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ORIGINAL ARTICLE

Circulating I-FABP as an Indicator of Intestinal Barrier Injury in Pediatric Type 1 Diabetes

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ABSTRACT

Background: **Intestinal barrier dysfunction has been implicated in the pathophysiology of pediatric type 1 diabetes mellitus (T1DM), but clinically useful circulating markers remain insufficiently characterized. The aim of this study was to evaluate circulating intestinal fatty acid-binding protein (I-FABP) and platelet-activating factor (PAF) levels in children with T1DM and examine their associations with glycemic control, diabetic ketoacidosis (DKA) burden, and disease duration.**

Methods: **In this case-control study, 43 children with T1DM and 43 age- and sex-matched healthy controls were enrolled. Serum I-FABP and PAF levels were measured using enzyme-linked immunosorbent assay (ELISA), and glucose and glycated hemoglobin (HbA1c) levels were assessed using standard biochemical methods.**

Results: **Serum I-FABP levels were significantly higher in patients than in controls (median 16.8 vs. 12.4 ng/mL; $p = 0.026$), whereas PAF levels did not differ significantly ($p = 0.389$). Both biomarkers correlated positively with HbA1c (I-FABP: $r = 0.552$; PAF: $r = 0.608$; $p < 0.001$ for both). Higher biomarker levels were also associated with a greater DKA frequency, whereas a shorter disease duration was associated with higher i-fabp and paf concentrations ($p < 0.001$).**

Conclusions: **Pediatric T1DM is associated with subclinical intestinal epithelial injury, as reflected by elevated circulating I-FABP levels. I-FABP may serve as a potential noninvasive marker of barrier dysfunction linked to glycemic burden; however, its modest diagnostic accuracy (area under the receiver operating characteristic curve [AUC] = 0.641) warrants cautious interpretation and further validation in larger cohorts. PAF appears less discriminative diagnostically but may reflect inflammatory activation during metabolic instability.**

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KEYWORDS

hyperglycemia, diabetic ketoacidosis, hemoglobin A, glycosylated

LIST OF ABBREVIATIONS

AUC - area under the receiver operating characteristic curve
BMI - body mass index
CV - coefficient of variation
DID - daily insulin dose
DKA - diabetic ketoacidosis
ELISA - enzyme-linked immunosorbent assay
FABP2 - fatty acid-binding protein 2 (intestinal)
HbA1c - glycated hemoglobin

I-FABP - intestinal fatty acid-binding protein
IQR - interquartile range
LPS - lipopolysaccharide
PAF - platelet-activating factor
RBS - random blood sugar
ROC - receiver operating characteristic
SCFA - short-chain fatty acid
T1DM - type 1 diabetes mellitus

INTRODUCTION

Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disease defined by T-cell-driven destruction of pancreatic β-cells, leading to complete insulin deficiency and permanent reliance on external insulin administration [1]. Worldwide, the incidence of T1DM among children and adolescents under 20 years of age reached 219,000 newly diagnosed cases in 2024, with an annual rate of 22.2 per 100,000 young individuals in the United States, indicating a 2.02% yearly rise since 2002 [2]. This increasing trend is particularly pronounced in pediatric populations, with rates increasing by up to 3.4% annually over three decades. Such an increase places a considerable burden on health systems, encompassing acute complications such as diabetic ketoacidosis and long-term microvascular and macrovascular risks, further aggravated by rapid urbanization and socioeconomic inequalities [3,4].

Beyond conventional autoimmune mechanisms, there is mounting evidence that the pathophysiology of T1DM in children is largely driven by gut barrier disruptions. The intestinal epithelial barrier, which consists of tight junctions, mucus layers, and enterocytes, normally prevents luminal antigens and microbial products from entering systemic circulation, thereby regulating systemic immune responses. In children with T1DM, intestinal hyperpermeability caused by dysbiosis is linked to increased production of lipopolysaccharide (LPS) and decreased butyrate synthesis. This environment encourages leaky gut and molecular mimicry, processes that ultimately accelerate the destruction of pancreatic β-cells. Recent cohort studies have linked gut barrier disruption to glycemic instability, independent of disease duration [5-9].

