

Association of microRNA-155, interleukin 17A, and proteinuria in preeclampsia

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Abstract

Background: This study aimed to explore the correlation between microRNA-155 (miR-155), interleukin 17A (IL-17), and late preeclampsia (PE) using biochemical parameters in maternal serum and urine.

Methods: Sixty patients with PE were recruited to this study and were divided into 3 groups according to levels of urinary protein: mild urinary protein group (group A); moderate urinary protein group (group B); and severe urinary protein group (group C). All subjects presented with late onset PE (≥ 32 weeks). In addition, 20 healthy pregnant women were recruited as a normal group (NP). Maternal serum and urine samples were obtained from all participants and assayed using immunofluorescence, transmission electron and immune electron microscopy, and western blot. Furthermore, placentas were also collected and miR-155 and IL-17 expression was measured using an enzyme-linked immunosorbent assay.

Results: Levels of miR-155 and IL-17 expression were found to be increased in PE placentas and serum, compared to the normal group ($P < .05$). IL-17 levels and podocytes had a positive association in the serum of patients with PE. In addition, over-expression of miR-155 resulted in increased IL-17 production by CD4+ T cells in vitro, and nephrin expression was decreased in podocytes. Furthermore, IL-17 reduced nephrin expression in podocytes and podocyte apoptosis in a dose and time-dependent manner.

Conclusion: The results of this study demonstrate a correlation between miR-155 and IL-17 in the formation of proteinuria during late onset PE.

Abbreviations: 24U-pro = quantitation of 24-hour urinary protein, CYR61 = cysteine-rich 61, DBP = diastolic pressure, group A = mild urinary protein group, group B = moderate urinary protein group, group C = severe urinary protein group, IL-17 = interleukin 17A, miR-155 = microRNA-155, miRNAs = microRNAs, MPE = mild PE group, NP = a normal group, PE = preeclampsia, SBP = systolic pressure, SPE = severe PE group, TBS = tris-buffered saline, VEGF = vascular endothelial growth factor.

Keywords: IL-17, miR-155, preeclampsia, proteinuria

1. Introduction

Preeclampsia (PE) is a pregnancy-specific disorder that affects ~7% of pregnancies.^[1-4] It is characterized by hypertension and proteinuria,^[5-7] which can significantly increase the risk of maternal complications and prenatal mortality.^[8] Previous reports have suggested that the presence of both proteinuria and hypertension during PE resulted in preterm deliveries and maternal mortality in 15% and 16% cases studied, respectively.^[9-10]

Previous studies reported that several factors can lead to PE, such as a loss of vascular endothelial growth factor (VEGF)

activity^[11-12] or glomerular endotheliosis.^[13] In addition, podocyturia (the presence of podocytes in the urine sediment) and nephrinuria (soluble nephrin in the urine) have been detected in the urine of patients with PE.^[14-16] Podocytes are glomerular epithelial cells that regulate filtration of circulating plasma proteins from the capillary lumen into Bowman's space.^[17-18] Nephrin is a podocyte-specific transmembrane protein that is predominantly localized to the glomerular slit diaphragm of podocytes.^[19] Thus, podocytes and nephrin expression are key factors in the assessment of proteinuria in patients with PE.

MicroRNAs (miRNAs) are a major class of tissue-specific regulators of gene expression.^[20] In 2007, Pineles et al^[21] reported a relationship between PE and aberrant miRNA expression in the placenta. Among miRNAs, microRNA-155 (miR-155) is a known oncogene and is upregulated in immune cells following exposure to inflammatory stimuli, suggesting an important role in immune regulation.^[21] Studies found that miR-155 expression is increased in the placentas of women with PE at the time of delivery.^[22-23] Another study revealed that miR-155 contributes to the pathogenesis of PE by downregulating cysteine-rich 61 (CYR61).^[23] It has also been reported that IL-17 expression is elevated in the circulation and placenta in PE, and could be involved in the pathogenesis of PE.^[24] We speculate that, in addition to CYR61, there may be other miR-155 and IL-17 targets in the occurrence of PE, because an individual miRNA has the potential to target multiple protein-coding genes.

