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Comparison of Clinical Methods With the Faecal Gluten Immunogenic Peptide to Assess Gluten Intake in Coeliac Disease

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ABSTRACT

Objectives: Detection of faecal gluten immunogenic peptides (GIP) is a biomarker of recent gluten consumption. GIP levels can be used to monitor gluten intake and compliment clinical methods to evaluate compliance to gluten-free diet (GFD). In the present study, recent gluten intake was measured by GIP in children with coeliac disease (CD) and compared to routine clinical measures to evaluate GFD compliance.

Methods: GIP was measured in 90 samples from 63 CD children (44 previously and 19 newly diagnosed with follow-up samples at 6 and 12 months on GFD). Compliance to GFD was evaluated based on clinical assessment, tissue transglutaminase (tTG) levels, and Biagi score.

Results: GIP was detectable in 16% of patients with previous CD diagnosis on GFD. Body mass index z score ($P = 0.774$), height z score ($P = 0.723$), haemoglobin concentration ($P = 0.233$), age ($P = 0.448$), sex ($P = 0.734$), or disease duration ($P = 0.488$) did not differ between those with detectable and nondetectable GIP. In newly diagnosed patients, on gluten-containing diet, GIP was detectable in 95% of them. Following GFD initiation, GIP decreased ($P < 0.001$); 17% and 27% had detectable levels at 6 and 12 months, respectively. Compared to GIP, the Biagi score, tTG, and clinical assessment presented sensitivity of 17%, 42%, and 17%, respectively. Likewise, GIP was detectable in 16%, 16%, and 14% of patients evaluated as GFD compliant according to the Biagi score, tTG, and clinical assessment, respectively. A combination of methods did not improve identification of patients who were noncompliant.

Conclusions: Inclusion of faecal GIP measurements is likely to improve identification of GFD recent noncompliance in CD management and could be incorporated into current follow-up strategies.

Key Words: clinical assessment, coeliac disease, gluten-free diet, gluten immunogenic peptides, tissue transglutaminase antibodies

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What Is Known

- Strict adherence to gluten-free diet is the only treatment of coeliac disease.
- The precision of current clinical methods to ascertain gluten-free diet compliance in coeliac disease remains unclear.
- Gluten immunogenic peptides in faeces have been proposed as a biomarker of gluten intake.

What Is New

- Gluten immunogenic peptide was detected in 16% of coeliac disease patients on recommendation to adhere to gluten-free diet.
- Using current clinical methods, almost 4 out of 5 children who do not comply with gluten-free diet will be potentially missed.
- Inclusion of gluten immunogenic peptide in routine practice may improve objective health professional judgment and management of coeliac disease.

Treatment of coeliac disease (CD) relies on a lifelong, strict gluten-free diet (GFD) which aims to ameliorate symptoms and induce mucosal healing. Adherence to GFD is challenging to maintain and can affect considerably a patient's social interaction and quality of life (1). Global assessment of gastrointestinal symptoms,

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anthropometry, haematological profile, diet, serological markers, and often validated questionnaires remains the standard approach to assess compliance to GFD in the current clinical practice. Endoscopy with small bowel biopsy is often reserved as last clinical resource to ascertain intermediate adherence to GFD but is invasive, requires general anaesthesia in paediatric patients, and interpretation findings may be difficult considering the lag time to complete healing.

Among serological markers, the serum tissue transglutaminase (tTG) titre is often used to ascertain compliance with GFD. Although tTG levels have prime validity for disease diagnosis they are relatively poor indicators of compliance with GFD (2). Similarly, self-reported compliance questionnaires, such as the Biagi score (3), based on the analysis of the strategy adopted by patients to avoid gluten consumption, may suffer from recall bias and rely on patient's awareness of gluten-containing meals, including those which use gluten as food additive and thus cannot preclude accidental exposure to gluten. Hence, there is a strong interest in the development of novel, objective biomarkers of GFD compliance. Among them, the serum concentration of alkylresorcinols, phenolic lipids which are present in the outer layers of wheat, rye, and barley grains, has been proposed as a biomarker of gluten exposure (4). Similarly, the average serum concentration of intestinal fatty acid–binding protein (iFABP), a marker of intestinal epithelial damage, is significantly elevated in patients with CD at diagnosis, associates positively with Marsh histological scoring and decreases after initiation of GFD (5). iFABP is also more sensitive than tTG and deamidated gliadin peptide antibodies (DGP) to short-term exposure to GFD (6), although other conditions can affect its serum titre (7). Antibodies to DGP have also shown higher sensitivity than tTG antibodies to monitor GFD compliance, but their screening validity for CD diagnosis is inferior to the latter (8).

