Measurement of Fecal Calprotectin and Zonulin as Biomarkers in Adults With Fish-Induced Food Protein-Induced Enterocolitis Syndrome

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Food protein-induced enterocolitis syndrome (FPIES) is a serious condition that primarily affects young children. However, the prevalence of FPIES in adults in Spain is increasing, with fish being one of the most common triggers of the disease [1].

Identification of useful biomarkers constitutes a major challenge in the diagnosis and management of FPIES. Fecal calprotectin and zonulin may be suitable candidates for further investigation [2,4].

Elevated fecal calprotectin indicates acute gastrointestinal inflammation [3]. Fecal zonulin regulates gut permeability by affecting tight junctions, serving as a biomarker of intestinal barrier integrity in various inflammatory diseases [4].

Exposure to trigger foods is thought to increase intestinal permeability, facilitating the entry of allergens and triggering the intestinal immune response. Measuring fecal zonulin and calprotectin before and after an oral food challenge (OFC) may provide information about the variation in the integrity of the intestinal barrier and the presence of an underlying inflammatory response.

We explored the usefulness of fecal calprotectin and zonulin in the identification of intestinal inflammation and altered intestinal permeability, as well as their potential to guide the clinical management of affected patients.

We conducted a prospective, observational study (Ethics Committee approval, PI-4670). Eight adult FPIES patients with >2 recorded reactions and a negative lymphocyte transformation test (LTT) result for the culprit fish [5] were invited to undergo an OFC.

Baseline blood and fecal markers were analyzed in all patients, although post-OFC data were collected only in those with a positive reaction (Table).

OFC was conducted in hospital by trained personnel using a modified protocol [6]. Placebo was given on day 1, and a stool sample was collected. After 48 hours, 25% of a masked full serving was administered. If the patient remained asymptomatic for 48 hours, a full masked portion was given. Symptomatic patients provided stool samples, and blood analysis was performed. Patients were observed for 4 hours after each dose. A follow-up call was made after a week, and an LTT with fish was repeated 24 hours after a positive OFC.

Fecal zonulin was analyzed in duplicate using the Zonulin Stool ELISA kit (DRG Instruments GmbH). The reference values provided by the supplier were a median (SD) of 61 ng/mL (46 ng/mL).

Fecal calprotectin was extracted using the Calprotectin Stool Extraction Device (Diasorin S.p.A., ref X0043) and analyzed on the LIAISON®XL analyzer following the manufacturer's protocol (DiaSorin S.p.A., ref 318960). Calprotectin concentrations were expressed as micrograms per gram of stool (µg/g, equivalent to mg C/kg). Reference values were 50 µg/g for fecal calprotectin.

Eight women participated in the OFC. The average age at onset of symptoms was 32 years, and the average time since the previous reaction was 78 months. During the challenge on the second day (25% serving), patient 3 had a positive reaction. The other patients continued the challenge on the third day, receiving 100% of the serving, and patients 1, 5, and 7 had a positive reaction. In total, 4 patients experienced a reaction. The average latency to onset of symptoms was 180 minutes, with an average duration of symptoms of 22 hours despite treatment.

After ingestion, 2 patients experienced vomiting, 3 had nausea, 1 experienced diarrhea, and all of them had abdominal pain with bloating. None of the patients experienced severe reactions, such as hypothermia, hypotension, lethargy, or pallor. No patients required emergency care.

Only 4 out of 8 patients had a positive OFC result, possibly depending on the kind of fish and patient variability. Of the patients who had a positive OFC, 2 ate salmon and 2 ate hake. However, of the patients who tolerated the full serving, 2 ate hake, 1 sea bass, and 1 anchovy (Table).

The C-reactive protein levels tested were always within the normal range. No significant changes were observed in the tryptase value. A slight increase in lymphocyte, leukocyte, and neutrophil levels was observed in OFC-positive patients, except in patient 5. There were no differences in baseline laboratory marker levels between the negative OFC and the positive ones.