The aim of this study is to test the hypothesis that miR-155 and IL-17 have a positive association in the formation of proteinuria during late onset PE.

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The authors have no conflicts of interest to disclose.

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2. Materials and methods

2.1. Study design and participants

The present study was performed using serum, urine, and placental tissues collected from 60 patients with PE and 20 normal pregnant women from the Second Xiangya Hospital, Central South University. The study was approved by the Medical Ethical Committee of the Second Xiangya Hospital and written informed consent was obtained from all participants. Normal pregnancy was defined as subjects who were normotensive during pregnancy and who, both previously and presently, had delivered a healthy neonate after 37 weeks of gestation. Late onset PE (≥ 32 weeks) was defined as those patients who displayed gestational hypertension and proteinuria without a history of hypertension, presenting with systolic blood pressure ≥ 140 mm Hg and/or diastolic blood pressure ≥ 90 mm Hg. Subjects who had complications of renal disease, spontaneous abortion, gestational diabetes, fetal chromosomal, or congenital abnormalities were excluded from this study.

Urinary samples were divided into 4 groups, according to levels of urinary protein: mild urinary protein group (group A); moderate urinary protein group (group B); severe urinary protein group (group C); and a normal group (NP).

2.2. Sample collection and preparation

Serum samples were collected at the time of delivery via cesarean section and centrifuged at 4000 rpm for 30 minutes at 4°C. Serum obtained was tested for liver and renal function, as well as IL-17 and miR-155 expression. Urine samples were obtained after every 24 hours from 3 days before cesarean section, and tested for urine protein excretion per 24 hours and podocyturia. Placental tissue was collected during cesarean section for testing IL-17 mRNA and miR-155 expression.

2.3. Immunofluorescence and microscopy

Frozen sections of placenta tissue (6 μm) were transferred onto slides and washed with cold phosphate buffer saline (PBS). Sections were fixed with chilled acetone for 10 minutes at -20°C and incubated with 0.1% Triton X-100 in PBS at 37°C for 15 x 200A; minutes. After washing with PBS, sections were blocked with 1% bovine serum albumin for 30 minutes and stained with goat anti-human nephrin (1:50; R&D Systems, Minneapolis, MN) for 12 hours at 4°C, followed by rhodamine-conjugated rabbit anti-goat immunoglobulin G (1:100; Santa Clara, CA). Control sections were stained with a nonassociated primary antibody instead of anti-nephrin. Sections were examined by fluorescence microscopy (BX251 microscope; Olympus, Tokyo, Japan). Images were captured using a spot charge-coupled device camera (Olympus). Exposure settings were kept constant for each primary antibody.

2.4. Transmission electron microscopy

Paraformaldehyde-glutaraldehyde-fixed 1-mm blocks of renal cortex were postfixed with 1% osmium in 0.1M cacodylate buffer for 1 hour, dehydrated through graded ethanol, embedded in Epon, sectioned, stained with uranyl acetate and lead citrate, and examined and photographed using a Hitachi H600 transmission electron microscope (Hitachi, Tokyo, Japan).

2.5. Immune electron microscopy

The paraformaldehyde-glutaraldehyde-fixed 1-mm blocks of renal cortex were washed with PBS, dehydrated through graded

ethanol, and embedded in Epon. Ultrathin sections were transferred to nickel grids and blocked with 1% bovine serum albumin and 1% normal goat serum in PBS. Sections were incubated with a primary polyclonal goat anti-human nephrin antibody against the intracellular domain of nephrin (1:50; R&D Systems), then with a secondary gold-conjugated (10 nm) rabbit anti-goat secondary antibody (1:100; DAKO, DK). Sections were postfixed with 1% glutaraldehyde, contrasted with uranyl acetate and lead citrate, and observed under an electron microscope and photographed for detailed analysis.