Beyond the blood biomarkers of compliance to GFD, detection of gluten immunogenic peptides (GIP) in faeces or urine has recently been proposed as a sensitive and specific marker to detect recent gluten intake (2) and has also been compared against endoscopy and mucosal healing outcomes (9). The 33-mer peptide from α2-gliadin is stable against breakdown by gastric, pancreatic, and intestinal brush border membrane enzymes and quantification in human faeces indicates recent gluten consumption (10). In a dose-response study, consumption of as little as 100 mg of gluten/day gave detectable levels of GIP in faeces (10). Monitoring GIP in faeces can therefore be used to monitor recent gluten intake and cross-check the validity of current clinical approaches to ascertain compliance to GFD.

The primary aim of the present study was to measure the concentration of faecal GIP, as an objective biomarker of recent gluten intake, in samples of children with CD before and while on treatment with GFD and compare this with serological markers, compliance questionnaires, and clinical assessment.

SUBJECTS AND METHODS

As part of a cross-sectional study, which explored the role of gut microbiota in CD, 90 fresh faecal samples were collected within 2 hours of passage and stored in −80°C from a convenience sample of 63 consecutive children with CD who attended our local outpatient clinic, between August 2011 and September 2013. Forty-four samples were from children with previously diagnosed CD and 19 from patients with newly diagnosed CD on gluten-containing diet. At follow-up, 12 and 15 of the newly diagnosed children provided repeat samples at 6 and 12 months, respectively, while on treatment with GFD. All patients were diagnosed according to the BSPGHAN guidelines in place at the time, including small bowel endoscopy with biopsy (1). Participants were not specifically aware that measurements of GIP would be performed on their faecal samples to minimize changes in their regular eating habits before sample collection.

Faecal GIP was measured with the iVYLISA GIP Stool ELISA kit (Biomedal, Spain) and according to the manufacturer's procedures. Patients were considered as compliant on GFD when the faecal concentration of GIP was below the level of quantification of the assay (ie, GIP <0.156 μg/g sample).

For patients on GFD, compliance to GFD was evaluated as ''good compliance'' or ''variable/noncompliant'' by the clinical dieticians (clinical assessment) running the outpatient clinic and adopting a structured approach including extensive diet history, changes in anthropometry, and recall of gastrointestinal symptoms (Supplemental Text, Supplemental Digital Content 1, *http://links.lww.com/MPG/B434*). Acquisition of diet history was performed by senior specialist dieticians and encompassed a 30-minute, face-to-face interview, with the child and carer, about dietary habits and transgressions with regard to compliance to GFD, using question prompting and a response-guided tactic. Although blood samples were obtained on the day of the clinical appointment for measurements of haematological profile and tTG titres, results were available only after clinic and so did not influence the dieticians' judgment on compliance.

After their routine clinical appointment, the patients were approached by a researcher, independent to the research team, who asked them to complete the Biagi score (3). A Biagi score equal to or greater than 3 was considered good compliance with GFD. For newly diagnosed patients assessments were repeated at 6 and 12 months postdiagnosis.

Statistical Analysis

Changes in the concentration of faecal GIP within groups were estimated using the general linear model with Box-Cox transformation and considering sample dependency. Sensitivity, specificity, and predictive values were calculated on cross-tabulated data. For the purposes of the present study, sensitivity (ie, proportion of patients with positive GIP correctly identified) and negative predictive value were considered as the most clinically relevant values to report. Interclass agreement between all methods and GIP levels was evaluated using Cohen κ statistics.

Ethical Considerations

Participants and their carers provided written informed consent to participate in the study. The study and its procedures were approved by the West of Scotland Research Ethics Committee (Ref: 11-WS-0006) and the National Health Service Research and Development office.

RESULTS

Gluten Immunogenic Peptide Levels Before and After Introduction of Gluten-free Diet

Participant characteristics are presented in Table 1. In 7 of the 44 (16%) patients with previously diagnosed disease, GIP was detectable indicating recent gluten consumption, and hence poor recent compliance with GFD. In this group of patients, there was no difference in body mass index z score ($P = 0.774$), height z score ($P = 0.723$), haemoglobin concentration ($P = 0.233$), age ($P = 0.448$), sex ($P = 0.734$), or disease duration ($P = 0.488$) between patients with detectable and nondetectable GIP.

