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Exploring the interplay of *MTHFR* and *FGG* polymorphisms with serum levels of adiponectin and leptin in pediatric lupus nephritis: a pilot study

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Abstract

Background Adiponectin and leptin are pivotal in the regulation of metabolism. Pediatric lupus nephritis (pLN), a manifestation of childhood systemic lupus erythematosus (SLE) affecting the kidneys, is associated with impaired adipokine levels, suggesting a role in pLN pathogenesis. The aim of this study was to explore the potential relationship between specific single-nucleotide polymorphisms (SNPs)—methylenetetrahydrofolate reductase (MTHFR) rs1801131 and fibrinogen gamma chain (FGG) rs2066865—and the serum levels of leptin and adiponectin in patients with pLN.

Methods Ninety-eight pLN patients and one hundred controls were enrolled in the study. Serum leptin and adiponectin levels were measured using ELISA. DNA extraction and real-time PCR genotyping were performed for *MTHFR* rs1801131 and *FGG* rs2066865 SNPs.

Results Compared to healthy controls, pLN patients exhibited significantly greater serum leptin (11.3 vs. 18.2 ng/mL, $p < 0.001$) and adiponectin (18.2 vs. 2.7 ug/mL, $p < 0.001$). Adiponectin levels were positively correlated with proteinuria ($p < 0.05$), while leptin levels positively correlated with proteinuria, SLE disease activity index-2000 (SLEDAI-2K), and cyclophosphamide usage (all $p < 0.05$). There was no significant association between *MTHFR* rs1801131 or *FGG* rs2066865 SNPs and pLN in either codominant or allelic models (all $p > 0.05$). However, the AG genotype of *FGG* gene rs2066865 SNP was significantly associated with high leptin levels (> 15 ng/mL) ($p = 0.01$).

Conclusion Serum adiponectin and leptin levels are associated with pathological manifestations of pLN. High leptin levels are associated with the AG genotype of *FGG* rs2066865 SNP in pLN patients, suggesting direct involvement in disease progression and potential utility as a disease biomarker.

Keywords Pediatric lupus nephritis, Adiponectin, Leptin, MTHFR, FGG, Polymorphism

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Introduction

Systemic lupus erythematosus (SLE) is a chronic, severe, and complex autoimmune disorder characterized by the loss of self-antigen tolerance, autoantibody production, immune complex deposition, and consequential end-organ damage [1]. Pediatric SLE (pSLE) shares similarities in clinical manifestations with adult-onset SLE; however, its earlier onset is concomitant with severe multiorgan involvement, particularly pediatric lupus nephritis (pLN), which manifests in up to 80% of cases. In Colombia, LN affects 75% of SLE pediatric patients [2].

Kidney disease is well established as a risk factor for atherosclerosis in the general population [3]. Studies have shown that increased levels of serum creatinine and the presence of proteinuria are strongly associated with cardiovascular events and mortality in LN [4–6]. As children and adolescents with SLE age, they face a lifelong burden of exposure to multisystem inflammatory disease with high atherogenic potential, placing them at particular risk [7].

Abnormal levels of adipokines and single-nucleotide polymorphisms (SNPs) have been implicated in the inflammatory processes observed in SLE, with potential roles in the modulation of innate and adaptive immunity [8–10]. Adiponectin and leptin are two adipokines involved in atherosclerosis. Adiponectin protects against atherogenesis, inflammation, and endothelial dysfunction via suppression of endothelial inflammatory reaction and stimulation of endothelial vasodilation [11]. In contrast, leptin induces endothelial dysfunction, stimulates the inflammatory response, and is pro-atherogenic, prothrombotic, and an angiogenic factor [8, 11].

Elevated homocysteine levels have been associated with premature atherosclerosis and thrombotic risk in both general and SLE populations [12]. Causes of hyperhomocysteinemia include renal impairment and genetic polymorphisms of the methylenetetrahydrofolate reductase (*MTHFR*) enzyme (c. 677C > T and c. 1298A > C) [13]. Moreover, the most common *MTHFR* gene polymorphism is the rs1801133 SNP, located in exon 4 on chromosome 1 in humans. This SNP has been correlated with an increased susceptibility to SLE [14].

The association between the *MTHFR* rs1801133 polymorphism and adipokine levels has been explored [15, 16]. Findings indicate that individuals with the TT or CT genotype of *MTHFR* rs1801133 display lower enzyme activity in comparison with those with the CC genotype. This lower activity is associated with dysregulated DNA methylation, impaired adipokine levels, and an elevated risk of cardiovascular disease [14–16].