2.6. Reverse transcription-polymerase chain reaction

Total RNA was isolated from placental tissues using Trizol reagent (Invitrogen Life Technologies, Carlsbad, CA). Total RNA (5g) was extracted from the renal cortex and was used to synthesize cDNA, which served as a template for amplification of nephrin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an endogenous standard. Sequences of forward and reverse primers (Shengong Company, Shanghai, China) were as follows: 5'-CTGTAAATGCTAATCGTGATAG-3' and 5'-GCAGGGTCCGAGGT-3' for miR-155 detection; 5'-CG-GACTGTGATGGTCAACCT-3' and 5'-GACCAGGATCTCT-TGCTGGA-3' for IL-17 detection; and 5'-GCACCGTCAA-GGCTGAGAAC-3' and 5'-TGGTGAAGACGCCAGTGGA-3' for GAPDH detection. AlphaEase FC image software (Panasonic WV-Bp 330/G, Japan) was used to analyze all those data.

2.7. Western blotting

Urine samples were homogenized on ice and proteins were extracted using a lysis buffer, centrifuged at 12,000g for 15 minutes at 4°C, and the resultant supernatant was collected. Protein concentrations were determined using a BCA protein assay kit (Pierce Biotechnology, Rockford, IL). Samples were separated on an 8% polyacrylamide gel then semidry blotted onto a PVDF membrane. The blotted membrane was incubated for 2 hours at room temperature in blocking solution (5% blocking agent in 1% nonfat milk) in tris-buffered saline (TBS) with 0.02% Tween 20 (T) then washed 3 times with a washing solution.

2.8. Cell culture

Podocytes were cultured at 37°C in a water-saturated atmosphere with 5% CO₂ for 5 days before initiation of experiments. Podocytes were separately exposed to Dkk1 (R&D Systems) or NS-398 (Sigma Chemical Co., St Louis, MO) in serum-free DMEM containing peptidase inhibitors (1 μM phosphoramidon, 4 $\mu\text{g}/\text{mL}$ bacitracin and 1 μM captopril; Sigma) for 15 minutes at 37°C in a water-saturated atmosphere with 5% CO₂. The cells were then treated with IL-17 and continuously stimulated with TWS119 (Cayman Chemical, Ann Arbor, MI) or Wnt-3a (R&D Systems) for 8, 12, or 24 hours in DMEM at 37°C. Podocytes were treated with different concentrations of IL-17.

2.9. Cell co-culture

The CD4⁺ T cells were transfected with a miR-155 mimic or a miR-155 mimic negative control; the CD4⁺ T cells and podocytes were co-cultured. Nephrin expression was measured using western blot. Podocyte apoptosis was assessed using flow cytometry (Annexin V-FITC Apoptosis Detection Kit I, BD Bioscience, San Jose, CA).

Table 1

Basic clinical characteristics among different preeclampsia groups.

Group	Gestational age, weeks	SBP, mm Hg	DBP, mm Hg	24U-pro, mg/24 h, median (IQR)
NP (n=20)	38.55 ± 1.37*	115.25 ± 9.78*	72.56 ± 7.01*	50.50* (26.25–107.56)
MPE (n=26)	36.12 ± 2.68†	152.21 ± 5.06†	97.39 ± 10.27†	1150.87† (730.67–1408.98)
SPE (n=34)	34.95 ± 1.86	170.61 ± 21.54	103.16 ± 13.73	2040.15 (648.25–5425.04)

DBP = diastolic blood pressure, IQR = interquartile range, MPE = mild preeclampsia, NP = normal group, SBP = systolic pressure, SPE = server preeclampsia, 24U = pro-quantitation of 24-hour urinary protein;
 * *P* < .05, means compared with groups MEP and SPE, respectively.
 † *P* < .05, means compared with the SPE group.

Table 2

Clinical indexes at different protein levels.