In 18 of 19 patients (95%) on gluten-containing diet under investigation for CD diagnosis, a concentration of GIP was detectable and in all except for 1 patient tTG levels were raised. In the

TABLE 1. Participant characteristics

	Newly diagnosed N = 19	Previously diagnosed N = 44
Sex, males (%)	10 (53)	18 (43)
Age, y	10 (3.2)	9.3 (3.1)
Height *z* score, SD	−0.05 (0.87)	0.01 (1.0)
BMI *z* score, SD	−0.27 (1.12)	0.12 (1.1)
Disease duration, y	n/a	4.6 (3.7)
Haemoglobin, g/dL	13.1 (9.4)	12.7 (8.6)
tTG antibodies, U/mL		
At recruitment	69.7 (43.1)	5.6 (10.4)
%Raised	90%	n/a
6-m follow up	9.8 (10)*	n/a
%Raised	66%	n/a
12-mo follow-up	6.9 (5.8)*	n/a
%Raised	18%	n/a

BMI = body mass index; SD = standard deviation; tTG = tissue transglutaminase antibodies.
*$P < 0.05$ compared with concentration at recruitment.

only patient with normal tTG the concentration of GIP was marginally above the limit of quantification of the assay. Following diagnosis and recommendation to adhere to GFD, the serum tTG decreased (Table 1) and as did the concentration of GIP; however, 2 of 12 patients (17%) and 4 of 15 (27%) patients had detectable levels of GIP at 6 and 12 months, respectively (Fig. 1).

Biagi Score Compared With Faecal Gluten Immunogenic Peptide Levels

Performance of the Biagi score was evaluated in a total of 65 data points from children with CD (previously diagnosed and newly diagnosed on GFD) on recommendation to adhere to a GFD. Based on the Biagi score, 61 (84%) of the assessments indicated compliance to GFD; in total 12 samples (18.5%) had detectable levels of GIP. The Biagi questionnaire presented a negative predictive value of 84% to predict recent compliance to a GFD but 17% (2/12) of all samples with detectable levels of GIP were identified correctly

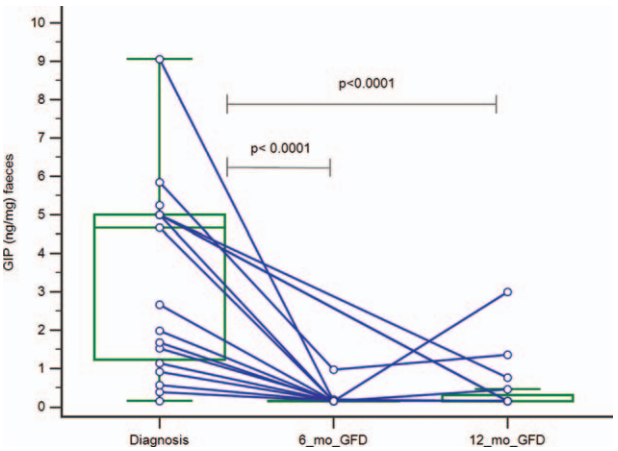

FIGURE 1. Changes in the concentration of faecal GIP at the time of diagnosis and at 6 and 12 month on gluten-free diet. 12_mo_GFD = 12 months on gluten-free diet; 6_mo_GFD = 6 months on gluten-free diet; GIP = gluten immunogenic peptide.

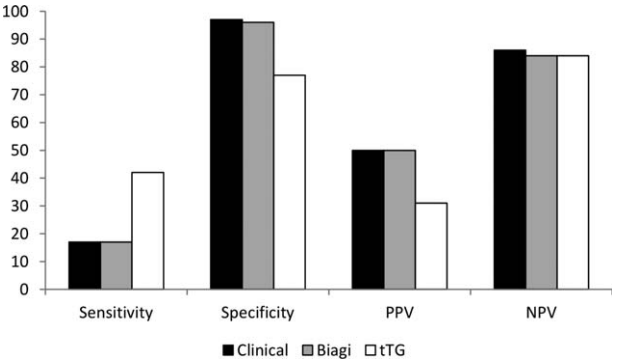

FIGURE 2. Specificity, sensitivity, positive, and negative predictive validity of clinical dietetic assessment, Biagi score, and tTG levels compared with faecal GIP. GIP = gluten immunogenic peptide; NPV = negative predictive value; PPV = positive predictive value; tTG = tissue transglutaminase antibodies.

(Fig. 2 and Supplemental Table, Supplemental Digital Content 2, *http://links.lww.com/MPG/B435*). The interclass agreement between the faecal GIP and the Biagi score was weak ($\kappa = 0.17$).