In patients with T1DM, increased intestinal permeability is often accompanied by varying degrees of enterocyte injury, underscoring the need for dependable biomarkers that can accurately represent the status of the intestinal barrier. The intestinal fatty acid binding protein (I-FABP; also known as FABP2) is a member of the FABP family of intracellular proteins with a molecular weight of 14 - 15 kDa [10]. These 126 - 134 amino acid proteins bind to long-chain fatty acids to promote signaling, metabolism, and intracellular transport [11]. I-FABP is involved in the uptake and trafficking of fatty acids in the small and large intestines and is mainly expressed by enterocytes at the intestinal villi tips. It is released into circulation when cell membrane integrity is compromised and has been used as a serum biomarker for a dysfunctional intestinal barrier [12].

In contrast, inflammatory mediators play a central role in connecting gut barrier dysfunction and vascular complications in T1DM. Platelet-activating factor (PAF) (1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine) [13,14] is a potent phospholipid mediator implicated in inflammatory signaling, leukocyte activation, and endothelial permeability. It has been proposed as both a biomarker and a mechanistic contributor to inflammatory disease [15,16]. Therefore, evaluating these markers together may provide complementary information on gut barrier damage and inflammation in children with T1DM. Accordingly, the aim of the present study was to assess circulating I-FABP and PAF levels in children with T1DM and examine their associations with glycemic control, diabetic ketoacidosis burden, and disease duration in comparison with healthy age- and sex-matched controls. We hypothesized that: 1) serum I-FABP and PAF levels would be significantly elevated in children with T1DM compared with healthy controls; 2) both biomarkers would correlate positively with indicators of poor glycemic control (HbA1c) and DKA burden; and 3) shorter disease duration would be associated with higher biomarker concentrations, reflecting more intense early intestinal injury.

MATERIALS AND METHODS

Study design and setting

This case-control study investigated intestinal barrier dysfunction in children with T1DM by measuring the circulating levels of I-FABP and PAF. Participants were recruited from the Endocrinology Unit of Al-Batoul Teaching Hospital, Diyala Governorate, Iraq, and laboratory analyses were performed at the Department of Chemistry and Biochemistry, College of Medicine, University of Babylon, Iraq.

A total of 90 participants were initially assessed for their eligibility. After excluding ineligible cases and removing incomplete datasets, 86 participants were included in the final analysis. The final study population comprised 43 children with confirmed T1DM and 43 apparently healthy controls matched for age and sex.

The sample size of 43 participants per group was determined based on feasibility constraints at a single center during the study period. No formal a priori power calculation was performed. However, a post-hoc analysis indicated that with 43 subjects per group, the study had approximately 80% power to detect a medium effect size (Cohen's d = 0.6) at a two-sided significance level of 0.05 for the primary comparison of I-FABP levels between groups. The absence of a prospective sample-size calculation is acknowledged as a limitation.

Participants

Children in the T1DM group were eligible if they were between 5 and 17 years of age, had a confirmed diag-

nosis of T1DM, and were receiving insulin. Healthy controls were recruited from the same community and matched with patients by age and sex.

Participants were excluded if they had recent diabetic ketoacidosis, acute infection, or chronic gastrointestinal disease (Figure 1). Additional exclusion criteria that should be reported, if applicable, include other autoimmune diseases, chronic inflammatory disorders, recent use of antibiotics, probiotics, corticosteroids, or immunosuppressive medications, and any condition known to affect intestinal permeability or inflammatory biomarkers.

Clinical and demographic data collection

Demographic and clinical data were recorded for each participant using a structured data collection form. These variables included age, sex, body mass index (BMI), BMI percentile, BMI Z-score, random blood sugar (RBS), glycated hemoglobin (HbA1c), and, for the patient group, disease duration, daily insulin dose (DID), and history of DKA episodes. The number of DKA episodes was categorized as 0, 1, or ≥ 2 episodes. Body weight was measured to the nearest 0.1 kg using a digital weighing scale (Seca 803, Seca GmbH, Hamburg, Germany), with participants wearing light clothing and no shoes. The scale was checked for zeroing before each measurement and calibrated regularly using standard weights. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (Seca 216, Seca GmbH, Hamburg, Germany), with participants standing barefoot, their heels together, and their heads positioned in the Frankfort horizontal plane. The mean of two consecutive readings was recorded. Body mass index (BMI; kg/m²) was calculated by dividing body weight in kilograms by height in meters squared.

BMI Z-scores and percentiles were derived according to age- and sex-specific CDC 2000 growth charts using a digital calculator. A BMI < 5th percentile was considered underweight, a BMI from the 85th to < 95th percentile was considered overweight, and a BMI ≥ 95th percentile was considered obese.