Group	Gestational age, weeks	SBP, mm Hg	DBP, mm Hg	UA, μmol/L	24U-pro, mg/24 h, median (IQR)	Albumin, g/L	Podocyte number (n/20HP) median (IQR)
NP (n=20)	38.55 ± 1.37*	115.25 ± 9.78*	72.56 ± 7.01*	303.48 ± 86.55*	50.50 (26.25–107.56)*	34.96 ± 2.16*	3.55 (2.61–4.78)*
A (n=20)	36.01 ± 3.12	160.56 ± 19.59	97.78 ± 9.47	376.14 ± 88.67	767.84 (494.75–1008.11)	32.72 ± 1.83	16.27 (15.13–20.37)
B (n=20)	35.42 ± 4.18	170.56 ± 22.27	103.67 ± 11.04	445.70 ± 77.53	2400.89 (2183.50–3602.17)	30.64 ± 2.38	28.45 (26.18–32.26)
C (n=20)	34.22 ± 2.06	160.61 ± 11.65	105.16 ± 13.73	446.42 ± 100.61	7159.24 (5150.05–12311.20)	28.55 ± 4.45	43.46 (36.45–49.24)

A = mild urinary protein group, B = moderate urinary protein group, C = severe urinary protein group, DBP = diastolic blood pressure, MPE = mild preeclampsia, SPE = server preeclampsia, NP = normal group, SBP = systolic pressure, UA = urine acid, 24U = pro-quantitation of 24-hour urinary protein;
 * *P* < .05, means compared with groups A, B, and C, respectively.