Serum Tissue Transglutaminase Antibodies Compared With Faecal Gluten Immunogenic Peptide Levels

Sixty data points from children with CD on GFD (previously diagnosed and newly diagnosed on GFD) were available to evaluate serum tTG as proxy of GFD compliance. Based on normal (<7 U/mL) tTG levels, 44 (73%) patients were assessed to be adherent on GFD, and in total 12 (20%) patients had detectable levels of faecal GIP. Classification based on tTG levels presented a negative predictive value of 84% to detect recent compliance with GFD and 42% (5/12) of all patients who were not compliant to GFD had raised levels of tTG (Fig. 2 and Supplemental Table, Supplemental Digital Content 2, *http://links.lww.com/MPG/B435*). There was no significant difference in the median concentration of tTG between patients with detectable and nondetectable GIP levels (tTG 3.0 vs 3.6 U/mL; $P = 0.432$). The interclass agreement between the faecal GIP and tTG was weak ($\kappa = 0.17$).

Clinical Dietetic Assessment Compared With Gluten Immunogenic Peptide Levels

Data from 37 previously diagnosed children on treatment with GFD (not including newly diagnosed patients at follow-up) were available to evaluate the agreement between the dietician's clinical assessment and GIP analysis. Thirty-five (97%) of the patients were assessed to be adherent on GFD based on the dieticians reports; 6 (16%) patients had detectable faecal GIP. Against this, clinical assessment had a negative predictive value of 86% to predict compliance on GFD but only 1 of 6 (17%) of all patients who were not compliant to GFD were assessed correctly (Fig. 2 and Supplemental Table, Supplemental Digital Content 2, *http://links.lww.com/MPG/B435*). The agreement between the faecal GIP and clinical assessment was negligible ($\kappa = 0.18$).

Composite Indices Versus Gluten Immunogenic Peptide Levels

Composite indices including any 2 or all 3 of the GFD compliance markers were produced for 33 patients on GFD and

with all data available and compared against GIP levels. Combination of indices did not increase identification of patients who had a recent consumption of gluten (ie, sensitivity) or the false negative ratio. In contrast, inclusion of tTG, alone or in combination with any of the other markers, decreased specificity and accordingly increased the proportion of children who were falsely assessed as noncompliant to recent consumption of gluten (Supplemental Figure, Supplemental Digital Content 3, *http://links.lww.com/MPG/B436*).

DISCUSSION

The present study shows that the current clinical approaches to ascertain intake of gluten are likely to underperform. Almost 4 out of 5 children who do not comply with GFD will be missed, and from the 10 children who will be assessed compliant with GFD, 2 will not be so. Inclusion of GIP may improve health professional judgment and change current practice for selected patients. Considering early evidence to show that faecal GIP is sensitive enough to detect small amounts of gluten intake (10), the results of this study are in support of its clinical use as biomarker of recent gluten intake and compliance to GFD, particularly when the aetiology of refractory disease and accidental exposure needs to be ruled out and before consideration of repeat endoscopy.

Faecal GIP levels were increased in almost all children who on clinical assessment were consuming gluten to confirm disease diagnosis, and as expected, GIP was below quantification level in the large majority of patients following introduction of GFD. Still approximately 18% of CD patients on recommendation to follow a GFD, had recent consumption of gluten, a figure which is <30% of participants with detectable GIP in a previous study in Spain of older participants, including adults with CD (11).

The mainstay approach to evaluate compliance to GFD in routine practice is clinical global assessment including serological measurement of tTG, with repeat small bowel biopsy reserved for a minority of the most difficult and refractory cases. In the research setting, this is done and in addition checked with the use of compliance questionnaires, such as the Biagi score, which we used in the present study. Although the findings of this study suggest that global clinical assessment, tTG, and Biagi score have a low false negative ratio to identify patients who do not consume gluten, their sensitivity to identify all patients who consume gluten was relatively poor. This prediction did not improve when we calculated a composite index.

Among the methods we evaluated in this study, tTG serology performed the best although more than 50% of the patients noncompliant on GFD still remained undetected. Interestingly, the specificity and positive predictive validity of tTG was very low. This may be due to the long half-time for tTG to normalize after the introduction of GFD, as also observed in the present study, or the different time-frame of exposure to consumption of gluten that each of these 2 biomarkers reflect. As detectable faecal GIP occurs after 3 days of gluten consumption (10) or within 4 to 6 hours when measurements in urine are performed (9) this will be a useful biomarker for recent or repeated incompliance to GFD. It will, however, not be good marker to indicate intermittent compliance unless this occurs close to the GIP test and for more than 3 days before faecal sampling. In this context a negative GIP cannot ascertain long-term compliance to GFD and serum tTG and clinical dietetic assessment will be needed to complement this to evaluate both long- and short-term adherence to GFD.

