent study [1] has demonstrated that: 1. the faecal amount of gluten reflects the ingested quantity; 2. gluten peptides become undetectable after 3-4 days of GFD and appear on day 3 during a gluten challenge.

We aimed to investigate the clinical usefulness of the new fecal test in children with coeliac disease (CD) and to compare this new method with traditional methods of evaluating the adherence to the GFD.

Materials and methods

CD children on a GFD for at least 6 months, healthy children on a normal diet and healthy controls on a GFD for one week were enrolled. A 3-day food diary (including report of quantities and brands of all the ingredients) was used to monitor the diet before enrollment. According to the diary, three classes of contamination risks (no evidence of contamination, possible risk of contamination, clear evidence of contamination) were identified. Furthermore, we evaluated the overall adherence to the standards suggested by the Italian Coeliac Society on the supply, preparation, and consumption of the GF foods, using a 16-point questionnaire. Evaluating the percentage of correct responses, adherence to the national standards was scored in three classes: excellent (> 80%), intermediate (60-80%) and poor (< 60%). Gastrointestinal symptoms were evaluated through the Gastrointestinal Symptoms Rating Scale (GSRS) [4] and coeliac serology (tTG IgA and DGP IgG antibodies) was collected within 1 month from the enrollment. The competitive ELISA iVYLISA GIP (Biomedal Diagnostic, Sevilla) designed to detect and quantify gluten immunogenic peptides (essentially peptides related to the 33-mer) and based on the G12 antibody

was used to analyse the stool samples collected after 3 days of food-record. The kit contains six standards (100, 50, 25, 12.5, 6.25, 3.12 ng/mL GIP) and the lower quantitation limit of the assay is 312 ng GIP/g sample (for a sample dilution of 1:10). Correlations between symptoms, food diary, and questionnaire analysis were analysed.

Results and discussion

Seventy-two CD children (mean age: 10.63 ys, SD: 4.78 ys), 16 controls on a normal diet (mean age: 7.97 ys, SD: 4.66 ys) and 4 healthy volunteers (medical doctors already trained on the GFD) following a GFD for at least one week were enrolled. Demographical and clinical data are outlined in Tab. 1.

Table 1. *Demographical and clinical features of the study group.*

Patients	**Age, years (mean ± SD)**	**Sex**	**GFD, years (mean ± SD)**	**National Celiac Society members (%)**
CD on a GFD (N=72)	10.63 (± 4.78)	20 M 52 F	3.43 (± 2.84)	67.27
Controls on a normal diet (N=16)	9.43 (± 6.45)	8 M 8 F	-	-
Controls on a GFD (N=4)	29.2 (± 3.2)	4 F	-	-

In CD children, the mean GFD duration was 3.43 ys (SD: 2.84 ys). Evaluation of the compliance to the GFD (including serological, 3-day food diary and questionnaire results) is summarised in Tab. 2.

Table 2. *Evaluation of adherence to the GFD measured by serological data, 3-day food diary and questionnaire.*

Adherence to the GFD	**Serology (IgA tTG and/or IgG DGP)**	**3-day food record**	**16-point questionnaire (based on the standards suggested by the National Coeliac Society)**
Good	Negative: 64%	No risk of contamination: 34%	Excellent adherence: 53%
Poor	Positive: 36%	Possible risk of contamination: 56% Evidence of contamination: 10%	Intermediate adherence: 37% Low adherence: 10%

Overall 47% of CD children were found to have detectable amounts of gluten in stools compared to 100% of controls on a normal diet. Mean GIP values in the CD group were significantly lower compared to the controls (Fig. 1).

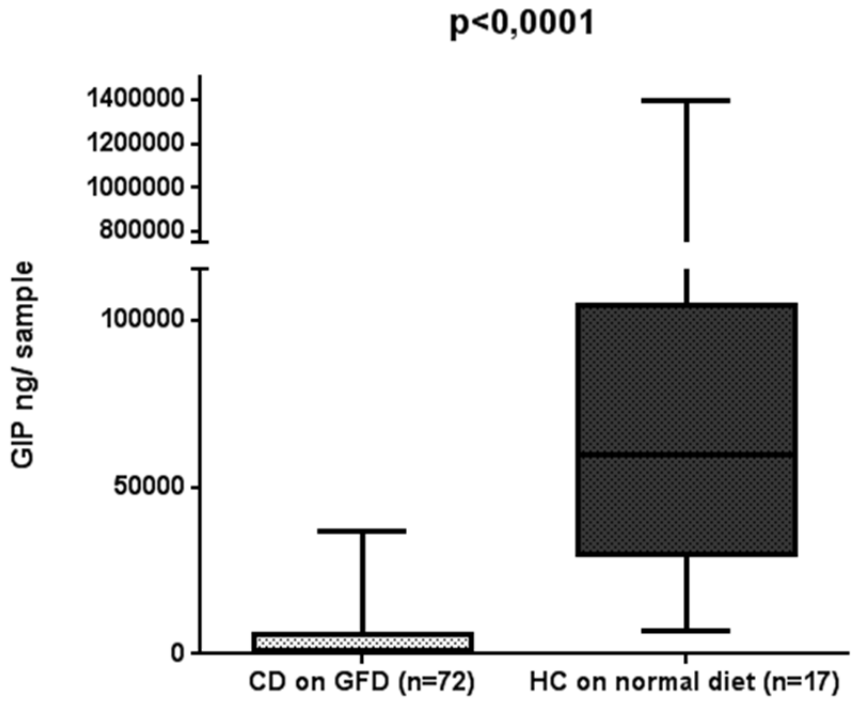

Figure 1. *Comparison between fecal GIP levels (ng/g sample) of CD children on a GFD and healthy controls (HC) on a normal diet*

No significant correlation was found between GIP levels and adherence to the diet (measured by the diary and the questionnaire). Both GI symptoms measured by the GSRS score and levels of "coeliac autoantibodies" were found to be positively correlated with GIP values (Fig. 2).