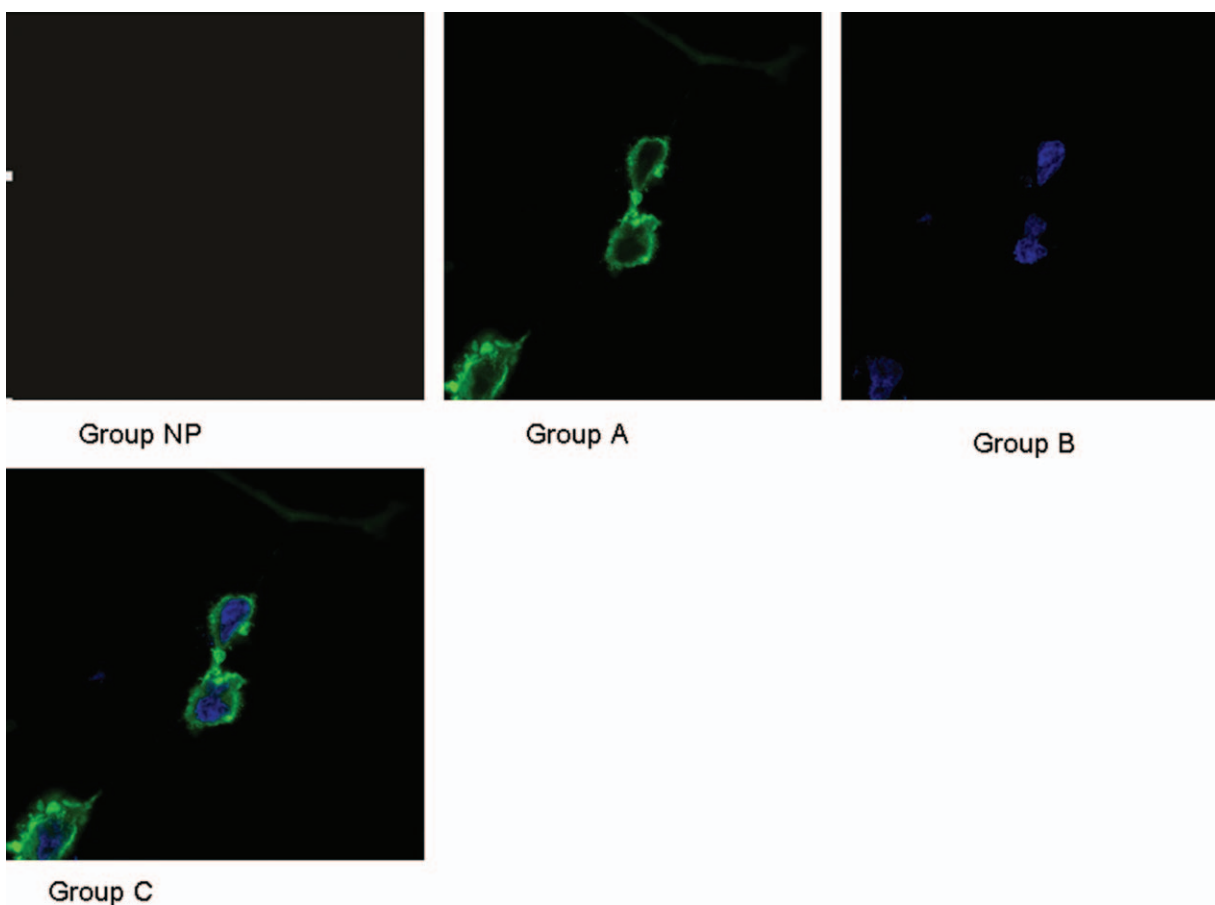


Figure 1. Podocyte in different urine protein level groups.

Table 3
Newborn outcomes at different urine protein levels.

Group	Placenta weight, g	Birth weight, g	1 min Apgar	5 min Apgar
NP (n=20)	576.72 ± 114.56	3245.65 ± 520.25	9.40 ± 0.74	10.00 ± 0.00
A (n=20)	522.18 ± 90.45*	3226.12 ± 587.67	9.20 ± 0.79	10.00 ± 0.00
B (n=20)	448.55 ± 86.35*	2755.45 ± 591.77*	8.50 ± 1.05*	9.53 ± 0.64*
C (n=20)	396.42 ± 81.41*	2268.57 ± 568.75*	8.10 ± 1.25*	9.20 ± 0.68*

A = mild urinary protein group, B = moderate urinary protein group, C = severe urinary protein group, NP = normal group.
* *P* < .05, means compared with groups A, B, and C, respectively.

2.10. Statistical analysis

Results were analyzed using SPSS 19.0 (SPSS Inc., Chicago, IL). Student’s *t*-test was used to ascertain the significance of differences between mean values of 2 continuous variables. A chi-squared test was used to analyze differences in proportions of categorical variables among different groups. Spearman correlation analysis was used to evaluate the relationship between 2 indexes. A *P*-value < .05 was considered significant.

3. Results

3.1. Basic clinical characteristics among different preeclampsia groups

Eighty patients were divided into 3 groups: a normal group (NP) (n=20), a mild PE group (MPE) (n=26) and a severe PE group (SPE) (n=34), according to the degree of PE. The basic characteristics of gestational age, systolic pressure (SBP), diastolic pressure (DBP), and quantitation of 24-hour urinary protein (24U-pro) were analyzed and compared among the different groups (Table 1). Significant differences in gestational age, SBP, DBP, and 24U-pro were found between the NP and MPE groups and the NP and SPE groups (*P* < .05, Table 1). Similarly, significant differences in gestational age, SBP, DBP and 24U-pro were found between the MPE and SPE groups (*P* < .05, Table 1).

3.2. Clinical indexes at different urine protein levels

Values of urine protein levels were used to divide the 80 samples into 4 groups: NP, A, B, and C. Gestational age and albumin levels were higher in group NP than in groups A, B, and C, while SBP, DBP, UA, 24U-pro values, and podocyte numbers were

significantly lower in group NP than in groups A, B, and C (*P* < .05, Table 2). Differences in all measurements were compared among groups A, B, and C using a one-way analysis of variance. The differences in gestational age among the 3 groups were significant (*F* = 3.27, *P* < .05, Table 2). A LSD-*t* test was used to compare between groups A, B, and C. Similarly, albumin levels showed significant differences among the 3 groups (*P* < .05, Table 2). A Kruskal–Wallis test was used to analyze the 24U-pro data, and results showed significant differences among groups A, B, and C (*P* < .05, Table 2). Results were similar for the podocyte numbers among the 3 groups (*P* < .05, Table 2, Fig. 1). However, no significant differences in SBP, DBP, and UA were found among the 3 groups.