The analytical costs of GIP may incur an additional expense to healthcare costs. Considering the overall treatment costs, time involved, and the performance of current clinical approaches to assess compliance to GFD, the introduction of GIP as an assessment tool of GFD compliance may, however, reduce health expenditure and improve patients care somewhat analogous to the introduction of faecal calprotectin in patients with inflammatory bowel disease. Both of these faecal tests are likely to reduce the need for endoscopy, although not to the same degree. In the present study we employed measurements of GIP as the criterion standard method to evaluate recent consumption of gluten and by inference a proxy marker of compliance to GFD. Although faecal GIP is a novel biomarker, additional research is needed to validate its usage in this context. Such research should include dose-response studies, explore factors influencing inter and intraindividual variations in GIP concentration, the effect of consumption of very low, safe concentrations of gluten from gluten-free products and comparison with mucosal biopsy healing.

Comino et al (11) observed a positive association between faecal GIP and DGP antibodies, although 87% of GIP-positive

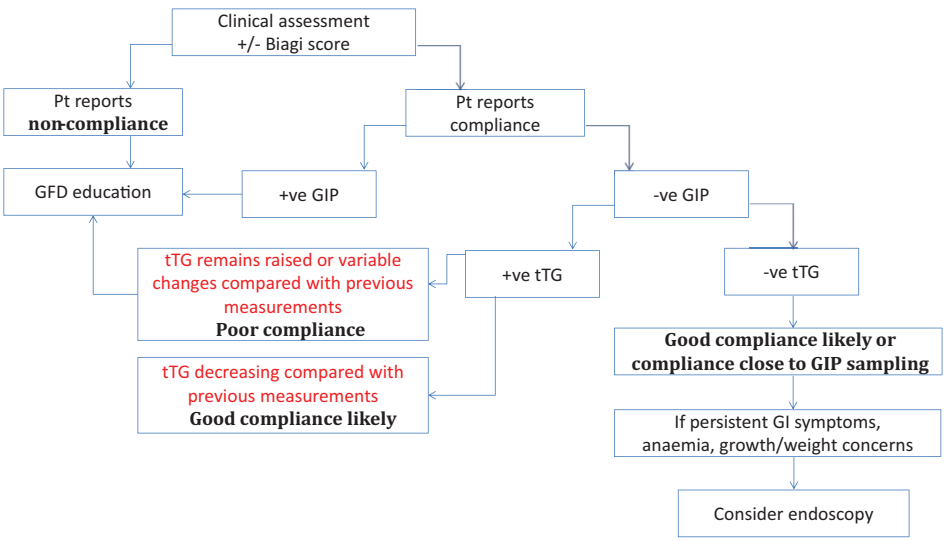

FIGURE 3. A decision pathway to monitor GFD compliance for patients with coeliac disease (CD) in routine practice. GFD = gluten-free diet; GI = gastrointestinal; GIP = gluten immunogenic peptide; tTG = tissue transglutaminase antibodies.

patients were negative for DGP antibodies. This suggests that use of different GFD biomarkers is not interchangeable but rather complementary. Hence, use of faecal GIP in conjunction with other novel blood biomarkers such as serum alkylresorcinols, iFABP, and DGP antibodies may improve evaluation of short-term and medium-term compliance to GFD and presence on enterocyte damage. This is particularly important to explore in future research as measurement of faecal GIP or serum alkylresorcinols will reflect recent dietary intake that patients may deliberately alter before testing. In contrast, other markers such as the serum iFABP and DGP antibodies will indicate medium-term adherence to GFD.

In the present study, noncompliance to a GFD was found to be much lower when compared to other studies in the literature (11,12). This may indicate sample selection bias; hence, the present study is unable to comment on the true prevalence of patients who do not comply with a GFD in routine clinical practice. Such outcome was, however, beyond the primary scope of this study, which was to assess the performance of routinely used GFD compliance methods, individually and all together, against the outcomes of GIP. For some patients, data were incomplete too, particularly for the follow-up clinical dietetic assessments. This was mainly due to 2 reasons. First some of the follow-up reviews of the newly diagnosed patients were performed in other general hospitals and by other health professionals, including general practitioners and paediatric gastroenterologists. Second as the primary aim of the present study was to explore the role of gut microbiota in CD and the effect of GFD, the timing of the follow-up sample collection from the newly diagnosed patients and hospital clinical assessments did not always match.

In conclusion, the current approaches of evaluating adherence to GFD could be improved and based on the novel findings of the present study we advocate for the introduction of faecal measurements of GIP to the routine management armamentarium of CD and propose a decision pathway to monitor compliance of GFD for patients with CD in routine practice (Fig. 3). Inclusion of faecal GIP measurements will assist health professional judgment based on objective measures and may reduce the need of additional tests.

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