Blood sampling and serum preparation

Venous blood samples were obtained from each participant under sterile conditions. A total of 4 mL of venous blood was collected in serum separator gel tubes for biochemical and immunoassay measurements, and an additional sample was collected in an EDTA tube for HbA1c analysis. After clot formation, serum samples were centrifuged at 2,000 × g for 15 minutes, separated promptly, and stored at -20 °C until analysis.

Biochemical and immunological assays

Measurement of I-FABP

Serum I-FABP concentrations were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Bioassay Technology Laboratory, China) according to the manufacturer's instructions. The human fatty acid-binding protein, intestinal (FABP2) ELISA Kit has catalog number E1541Hu. The assay detection range (standard curve range) was 0.5 - 150 ng/mL, and the lower limit of detection (sensitivity) was 0.29 ng/mL. The intra-assay coefficient of variation (CV) was ≤ 8%, based on three samples of known concentrations tested on one plate. The inter-assay CV was ≤ 10% from three samples tested in separate assays.

Measurement of PAF

Serum PAF levels were measured using a commercial ELISA kit (Bioassay Technology Laboratory, China) according to the manufacturer's instructions. The human platelet activating factor (PAF) ELISA kit used in this study had the catalog number E1156Hu. The analytical sensitivity (minimum detectable concentration) of this assay was 1.17 ng/mL. The assay had a detection range of 2 - 600 ng/mL. According to the manufacturer, the intra-assay and inter-assay coefficients of variation (CVs) were < 8% and <10 %, respectively.

Measurement of HbA1c and glucose

HbA1c was measured using an automated clinical chemistry analyzer (Biorex Diagnostics, United Kingdom) with the corresponding reagent kit, according to the manufacturer's instructions. Serum glucose levels were measured using the same analyzer with a glucose reagent kit based on the enzymatic colorimetric method.

Measures to address potential sources of bias

Several measures were implemented to minimize potential sources of bias. Selection bias was reduced by enrolling consecutive eligible patients from the endocrinology clinic and matching controls by age and sex from the same community. Measurement bias was limited by analyzing all samples in a single batch using the same ELISA kits and by blinding laboratory personnel to group allocation during biomarker measurement. Although fasting status could not be standardized for all participants (particularly younger children), all blood samples were collected during morning clinic visits to reduce diurnal variability. The non-fasting status of some participants is acknowledged as a potential source of biological variability.

Ethical considerations

This study was conducted in accordance with the principles of the Declaration of Helsinki and applicable national guidelines for biomedical research involving human participants. Written informed consent was obtained from the parents or legal guardians of all the children before enrollment. Ethical approval was granted by the Ethics Committee of the College of Medicine, University of Babylon (Approval No. 5-76).

Statistical analysis

Data were analyzed using IBM SPSS Statistics version 25.0. The normality of the continuous variables was assessed using the Shapiro-Wilk test. Normally distributed variables are expressed as mean ± standard deviation

Table 1. Demographic, anthropometric, and biochemical characteristics of children with T1DM and healthy controls.

Variable	Group	Value	p-value
Age (years)	patient	11.16 ± 2.88	0.561
	control	10.74 ± 3.72	
BMI percentile (%)	patient	67.30 (33.25 - 91.65)	0.543
	control	82.00 (44.25 - 91.45)	
BMI Z score	patient	0.45 (-0.44 - 1.38)	0.540
	control	0.91 (-0.15 - 1.37)	
RBS (mg/dL)	patient	206 (148 - 291)	< 0.001
	control	101 (91 - 113.2)	
HbA1c (%)	patient	8.1 (7.4 - 9.3)	< 0.001
	control	4.9 (4.8 - 5.1)	
I-FABP (ng/mL)	patient	16.8 (12.5 - 21.4)	0.026
	control	12.4 (6.85 - 21.98)	
PAF (ng/mL)	patient	58.70 (45.10 - 73.60)	0.389
	control	53.50 (34.20 - 79.50)	

Table 2. Diagnostic performance of I-FABP and PAF for distinguishing children with T1DM from healthy controls.

Variable	Cutoff	AUC (95% CI)	SE	Sensitivity	Specificity	p-value
IFABP (ng/mL)	10.7	0.641 (0.518 - 0.764)	0.063	86%	46.3%	0.026
PAF (ng/mL)	35.4	0.555 (0.428 - 0.682)	0.065	92.9%	26.8%	0.386

Table 3. Comparison of serum I-FABP and PAF levels according to disease duration in children with T1DM.