Elevated plasma fibrinogen levels are linked to heightened platelet aggregation and plasma viscosity. Furthermore, there is an increased risk of deep vein thrombosis associated with the fibrinogen gamma chain (FGG) rs2066865 polymorphism in Hispano-Americans [17]. The rs2066865 SNP within the *FGG* gene is located in exon 3 on chromosome 4 in humans. [18]. Studies suggest a correlation between *FGG* gene polymorphism and elevated plasma fibrinogen levels, which may indirectly impact adipokines. This association is plausible given the involvement of fibrinogen in inflammatory processes and the increased rate of microvascular thrombosis. Adipokines are recognized to be linked with inflammatory processes, further connecting the genetic variations in the *FGG* gene with potential implications for inflammatory pathways and adipokine regulation [19, 20].

Given that children and adolescents with SLE present fewer cardiovascular risk factors and comorbidities than their adult counterparts, this population offers a unique opportunity to further understand the role of *MTHFR* and *FGG* polymorphisms with serum adipokines in the pathogenesis of atherosclerosis. This study aims to explore the potential association between the *MTHFR* rs1801131 and *FGG* rs2066865 SNPs and serum leptin and adiponectin levels in pLN patients.

Methods

Study population

We conducted a case–control analysis that involved patients diagnosed with pLN ($n = 98$) and healthy controls ($n = 100$). The study participants were selected through simple random sampling from the Nephrology Department of a tertiary hospital in Barranquilla, Colombia, located on the northern Caribbean coast. The diagnosis of SLE was confirmed according to the American College of Rheumatology (ACR) diagnostic criteria [21]. Renal diagnoses were established following the consensus of the Systemic Autoimmune Diseases Group (GEAS) of the Spanish Society of Nephrology (SEN) [22]. The control group consisted of children attending the hospital for clinical reasons other than autoimmune or renal diseases, selected from the same medical facility where patients with pLN were enrolled. Exclusion criteria encompassed patients with polyautoimmunity or end-stage kidney disease (ESKD). All participants were unrelated by familial ties.

DNA extraction and SNP genotyping

The DNA extraction process involved a modified salting-out method, and the extracted DNA was preserved in TE buffer at − 20 °C until analysis. The purity and concentration of each DNA sample were assessed using spectrophotometry with a NanoDrop 2000 from Thermo Scientific. Real-time PCR (RT-PCR) was employed for genotyping of *MTHFR* rs1801131 and *FGG* rs2066865 single-nucleotide polymorphisms (SNPs). Commercial

TaqMan® SNP genotyping kits obtained from Applied Biosystems were employed in a qPCR 7500 instrument from the same manufacturer. Each reaction was prepared with a total volume of 20 µL, comprised of 6 µL of DNA (~ 27 ng/µL), 10 µL of Master Mix-2x, 0.5 µL of TaqMan genotyping-40 × probes specific to each SNP, and 3.5 µL of deionized water. The cycling program initiated with a 10-min step at 95 °C, followed by 40 cycles involving 15 s at 92 °C and 1 min at 60 °C per cycle. Automatic genotyping assignment was carried out using the allelic discrimination application provided by Applied Biosystems, with an amplification quality threshold set at ≥ 90% per sample.

Quantification of serum leptin and adiponectin levels

Serum levels of leptin and adiponectin were quantified using the Millipore ELISA kit. The reference range for serum leptin levels in children, typically ranging from 1 to 15 ng/mL, was established by referring to the values reported by Simon E. et al. [23]. Simultaneously, the reference range for adiponectin concentrations in children, generally falling within the range of 5–30 µg/mL, was determined based on the findings reported by Chandran M. et al [24].