3.3. Newborn outcomes at different urine protein levels

Placenta weight in the NC group was significantly higher in groups A, B, and C (*P* < .05, Table 3). Moreover, birth weight, 1-minute Apgar score,^[25] and 5-minute Apgar score were significantly higher in the group NC than in groups A and B (*P* < .05, Table 3). The LSD-*t* test was used to analyze the placenta weight, birth weight, 1-minute Apgar score, and 5-minute Apgar score data were significant differences among groups A, B, and C (*P* < .05, Table 3).

3.4. Nephrin expression at different urine protein levels

Results of western blot of urine nephrin expression at different urine protein levels showed that the relative mean value was 1.071 in the NC group, while they were 3.450, 6.500, and 8.783 in the group A, B, and C, respectively. Significant differences were found among the 4 groups (*P* < .05, Fig. 2A–C).

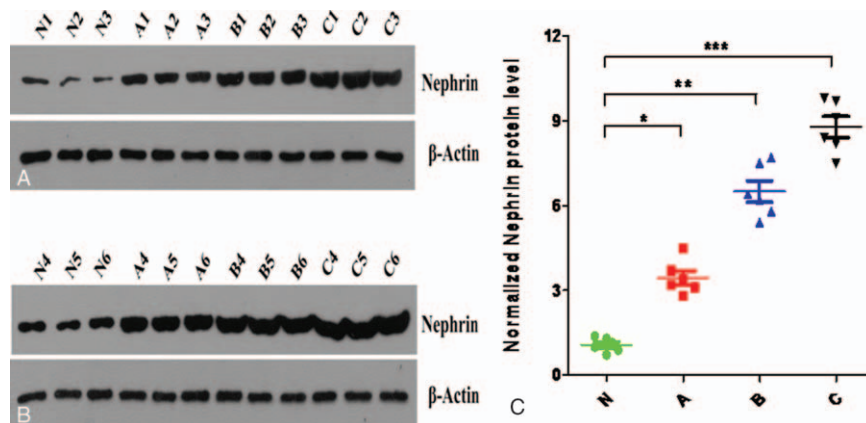


Figure 2. (A–C) Urine nephrin expression at different urine protein levels.

Table 4

Correlation between 24-hour urine protein and different indexes.

Index (n=80)	r	P value
Podocyte in urine	0.826	<.001
Nephrin protein in urine	0.616	<.001
Albumin	-0.765	<.001
Placenta weight	-0.798	<.001
Birth weight	-0.722	<.001
UA	0.305	.058
SBP	0.277	.088
DBP	0.291	.090

DBP = diastolic blood pressure, SBP = systolic pressure, UA = urine acid.

3.5. Correlation analysis between 24-hour urine protein and different indexes

Positive correlations were found between 24-hour urine protein levels, the number of podocytes, and nephrin expression in the urine ($P < .001$, Table 4). However, negative correlations were noted between 24-hour urine protein levels and placental weight, serum albumin, and birth weight ($P < .001$, Table 4).

3.6. miR-155 and IL-17 mRNA expressions in serum and placenta

Multiple methods were used to compare the expressions of miR-155 and IL-17 in serum and placenta (Table 5). Significant differences in miR-155 and IL-17 expressions in the serum and placenta were found among the 4 groups ($P < .01$ Table 4, Figs. 3–6).

3.7. Correlation analysis between IL-17 mRNA expression and other indexes in serum

A positive correlation was found between IL-17 levels in the serum and placenta ($P < .001$, Table 6). In addition, a positive correlation was found between IL-17 levels in the serum and miR-155 expression in both the serum and placenta ($P < .001$, Table 6). Moreover, a positive correlation was found between the level of IL-17 in the serum, nephrin expression, and the number of podocytes in the urine ($P < .001$, Table 6).