Variable	Disease duration	n	Median (interquartile ranges)	p-value
IFABP (ng/mL)	group I (< 3years)	25	19.9 (16.8 - 23.0)	< 0.001
	group II (≥ 3 years)	18	12.6 (10.3 - 14.2)	
PAF (ng/mL)	group I (< 3years)	25	71.5 (59.6 - 85.8)	< 0.001
	group II (≥ 3 years)	18	45.1 (37.3 - 48.8)	

Table 4. Comparison of serum I-FABP and PAF levels according to the frequency of diabetic ketoacidosis episodes in children with T1DM.

Variable	DKA episodes	No.	Median (IQR)	p-value
IFABP (ng/mL)	0	10	11.90 (10.10 - 15.20)	0.002
	1	11	17.20 (12.30 - 20.95)	
	≥ 2	22	19.15 (14.20 - 26.10)	
PAF (ng/mL)	0	10	39.90 (36.80 - 49.70)	0.023
	1	11	48.80 (44.65 - 67.81)	
	≥ 2	22	71.50 (57.70 - 85.80)	

Table 5. Spearman's correlations of I-FABP and PAF with clinical and metabolic variables in children with T1DM.

Variable	Age	BMI	IFABP	PAF	HbA1c	RBS	Disease duration	DKA episodes	DID
IFABP (ng/mL)									
R	-0.38	0.17	-	0.794	0.552	0.280	-0.651	0.410	-0.230
95% CI	-0.61 to -0.09	-0.14 to 0.45	-	0.65 to 0.88	0.30 to 0.73	-0.03 to 0.54	-0.80 to -0.43	0.12 to 0.63	-0.50 to 0.08
Sig.	0.012	0.288	-	0.000	0.000	0.069	0.000	0.006	0.137
PAF (ng/mL)									
R	-0.31	-0.25	0.794	-	0.608	0.342	-0.681	0.514	-0.167
95% CI	-0.56 to 0.00	-0.52 to 0.06	0.65 to 0.88	-	0.37 to 0.77	0.05 to 0.58	-0.82 to -0.48	0.25 to 0.70	-0.45 to 0.14
Sig.	0.042	0.101	0.000	-	0.000	0.027	0.000	0.001	0.290

(SD), whereas non-normally distributed variables are presented as median and interquartile range (IQR). Comparisons between two independent groups were performed using the independent-samples *t*-test for normally distributed variables and the Mann-Whitney U test for non-normally distributed variables. Comparisons across more than two groups were conducted using the Kruskal-Wallis test. The correlations between continuous variables were assessed using Spearman's rank correlation coefficient. Receiver operating characteristic (ROC) curve analysis was used to evaluate the discriminatory performances of I-FABP and PAF.

Linear regression analyses were performed to identify independent predictors of I-FABP and PAF levels. Three separate univariable regression models were constructed for each biomarker, with HbA1c, glucose, and disease duration entered individually as predictors. Regression coefficients (B) are reported with standard errors and 95% confidence intervals. The primary prespecified analyses included the between-group comparison of I-FABP and PAF levels and their correlations with HbA1c, DKA frequency, and disease duration. Subgroup analyses by disease duration category and DKA episode frequency were considered exploratory and are presented without adjustment for multiplicity. Missing data were minimal (< 5% for any variable); cases with missing biomarker values were excluded from the respective analyses. Matching of cases and controls by age and sex was accounted for in the study design; no additional statistical adjustment for matching was applied in the primary analysis. Statistical significance was set at $p < 0.05$.

RESULTS

Table 1 presents the demographic, anthropometric, and biochemical characteristics of the children with T1DM and the healthy controls. The two groups were comparable in terms of age, BMI percentile, and BMI Z score, with no statistically significant differences, indicating appropriate matching between patients and controls. In contrast, children with T1DM had significantly higher random blood sugar (RBS) and HbA1c levels than controls (both $p < 0.001$), reflecting a poorer glycemic status. Serum I-FABP levels were significantly elevated in the T1DM group ($p = 0.026$). However, although PAF levels were numerically higher in patients than in controls, the difference did not reach statistical significance ($p = 0.389$).