Statistical analysis

The normality of the data was evaluated using the Kolmogorov–Smirnov test. Descriptive statistics for serum concentrations of leptin and adiponectin were presented as median and interquartile range. Gender and categorical variables of leptin and adiponectin, based on reference parameters, were summarized using frequency tables. To compare categorized concentrations of leptin and adiponectin between study groups, the Pearson's Chi-squared test or Fisher's exact test was applied using SPSS v24 statistical software (IBM® SPSS® Statistics 24, IBM Corp., USA). Spearman's rho correlation coefficients (rs) were employed to assess the correlation between adiponectin and leptin levels and disease-specific characteristics (disease duration, proteinuria, Systemic Lupus Erythematosus Disease Activity Index 2000—SLEDAI-2K, cyclophosphamide—CYP, and mycophenolate—MMF). Allelic and genotypic frequencies were estimated using Arlequin v3.5 software, and the Hardy–Weinberg genetic equilibrium was assessed. Association analysis at the allele and genotype levels was performed using logistic regression models, with odds ratios estimated and 95% confidence intervals adjusted for gender in SPSS v24 software. The Kruskal–Wallis test, with Bonferroni corrections for multiple comparisons, was used to compare serum concentrations of leptin and adiponectin between *MTHFR* rs1801131 and *FGG* rs2066865 SNPs. Statistical significance was considered at $p < 0.05$.

Results

Characteristics of the study sample

General characteristics of patients and controls are shown in Table 1. The patient and control groups were matched by age (mean age of pLN patients: 14.1 ± 2.4; mean age of controls: 13.5 ± 1.7 years).

Leptin and adiponectin serum levels

Mean serum levels of leptin were significantly higher in patients with pLN than in controls (11.3 ng/mL ± 12.1; vs. 18.2 ng/mL ± 18.5, $p < 0.001$). Similarly, mean adiponectin levels were higher in pLN compared to controls (18.2 ug/mL ± 18.5; vs. 2.7 ug/mL ± 3.2, $p < 0.001$) (Table 1) (Fig. 1). In 70.1% of pLN patients and 90% of the control group, normal levels of leptin (1–15 ng/mL) were observed. Notably, pLN patients demonstrated a significantly higher frequency of elevated adiponectin levels (> 30 µg/mL) and elevated leptin levels (> 15 ng/mL) compared to the control group (28.9% vs. 0%; 24.7% vs. 0%; $p < 0.05$). No significant association was found between serum leptin and adiponectin levels ($p = 0.185$) (Table 2).

Correlation between serum Adipokines and disease-specific characteristics

Table 3 presents the correlation between serum levels of adiponectin and leptin and disease-specific characteristics in pLN patients. Leptin levels showed a strong

Table 1 Characteristics of pLN patients and controls

Parameter	pLN ($n = 98$)[1]	Control ($n = 100$)[1]	*p*-value
Age, years	14.1 ± 2.4	13.8 ± 1.7	0.06[2]
Gender			0.001[3]
Female	78 (79%)	58 (58%)	
Male	20 (21%)	42 (42%)	
Disease duration, years	3.4 ± 4.5		
SLEDAI-2K	15.6 ± 9.9		
Proteinuria (g/day)	2.8 ± 3.5		
Medication usage			
Steroids	55 (56%)		
MMF	28 (28%)		
CYP	62 (63%)		
Antimalarials	76 (77%)		
Biologic	7 (7%)		
Serum adipokines			
Leptin (ng/mL)	11.3 ± 12.1	2.5 ± 2.8	< 0.001[2]
Adiponectin (ug/mL)	18.2 ± 18.5	2.7 ± 3.2	< 0.001[2]

[1] Mean ± SD; *n* (%)

[2] Welch two-sample *t*-test

[3] Pearson's Chi-squared test

SLEDAI-2K, Systemic Lupus Erythematosus Disease Activity Index; pLN, pediatric lupus nephritis; MMF, mycophenolate; CYP, cyclophosphamide