Table. Laboratory Data.

Patient	1		2	3		4	5		6	7		8
Total IgE, kU/L	35.3		120	<2		62.9	10.6		32.4	8.42		32.2
Fish sIgE, kU/L	0		0	0		0	0		0	0		0
Months since last reaction	18		6	12		24	48		204	192		120
OFC fish	Salmon		Seabass	Hake		Hake	Salmon		Hake	Hake		Anchovy
OFC result	100% serving		Neg	25% serving		Neg	100% serving		Neg	100% serving		Neg
	Baseline	**After OFC**	**Baseline**	**Baseline**	**After OFC**	**Baseline**	**Baseline**	**After OFC**	**Baseline**	**Baseline**	**After OFC**	**Baseline**
Tryptase, mg/dL	0	0	3.25	7.86	6.62	5.31	3.67	1.91	3.95	7.78	6.36	-
Leukocytes, 10^3/µL	7.60	8.79	7.34	7.67	9.48	7.90	6.03	4.47	5.85	8.02	11.73	-
Lymphocytes, 10^3/µL	2.37	2.46	2.74	4.31	5.21	2.66	1.82	0.94	0.92	2.86	4.15	-
Neutrophils, 10^3/µL	4.64	5.72	3.96	2.64	3.40	4.14	3.71	3.10	4.37	4.43	6.51	-
Eosinophils, 10^3/µL	0.10	0.09	0.07	0.12	0.17	0.30	0.05	0.02	0.09	0.12	0.19	-
Platelets, 10^3/µL	197	175	307	280	286	276	413	308	194	333	293	-
C-reactive protein, mg/dL	0	0	0	-	4.60	-	-	-	-	0	-	-
Fecal calprotectin, µg/g	0	27.2	0	25.3	25.2	67.7	0	82.5	6	62.4	200	9.8
Fecal zonulin, ng/mL	89	186	71	187	410	78	74	222	96	110	597	230
LTT	Neg	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg

Abbreviations: Neg: negative; OFC, oral food challenge; Pos, positive; LTT, lymphocyte transformation test.
The OFC was positive in patients 1, 3, 5, and 7.

Fecal calprotectin and zonulin values were evaluated, since their concentrations are known to correlate, respectively, with the level of intestinal mucosal inflammation [7] and increased intestinal permeability [8]. Calprotectin increased from an average of 21.93 µg/g to 83.72 µg/g after the reaction (average increase of 61.8 µg/g) in 3 patients. Zonulin levels increased in all patients from an average of 116.9 ng/mL at baseline to an average of 353.7 ng/mL after the reaction (average increase of 236.8 ng/mL) (Supplementary Figures 1 and 2).

Kolho et al [9] reported a higher increase in calprotectin values in patients with cow's milk–positive OFC results than in those with negative OFC results and controls. Our results indicate that levels of these markers changed after exposure to the offending fish when the patient had a reaction. The low number of patients included precludes a statistical analysis, although the results show that larger studies are warranted. The increase in zonulin levels is striking: all of the patients started with elevated baseline zonulin, although only 4 had a positive OFC result. Regarding calprotectin, 2 out of 3 patients with increased levels after the OFC had levels within the normal range.

Nagata et al [2] found that fecal marker levels increased significantly after antigen ingestion in FPIES patients, with no correlation between the amount of egg yolk ingested and the fecal markers. A lymphocyte proliferation assay performed in 26 children showed positivity with egg yolk but not with fish. We performed the LTT at baseline (all negative) and after a positive OFC result. The result turned positive in the second LTT in only 1 patient despite 4 positive OFC results. The higher sensitivity of the salmon LTT than the hake LTT might be due to the extract used or unknown factors.

Research on adult FPIES remain limited, although as awareness of this condition increases, it is crucial to investigate and validate biomarkers that aid in its diagnosis and management. In this context, calprotectin and zonulin seem promising options. However, further research is needed to validate their clinical utility and establish clear guidelines for their application in daily clinical practice.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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