Table 2 summarizes the ROC curve analysis evaluating the discriminatory ability of I-FABP and PAF between children with T1DM and healthy controls. I-FABP showed modest but statistically significant diagnostic performance, with an area under the receiver operating characteristic curve (AUC) of 0.641 (95% confidence interval [CI]: 0.518 - 0.764; $p = 0.026$). At a cutoff value of 10.7 ng/mL, I-FABP achieved a sensitivity of 86.0% and specificity of 46.3%, indicating good sensitivity but limited specificity. In comparison, PAF demonstrated poor discriminatory capacity, with an AUC of 0.555 (95% CI: 0.428 - 0.682), which was not statistically significant ($p = 0.386$). Although PAF showed high sensitivity (92.9%) at the selected cutoff value of 35.4 ng/mL, its specificity was low (26.8%), limiting its clinical utility as a standalone diagnostic marker.

Table 3 compares the serum I-FABP and PAF concentrations in children with T1DM according to disease duration. Patients with a disease duration of less than 3 years had significantly higher median I-FABP levels than those with a duration of ≥ 3 years (19.9 [16.8 - 23.0] vs. 12.6 [10.3 - 14.2] ng/mL, $p < 0.001$). Likewise, PAF levels were significantly higher in the shorter-duration group than in the longer-duration group (71.5 [59.6 - 85.8] vs. 45.1 [37.3 - 48.8] ng/mL, $p < 0.001$).

Table 4 shows the relationship between biomarker levels and the number of previous DKA episodes in children with T1DM. Serum I-FABP levels increased significantly across DKA categories ($p = 0.002$), with the lowest median levels observed in patients without prior DKA, intermediate levels in those with one episode,

Table 6. Univariable linear regression analysis of HbA1c, random blood glucose, and disease duration in relation to serum I-FABP levels in children with T1DM.

Model		B	Std. error	95% CI	Sig.
1	HbA1c	2.111	0.558	1.002 - 3.221	0.0001
2	glucose	0.052	0.015	0.021 - 0.083	0.001
3	disease duration	-3.062	0.067	-5.216 to -0.908	0.006

Table 7. Univariable linear regression analysis of HbA1c, random blood glucose, and disease duration in relation to serum PAF in children with T1DM.

Model		B	Std. error	95% CI	Sig.
1	HbA1c	4.443	1.874	0.714 - 8.172	0.020
2	glucose	0.107	0.051	0.005 - 0.208	0.039
3	disease duration	-11.814	2.976	-17.8 to -5.8	0.000