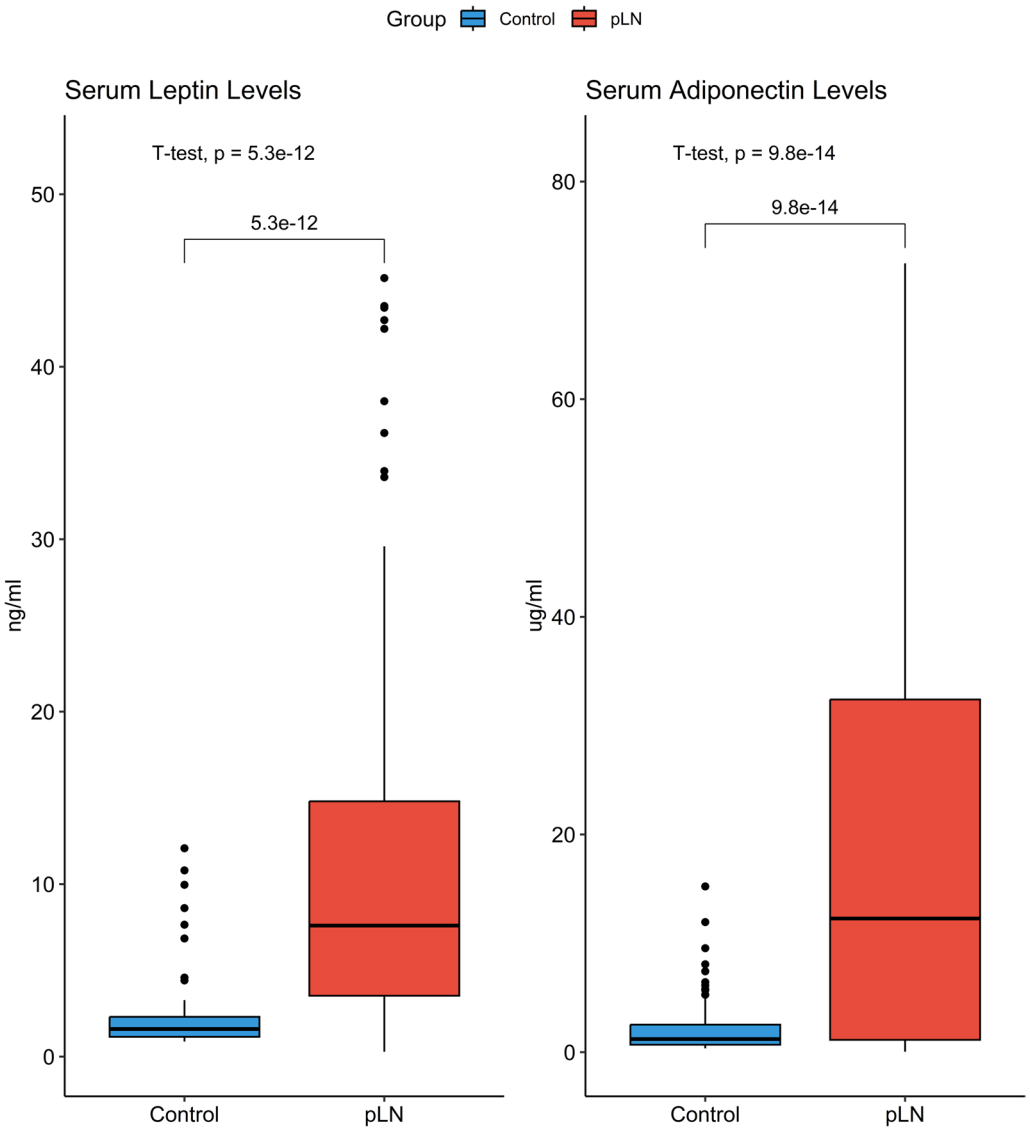

Fig. 1 Serum leptin and adiponectin levels in pediatric lupus nephritis patients and controls

Table 2 Correspondence analysis between serum levels of leptin and adiponectin in pediatric lupus nephritis patients

Leptin	Adiponectin				*p*-value
	Low	Normal	High	Total	
Low	1	1	3	5	
Normal	28	25	16	69	0.185[1]
High	11	4	9	24	
Total	40	30	28	98	

[1] Fisher's exact test

positive correlation with proteinuria (Spearman $r=0.76$, $p<0.05$), SLEDAI-2K (Spearman $r=0.78$, $p<0.05$) and the usage of immunosuppressive medications, including CYP (Spearman $r=0.66$, $p<0.05$). On the other hand, adiponectin exhibited a moderate positive correlation with proteinuria (Spearman $r=0.45$, $p<0.05$).

Genotypic frequency of *MTHFR* rs1801131 and *FGG* rs2066865 polymorphisms

The frequency of *MTHFR* rs1801131 and *FGG* rs2066865 genotypes is presented in Table 4. There was no significant association between *MTHFR* rs1801131 or *FGG* rs2066865 SNPs and pLN, neither in codominant nor in allelic models (all $p>0.05$). No significant

Table 3 Correlation between adipokine serum levels and disease-specific characteristics of pLN