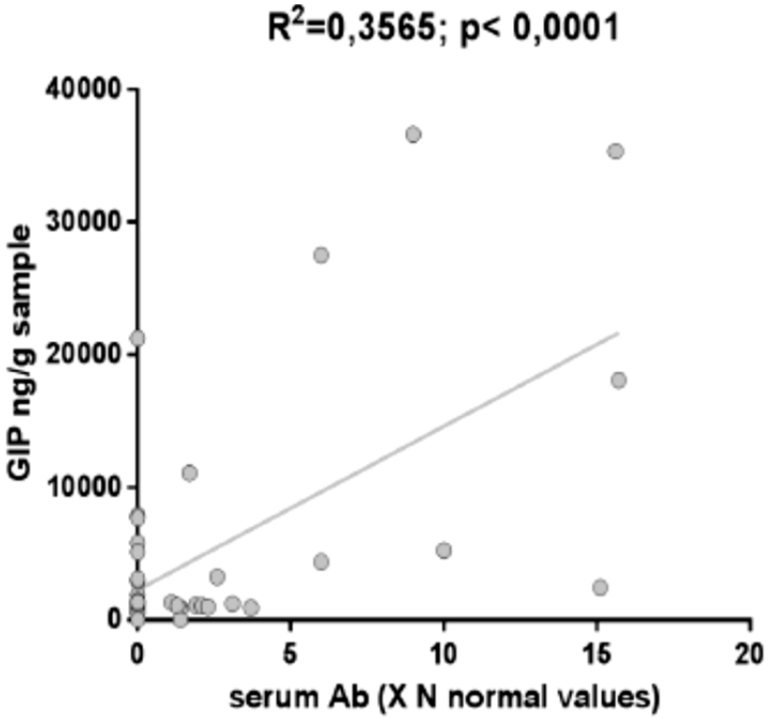

Figure 2. *Positive correlation between faecal GIP levels and serum antibodies in coeliac patients*

Analysing serial samples collected from the group of healthy volunteers during 7 days of GFD, further results were obtained: 1. In some subjects faecal gluten disappeared more slowly than expected (more than 3 days, as previously described [1]) 2. Some subjects continued to eliminate gluten despite the GFD, 3. Levels of GIP in stools can vary more quickly than previously thought (Fig. 3).

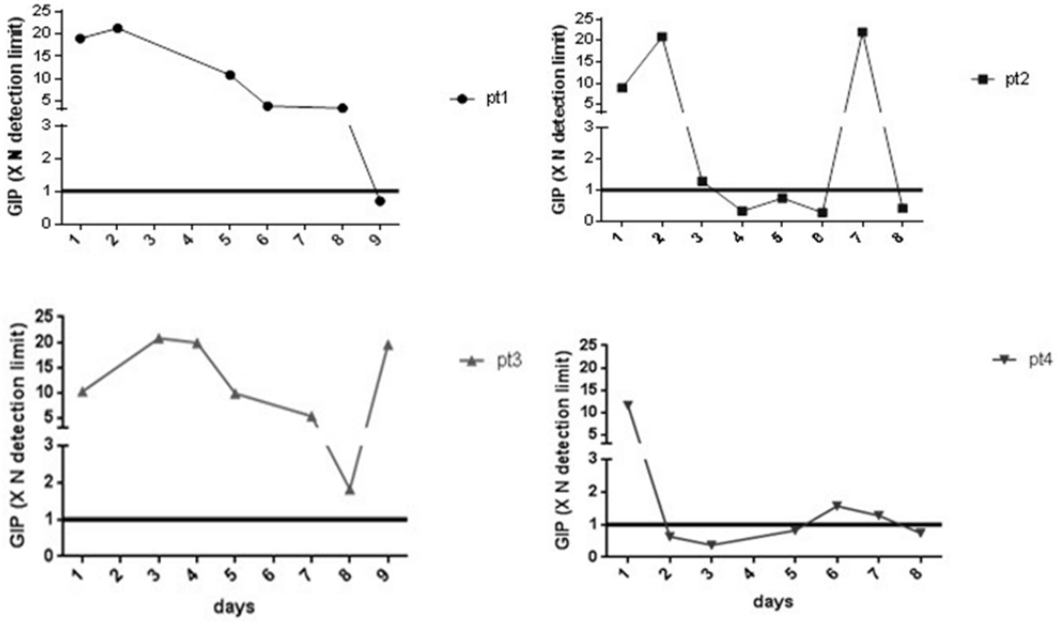

Figure 3. *Time to elimination of ingested gluten in the 4 healthy controls well-trained on the GFD*

These final results raised some questions about the specificity of the test and the need to consider possible confounding environmental factors occurring during sample collection or the analysis.

Conclusions

The iVYLISA GIP test is a non-invasive, very sensitive, and promising test to assess the compliance to the GFD, especially in children. Our results show that a high percentage of CD children have detectable traces of gluten in faeces. This may indicate incomplete adherence to the GFD and furthermore, we found a significant correlation with both clinical and serological data. Our preliminary findings need to be replicated in other centres and possibly compared to a larger group of healthy controls. However, the presence of gluten in control samples (collected from well-trained subjects on the GFD) could reflect a low sensitivity of the test. The technique itself is not particularly challenging, but the analysis is quite long (5 - 6 hours) and can present some minimal technical problems.

References

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