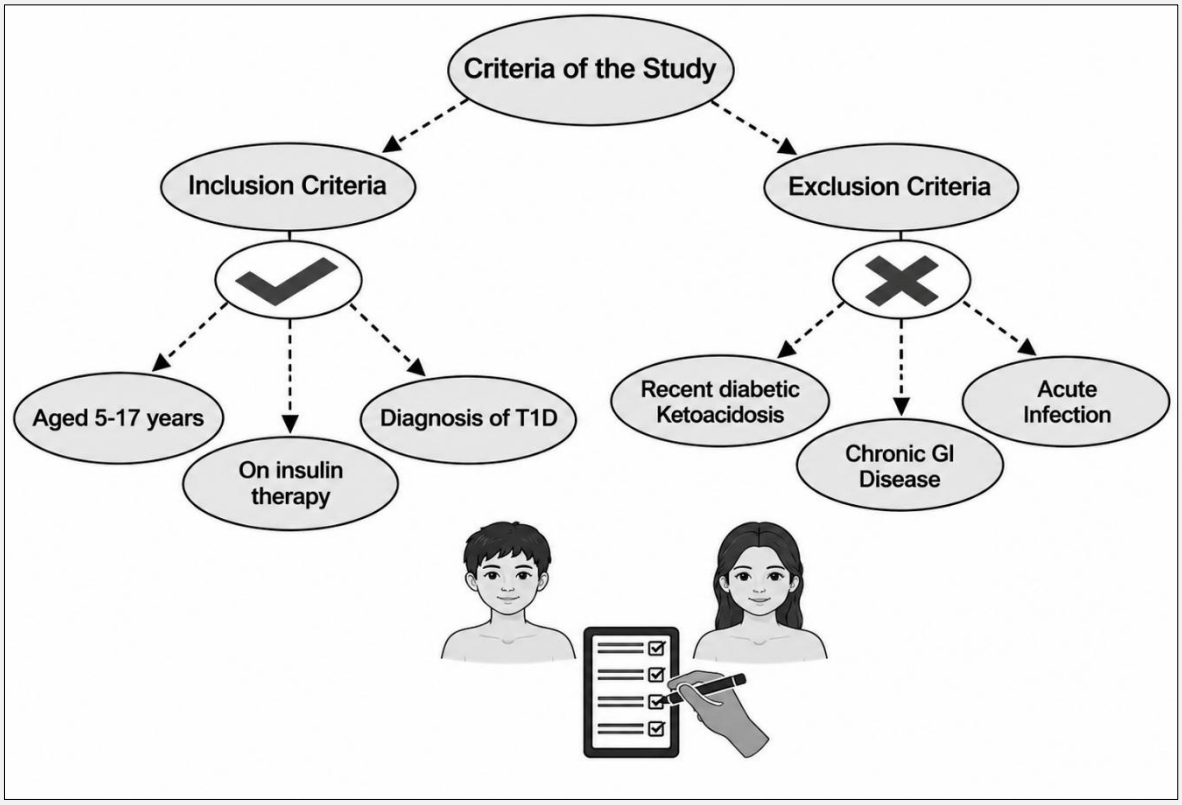


Figure 1. Inclusion and exclusion criteria.

and the highest levels in those with two or more episodes (≥ 2). A similar trend was observed for PAF, which also increased significantly with a greater DKA burden ($p = 0.023$).

Table 5 presents the correlations between I-FABP and PAF and the selected clinical and biochemical variables in the T1DM group. I-FABP showed a strong positive correlation with PAF ($r = 0.794$, $p < 0.001$) and a moderate positive correlation with HbA1c ($r = 0.552$, $p < 0.001$), while its association with random blood glucose was weak and not statistically significant. In addition, I-FABP levels were negatively correlated with disease duration ($r = -0.651$, $p < 0.001$) and positively correlated with the frequency of DKA episodes ($r = 0.410$, $p = 0.006$). Similarly, PAF was positively correlated with HbA1c, random blood glucose, and DKA frequency but negatively correlated with disease duration.

Tables 6 and 7 present the univariable linear regression analyses for predictors of serum I-FABP and PAF levels, respectively. Each predictor (HbA1c, glucose, and disease duration) was entered in a separate univariable model. For I-FABP (Table 6): HbA1c ($B = 2.111$, 95% CI: 1.002 - 3.221, $p < 0.001$), glucose ($B = 0.052$, 95% CI: 0.021 - 0.083, $p = 0.001$), and disease duration ($B = -3.062$, 95% CI: -5.216 to -0.908, $p = 0.006$). For PAF (Table 7): HbA1c ($B = 4.443$, 95% CI: 0.714 - 8.172, $p = 0.020$), glucose ($B = 0.107$, 95% CI: 0.005 - 0.208, $p = 0.039$), and disease duration ($B = -11.814$, 95% CI: -17.8 to -5.8, $p < 0.001$).

DISCUSSION

The present study investigated the circulating levels of I-FABP and PAF in children with T1DM and explored their associations with glycemic control, DKA burden, and disease duration. The principal findings were that children with T1DM had significantly higher circulating I-FABP concentrations than healthy controls, whereas the difference in PAF was not statistically significant. This pattern supports the interpretation that pediatric T1DM is associated with subclinical intestinal epithelial injury, whereas the inflammatory signal represented by PAF may be more variable and dependent on disease activity, metabolic stress, or timing of sampling.

The elevation of I-FABP levels observed in our cohort is scientifically plausible and consistent with the current literature. I-FABP is released from mature enterocytes when epithelial integrity is disrupted and is therefore considered a marker of enterocyte damage rather than a disease-specific marker of any single gastrointestinal disorder [17]. In pediatric patients, Ochocińska et al. reported significantly higher I-FABP concentrations in children with T1DM than in healthy controls, indicating that epithelial barrier injury may already be present in this population, even in the absence of overt intestinal symptoms [18]. Importantly, that study also showed that although I-FABP reflects intestinal epithelial damage, it is not sufficiently specific to function as an early marker of celiac disease in the T1DM population [18]. Accordingly, the increase in I-FABP levels in our study should be interpreted primarily as evidence of intestinal epithelial injury and barrier disturbance, rather than as a direct and exclusive test of intestinal permeability.