Serum Adipokines	Mean SD	Spearman *r* correlation with disease duration	Spearman *r* correlation with proteinuria	Spearman *r* correlation with SLEDAI-2K	Spearman *r* correlation with CYP	Spearman *r* correlation with MMF
Adiponectin (ug/mL)	18.2 ± 18.5	−0.2	0.45*	0.59	0.35	0.45
Leptin (ng/mL)	11.3 ± 12.1	−0.15	0.76*	0.78*	0.66*	0.14

SLEDAI-2K, Systemic Lupus Erythematosus Disease Activity Index 2000; CYP, cyclophosphamide; MMF, mycophenolate; pLN, pediatric lupus nephritis

*$p < 0.05$

Table 4 Comparison of genotypic and allelic frequency of *MTHFR* and *FGG* polymorphisms in pediatric lupus nephritis patients and control group

	pLN ($n = 98$)	ω H-W	Control ($n = 100$)	ω H-W	*p-value	OR (95% CI)	σp-value
rs1801131 (MTHFR)							
GG, *n* (%)	6 (6)	0.12	13 (13)	0.00	0.35	0.6 (0.29–1.96)	0.26
GT, *n* (%)	22 (22)		16 (16)			1.5 (0.74–3.10)	0.44
TT, *n* (%)	70 (72)		71 (71)		0.98σ	1	
G, *n* (%)	34 (17)		36 (18)			0.9 (0.50–1.50)	0.72
T, *n* (%)	162 (83)		164 (82)			1	
rs2066865 (FGG)							
AA, *n* (%)	4 (4)	0.38	4 (4)	0.22	0.92	2.2 (0.62–12.1)	0.65
AG, *n* (%)	37 (38)		41 (41)			1.2 (0.62–1.81)	0.68
GG, *n* (%)	57 (58)		55 (55)		1σ	1	
A, *n* (%)	45 (23)		47 (23)			2.3 (0.85–1.98)	0.91
G, *n* (%)	151 (77)		153 (77)			1	

*: $p < 0.05$ Pearson's Chi-squared test

σ: Yates correction

ω, Hardy–Weinberg genetic equilibrium; OR, odds ratio; CI, confidence interval; OR [95%IC]; pLN, pediatric lupus nephritis; MTHFR, methylenetetrahydrofolate reductase; FGG, fibrinogen gamma chain. Logistic regression models were adjusted by gender

differences were observed when analyzing allelic and genotypic frequencies based on age (all $p > 0.05$) (Data not shown). Logistic regression models were adjusted by gender (Table 4).

Association of serum leptin/adiponectin and *MTHFR/FGG* polymorphisms

Serum levels of leptin and adiponectin were compared with *MTHFR* and *FGG* SNPs in patients with pLN and the control group, as detailed in Table 5. Following Bonferroni correction for multiple tests, the AG genotype of *FGG* rs2066865 SNP exhibited a significant association with elevated serum leptin levels (> 15 ng/mL) ($p = 0.01$). Likewise, the AG genotype of *FGG* rs2066865 was the only genotype displaying statistically significant differences in adiponectin levels between pLN patients and the control group ($p = 0.02$). However, these differences did not retain statistical significance after the Bonferroni correction ($p = 0.45$).

Discussion

To the best of our knowledge, this study represents the initial investigation of potential links between serum adipokines and *MTHFR* rs1801131/*FGG* rs2066865 SNPs in pLN patients from Colombia. Our findings revealed significantly elevated serum leptin and adiponectin levels in pLN patients. Furthermore, a significant positive correlation was observed between serum leptin levels and disease-specific characteristics of pLN. Additionally, the AG genotype of the *FGG* gene rs2066865 SNP was associated with elevated serum leptin levels. This finding is particularly noteworthy, given the previously established link between leptin and pro-inflammatory mechanisms in autoimmune diseases, such as rheumatoid arthritis [25].

Lupus nephritis has a significant prevalence in women, with a female-to-male ratio ranging between 8:1 and 15:1 [26]. In this study, 79% pLN patients were female with a female-to-male ratio of 8:2. The overall incidence rate of SLE is higher in women compared to men, with an incidence rate of 4.8 per 100,000 inhabitants for women as

Table 5 Comparison between genotypic frequency of *MTHFR/FGG* polymorphisms and leptin/adiponectin levels in pediatric lupus nephritis patients and control group

Leptin levels	**pLN (*n* = 98)**				**Control (*n* = 100)**			
	< 1 ng/mL	**1–15 ng/mL**	**> 15 ng/mL**	***p*-value**	**< 1 ng/mL**	**1–15 ng/mL**	**> 15 ng/mL**	**¥*p*-value**
rs1801131 (MTHFR)								
GG, *n* (%)	0 (0)	4 (5)	2 (8)	0.16	5 (35)	8 (9)	0 (0)	0.86
TT, *n* (%)	5 (100)	52 (76)	13 (54)		8 (58)	63 (74)	0 (0)	
GT, *n* (%)	0 (0)	13 (19)	9 (38)		1 (7)	15 (17)	0 (0)	
rs2066865 (FGG)								
AA, *n* (%)	1 (20)	3 (4)	0 (0)	0.10	0 (0)	4 (4)	0 (0)	0.01
AG, *n* (%)	3 (60)	24 (35)	10 (42)		0 (0)	41 (46)	0 (0)	
GG, *n* (%)	1 (20)	42 (61)	14 (58)		10 (100)	45 (50)	0 (0)	
Adiponectin levels	**pLN (*n* = 98)**				**Control (*n* = 100)**			
	< 5 µg/mL	**5–30 µg/mL**	**> 30 µg/mL**	***p*-value**	**< 5 µg/mL**	**5–30 µg/mL**	**> 30 µg/mL**	**¥*p*-value**
rs1801131 (MTHFR)								
GG, *n* (%)	2 (5)	1 (3)	3 (10)	0.85	9 (11)	4 (23)	0 (0)	0.32
TT, *n* (%)	29 (74)	23 (77)	18 (62)		61 (73)	10 (59)	0 (0)	
GT, *n* (%)	8 (21)	6 (20)	8 (28)		13 (16)	3 (18)	0 (0)	
rs2066865 (FGG)								
AA, *n* (%)	0 (0)	0 (0)	4 (14)	0.02	2 (2)	2 (10)	0 (0)	0.45
AG, *n* (%)	18 (46)	13 (43)	6 (21)		34 (42)	7 (37)	0 (0)	
GG, *n* (%)	21 (54)	17 (57)	19 (65)		45 (56)	10 (53)	0 (0)	

*: $p < 0.05$ Pearson's Chi-squared test

¥: Bonferroni correction

pLN, Pediatric lupus nephritis; MTHFR, methylenetetrahydrofolate reductase; FGG, fibrinogen gamma chain

opposed to 1.2 per 100,000 inhabitants for men. Additionally, up to 90% of patients with SLE are female, highlighting the gender disparity associated with this autoimmune disease [26, 27].

Adipose tissue is currently recognized as an important endocrine organ that produces various adipokines involved in immune and neuroendocrine functions. Adipokines such as leptin and adiponectin are responsible for pathological changes associated with cardiovascular disease [28, 29]. In addition, these adipokines could affect metabolism and play significant roles in inflammatory, autoimmune, and rheumatic diseases [9, 30]. There is a growing interest in the study of adipokines as immunomodulators of adipocyte-derived cytokines in SLE and LN patients [28].

Adiponectin has anti-inflammatory and vasoprotective properties. It can inhibit TNFα-induced adherence of monocytes to endothelial cells by attenuating surface expression of the adhesion molecules vascular cellular adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and E-selectin [31]. However, other studies indicate that adiponectin may have an inflammatory effect by inducing the production of cytokines and pro-inflammatory chemokines, including IL-8 and monocyte chemoattractant protein-1 (MCP-1) [28].

Multiple studies have reported an association between increased levels of adiponectin and other autoimmune diseases [28, 32, 33]. This observation may be related to the differing isoforms of adiponectin, with high molecular weight adiponectin having pro-inflammatory effects [34], while low molecular weight adiponectin does not appear to affect inflammatory cytokine production [11]. Our study revealed that pLN patients have significantly higher levels of adiponectin compared to the control group ($p < 0.001$), which is consistent with the findings of Hutchenson et al [35], who observed a 3.5-fold increase in serum adiponectin levels in LN patients ($p < 0.001$) compared to normal controls. Further studies are needed to elucidate the relationship between adiponectin isoforms and complex diseases such as pLN.