Our findings are also supported by broader evidence showing that intestinal barrier dysfunction is a relevant component of T1DM pathophysiology. A comprehensive review published in 2021 concluded that intestinal permeability is generally increased in T1DM across several human and experimental methods, although the authors emphasized that blood-based molecular markers should be interpreted cautiously and in context [17]. This is an important distinction for interpreting our results. Although elevated I-FABP levels strongly support epithelial injury, they do not define the degree of permeability impairment. Nevertheless, when combined with current mechanistic knowledge and pediatric clinical data, our results reinforce the concept that the intestinal barrier is compromised in children with T1DM [17,19].

Recent studies have further strengthened this interpretation by linking gut barrier abnormalities with microbial dysbiosis and metabolic dysfunction. In a prospective cohort study, Harbison et al. demonstrated that children with multiple islet autoantibodies or recent-onset T1DM had higher small intestinal permeability, reduced microbial diversity, and lower abundance of short-chain fatty acid-producing bacteria [20]. Particularly relevant was the finding that children who progressed from islet autoimmunity to overt diabetes had more pronounced permeability abnormalities than non-progressors, suggesting that barrier dysfunction may arise early in the disease process rather than merely reflecting late metabolic consequences [21]. This is important for the present study because it supports the possibility that the elevated I-FABP levels detected in our patients reflect not only the effect of chronic hyperglycemia but also a more fundamental component of autoimmune disease development.

The mechanistic explanation for these observations has become clearer in recent pediatric microbiome studies. Yuan et al. demonstrated that children with new-onset T1DM show reduced microbial richness and diversity, decreased pathways involved in butyrate production, and increased microbial genes associated with lipopolysaccharide biosynthesis [6]. These findings are biologically meaningful because butyrate is a key metabolite for epithelial health and tight junction support, whereas increased lipopolysaccharide-related signaling may promote mucosal inflammation and systemic immune activation [6]. In addition, a systematic review concluded that pediatric T1DM is consistently associated with reduced microbial diversity, depletion of beneficial short-chain fatty acid-producing bacteria, and increased markers of intestinal permeability, even though heterogeneity remains in the specific microbial taxa reported by different studies [5]. Taken together, these studies provide a stronger mechanistic context for our results and sup-

port the view that intestinal epithelial damage in pediatric T1DM may occur within a broader environment of dysbiosis, impaired protective microbial metabolism, and inflammation.

Another important finding of our study was the significant positive association between both I-FABP and PAF with HbA1c, along with their association with the burden of diabetic ketoacidosis. These relationships suggest that poor glycemic control and recurrent metabolic decompensation may aggravate epithelial injury and inflammatory activation. Chronic hyperglycemia increases oxidative stress, disturbs tight junction regulation, and amplifies low-grade inflammation, thereby creating conditions that may promote intestinal barrier dysfunction [17]. From this perspective, I-FABP may reflect not only autoimmune-related mucosal damage but also the cumulative burden of prolonged glycemic instability. The association with DKA episodes in our cohort further supports the notion that intestinal injury may worsen during periods of acute metabolic stress.

In contrast to I-FABP, the interpretation of PAF in pediatric T1DM should remain cautious. PAF is a potent phospholipid mediator involved in leukocyte activation, endothelial permeability, and amplification of inflammatory responses. Recent reviews continue to confirm its broad importance in inflammatory disease biology [16]. However, direct contemporary evidence regarding its exact role in pediatric T1DM is limited. Although earlier reports suggested increased circulating PAF, especially near disease onset, recent pediatric evidence indicates a more complex and less stable pattern. In the 2023 study by Ochocińska et al., both I-FABP and PAF levels differed between children with T1DM and healthy controls; however, the effect of disease duration appeared clearer for PAF than for I-FABP, and the direction of the duration-related change was not fully consistent with the pattern observed in our cohort [22]. Therefore, our finding that the PAF was higher in children with shorter disease duration and greater DKA burden should be interpreted as a potentially important but still hypothesis-generating observation that requires confirmation in larger longitudinal studies.

The inverse association between disease duration and I-FABP levels observed in our patients is of particular interest. One possible explanation is that intestinal epithelial injury is more pronounced during the early years after diagnosis, when autoimmune activity, glycemic instability, and inflammatory stress may be the greatest. This interpretation is partly consistent with prospective pediatric data showing that barrier dysfunction can be detected before or around the time of diabetes onset [20]. However, because larger pediatric studies have not consistently demonstrated a duration effect for I-FABP, this aspect of our findings should be discussed carefully [22]. It is possible that I-FABP behaves as an early injury marker that declines over time because of metabolic stabilization, treatment effects, mucosal adaptation, or reduced acute epithelial shedding; however, these possibilities remain speculative.

The observed relationship between biomarker levels and DKA history warrants further investigation. Recurrent DKA is accompanied by marked metabolic stress, dehydration, oxidative injury, and systemic inflammation, all of which may worsen intestinal epithelial integrity and increase the release of injury-related and inflammatory mediators into circulation. The progressive increase in both I-FABP and PAF across DKA categories in our cohort supports the idea that intestinal barrier dysfunction in T1DM is dynamic and may intensify during episodes of metabolic decompensation. This interpretation is compatible with the broader concept that gut dysfunction in T1DM is not a static phenomenon but rather one that may fluctuate in relation to disease activity and glycemic burden [17,20].

Overall, the present findings support a model in which pediatric T1DM is associated with intestinal epithelial injury, likely occurring within a wider network of dysbiosis, reduced short-chain fatty acid production, increased inflammatory signaling, and poor metabolic control. Among the two biomarkers examined, I-FABP appears to have a stronger and more biologically coherent relationship with gut barrier injury. However, the modest diagnostic performance of I-FABP (AUC = 0.641; specificity = 46.3%) indicates that it should not be considered a definitive diagnostic tool in its current form but rather a potential research biomarker that requires further validation. In contrast, PAF may still be relevant as an inflammatory mediator, but its role in pediatric T1D appears less stable and requires further clarification through larger longitudinal studies that combine serum biomarkers with direct intestinal permeability testing and microbiome analysis.

Study limitations

This study had several limitations. First, it was conducted at a single center with a relatively small sample size (n = 43 per group) without a formal a priori power calculation, which may limit the generalizability of the findings to the broader pediatric population with T1DM. Second, the cross-sectional design restricts the ability to determine causality or temporal sequence; therefore, it cannot establish whether intestinal barrier injury precedes worsening glycemic control or develops as a consequence. Third, intestinal barrier dysfunction was inferred indirectly from circulating biomarkers because direct functional tests, such as lactulose-mannitol or lactulose-rhamnose permeability assays, were not performed. Fourth, no stool microbiome analysis, endoscopic evaluation, or histopathological assessment of the intestinal mucosa was included, which limits the mechanistic interpretation of the observed biomarker changes. Fifth, fasting blood sampling could not always be standardized because many participants were young children who were apprehensive about prolonged fasting, which may have introduced additional biological variability. Sixth, the modest discriminatory performance of I-FABP (AUC = 0.641) and the exploratory nature of the subgroup analyses limit the strength of di-

agnostic conclusions that can be drawn from this study. Finally, because recent literature shows some inconsistency regarding the behavior of inflammatory markers such as PAF across disease durations, the present findings should be interpreted cautiously until they are confirmed in larger, multicenter, longitudinal studies.

CONCLUSION

In conclusion, the present study provides evidence that children with T1DM exhibit signs of subclinical intestinal barrier injury, as reflected by the significant elevation of circulating I-FABP levels. The positive associations of I-FABP and PAF with HbA1c and diabetic ketoacidosis burden further suggest that poor glycemic control and recurrent metabolic stress may contribute to intestinal epithelial damage and inflammation. Although PAF did not differ significantly between patients and controls, its correlations with glycemic indices and acute complications indicate that it may still participate in inflammatory responses during disease instability. These findings suggest that I-FABP warrants further investigation as a potential noninvasive marker of intestinal epithelial injury in pediatric T1DM; however, its modest diagnostic accuracy precludes definitive clinical recommendations at this stage. Future longitudinal studies integrating serum biomarkers, direct permeability testing, and gut microbiome profiling are needed to clarify the temporal and mechanistic relationships between intestinal dysfunction and the course of T1DM.

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Ethical Considerations:
The study was conducted in accordance with the principles of the Declaration of Helsinki and the applicable national guidelines for biomedical research involving human participants. Written informed consent was obtained from the parents or legal guardians of all children before enrollment. Ethical approval was granted by the Ethics Committee of the College of Medicine, University of Babylon (Approval No. 5-76).

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The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable requests.

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Artificial intelligence tools were used during the preparation of this manuscript to improve sentence structure and clarity. The authors reviewed and edited all AI-assisted output and take full responsibility for the content of the published article.

Declaration of Interest:
The authors declare no conflicts of interest.

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