The involvement of adiponectin in the renal manifestations of glomerulonephritis has not been consistently established, as conflicting findings regarding adiponectin levels and proteinuria have been documented across various diseases [36, 37]. Notably, serum adiponectin levels show a significant increase in end-stage kidney disease, and despite a decline post-successful

kidney transplantation, they do not return to normal levels [32, 34]. In this study, a positive correlation was identified between adiponectin levels and proteinuria in pLN patients. Adiponectin has been documented to be present in the lining of glomerular capillaries and other endothelial surfaces in the kidney [34]. Rovin et al. [38] suggest that the kidney endothelium may have unique adiponectin binding properties and may modulate renal inflammation in SLE. The inflammatory environment might contribute to the release of adiponectin from endothelial surfaces [38].

Leptin modulates innate and adaptive immunity, enhancing monocyte cell proliferation and activation, while inhibiting natural killer cells on innate immunity, and increasing T and B-cells proliferation, as well as enhancing the production of pro-inflammatory cytokines on adaptive immunity [38]. Patients with SLE usually demonstrate elevated serum leptin, especially patients with associated LN [39]. This study found increased serum leptin levels in almost one-third of pLN patients, which is consistent with previous studies in adults with SLE [40, 41]. A meta-analysis comprising 1333 patients and 1048 controls found increased leptin levels among SLE patients irrespective of ethnicity [42]. However, some studies have not reported significant differences in leptin levels [40, 41]. Variations in sample size, patient demographics, clinical heterogeneity, and therapeutic effects could contribute to these disparities [41].

High levels of circulating leptin correlate with vascular stiffness parameters, increased risk for atherosclerosis, and increased inflammatory atherosclerotic biomarkers in SLE patients [43, 44]. Similarly, leptin levels have been positively correlated with pLN disease activity and SLEDAI-2K score [39]. Furthermore, Barbosa et al [31] found that renal involvement is the only clinical manifestation linked to elevated levels of leptin. In the present study, a strong positive correlation was identified between leptin levels and disease-specific pLN characteristics such as proteinuria, SLEDAI-2K, and CYP usage (all $p < 0.05$). These observations substantiate the involvement of leptin in the pathophysiology of pLN and underscore its potential value as both a diagnostic biomarker and a therapeutic target.

Disease duration, low folate levels, steroid treatment, and *MTHFR* (c. 677C > T and c. 1298A > C) SNPs have been found to be related to high homocysteine levels [45]. LN patients are at a higher risk (66.6%) for developing elevated homocysteine levels [46]. Hyperhomocysteinemia has been shown to increase LDL nitration and scavenger receptor uptake from monocytes and macrophages [47, 48]. It is postulated that homocysteine exerts negative effects on the endothelium through oxidative damage. Homocysteine plasma autoxidation generates reactive oxygen species, which in turn induce lipid peroxidation on the endothelial cell membrane and lipoprotein particle peroxidation in plasma [45, 48].

In LN patients, subclinical cardiovascular diseases such as arterial wall thickening (intima media thickness > 0.9 mm), carotid plaque formation, coronary artery calcification, arterial stiffness, and atherosclerosis progression have been associated with high homocysteine levels [48, 49]. *MTHFR*677TT (but not 1298CC) has been identified as a predictor for plaque formation and arterial wall thickening in SLE patients [48]. An Italian study reported an increased frequency of *MTHFR*677TT genotype in SLE patients with a high incidence of thrombotic events [48].

Fibrinogen is a plasma glycoprotein that comprises three pairs of nonidentical polypeptide chains (Aα, Bβ, and γ), each encoded by a different gene (fibrinogen alpha [FGA], fibrinogen beta [FGB], and [*FGG*]) [48]. Thrombin-induced conversion of fibrinogen to fibrin is necessary to stabilize thrombi [3].

Elevated plasma fibrinogen levels are associated with increased platelet aggregation and plasma viscosity [45]. A SNP characterized by a C-to-T substitution at nucleotide 10,034 of the *FGG* (10034C > T, rs2066865) has been proposed as a novel risk factor for deep venous thrombosis [45]. Only one study has explored the *FGG* rs2066865 SNP role in cardiovascular events in SLE. In a meta-analysis of 1698 patients with SLE, Kaiser et al. [17] found an association between *FGG* rs2066865 SNP and venous thrombosis risk in European Americans (OR 1.49, $p = 0.02$) and arterial thrombosis risk in Hispanics (OR 2.19, $p = 0.003$).

Studies have reported an association between *FGG* gene polymorphism and elevated plasma fibrinogen levels, suggesting a potential indirect impact on adipokines [19, 20]. In this study, high serum leptin levels were associated with the AG genotype of *FGG* rs2066865 SNP in pLN patients. This finding may be attributed to the pleiotropic effects of this genetic variant on both fibrinogen levels and leptin regulation. Fibrinogen, as an inflammatory marker, may contribute to chronic low-grade inflammation, which is associated with increased leptin production [20]. Leptin, in the other hand, is recognized to be linked with inflammatory processes [8, 11], establishing a further connection between genetic variations in the *FGG* gene and potential implications for inflammatory and adipokine regulation. Further research may be needed to explore specific molecular pathways between *FGG* gene polymorphism, fibrinogen levels, and adipokines such as leptin.

There are few studies that evaluate susceptibility to SLE with *MTHFR* SNPs and none with the *FGG* gene [6, 46]. Salimi et al. [46] observed an association between

AC + CC genotypes of the *MTHFR* gene and susceptibility to LN in a cross-sectional study of 150 Iranians LN patients and 160 controls. The most common *MTHFR* gene polymorphism among SNPs is rs1801133 (677C > T) [14]. This specific SNP entails a C-to-T transition at nucleotide 677 in exon 4, resulting in an alanine (C) to valine (T) substitution, leading to decreased enzymatic activity. This variant may affect the folate/homocysteine metabolic pathway, influencing critical immune function processes, including DNA methylation and synthesis [10, 14, 16].

In a meta-analysis involving 882 cases and 991 controls, a significant association was observed between the *MTHFR* rs1801133 T carrier and an increased risk of SLE when compared to the C allele [odds ratios were 1.766 (1.014–3.075) for T carrier vs CC, $p = 0.04$].

However, in our study, we did not find an association between the *MTHFR* and *FGG* SNPs with susceptibility to pLN. Functional studies are necessary to ascertain its role in LN. In this study, the MTHFR gene SNP did not adhere to the genetic equilibrium of Hardy–Weinberg, potentially attributed to the impact of migratory flows in the region [47]. However, genetic ancestry was not considered, and analyses using InDEL markers were not conducted.

To our limitation, firstly, this study was retrospective with a small sample size, and no size/power analysis was conducted. Secondly, there is a lack of long-term assessment of renal and cardiovascular disease risk factors and outcomes, and thirdly, the serum concentrations of MTHFR and FGG were not measured. Further prospective studies with a larger sample size are required not only to substantiate our results but also to explore the correlation between adiponectin/leptin serum levels, MTHFR/FGG serum concentrations, and *MTHFR/FGG* polymorphisms with cardiovascular and renal biomarkers in pLN patients, considering the histopathological profile (proliferative vs. non-proliferative classes) and immunosuppressive treatment regimens (CYP vs. MMF).

Conclusions

Serum adiponectin and leptin levels are significantly increased in pLN patients compared to healthy controls. Adiponectin levels exhibit a positive correlation with proteinuria, while leptin levels show positive correlations with disease severity as measured by the SLEDAI-2K, proteinuria, and CYP usage. Notably, high leptin levels were found to be associated with *FGG* rs2066865 SNP in pLN patients, suggesting a direct involvement in disease pathology and potential utility as a biomarker. Further studies using larger epidemiological cohorts are recommended to better understand the role of these adipokines in the development of atheromatosis, cardiovascular disease, and/or impairment in renal function in pLN patients.

Abbreviations

SLE	Systemic lupus erythematosus
pSLE	Pediatric systemic lupus erythematosus
pLN	Pediatric lupus nephritis
SNPs	Single-nucleotide polymorphisms
SLEDAI-2K	SLE disease activity index-2000
MTHFR	Methylenetetrahydrofolate reductase
CYP	Cyclophosphamide
MMF	Mycophenolate
FGG	Fibrinogen gamma chain
ACR	American College of Rheumatology
GEAS	Group of systemic autoimmune diseases
SEN	Spanish Society of Nephrology
ESKD	End-stage kidney disease
VCAM-1	Vascular cellular adhesion molecule-1
ICAM-1	Adhesion molecule-1

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Author contributions

GG and EE conceptualized and designed the study. GA, ZE, JD, CM, AI, and AM carried out the clinical data collection. LF, AD, and NP analyzed and interpreted the clinical data. LF and AD wrote the first draft of this manuscript. GG, NP, JD, GA, ZE, CM, AI, GL, and EE reviewed and revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of Universidad del Norte, Barranquilla, Colombia (Approval # 00029, 13 October 2019).

Consent for publication

Not applicable.

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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