3.8. Nephrin and podocyte apoptosis following miRNA transfection

After co-culture, nephrin expression was higher in the group transfected with a miR-155 mimic than in the group transferred with a miR-155 mimic negative control. Podocyte apoptosis was lower in the group transferred with miR-155 mimic than in the group transferred with a miR-155 mimic negative control ($P < .05$, Table 7).

Table 5

MiRNA-155 and IL-17 expressions in serum and placenta.

Group	Serum ($2^{-\Delta\Delta Ct}$)		Placenta ($2^{-\Delta\Delta Ct}$)	
	miRNA-155	IL-17, pg/mL	miRNA-155	IL-17
NP (n=20)	0.1772 ± 0.0091*	40.01 ± 2.28*	0.3284 ± 0.0250*	0.0071 ± 0.0007*
A (n=20)	0.2161 ± 0.0165	66.11 ± 8.93	0.4752 ± 0.0416	0.0117 ± 0.0016
B (n=20)	0.3056 ± 0.0212	78.80 ± 6.54	0.6315 ± 0.0705	0.0165 ± 0.0014
C (n=20)	0.4225 ± 0.0373	88.77 ± 8.75	0.8392 ± 0.0855	0.0233 ± 0.0019

A = mild urinary protein group, B = moderate urinary protein group, C = severe urinary protein group, IL-17 = interleukin 17, miRNA-155 = microRNA-155, NP = normal group

* $P < .05$, means compared with groups A, B, and C, respectively.

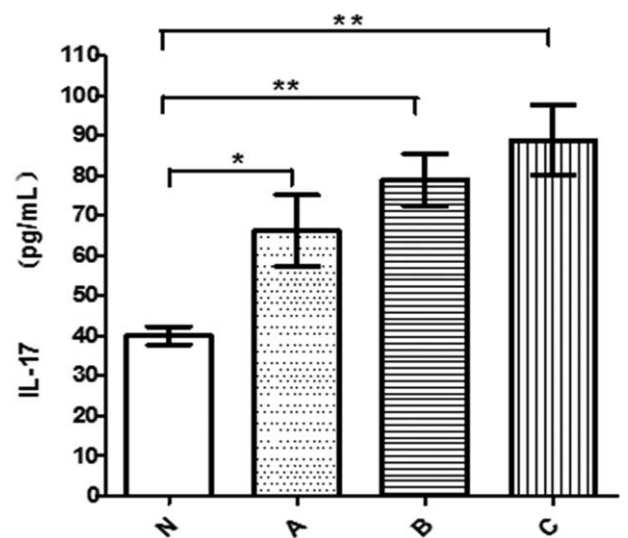


Figure 3. IL-17 in serum in different groups. IL-17 = interleukin 17.

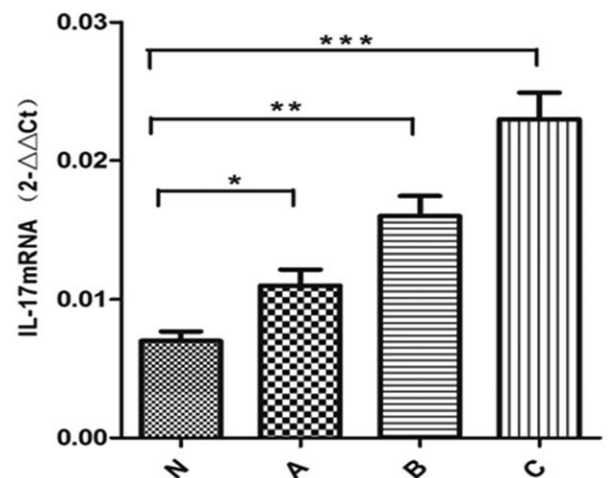


Figure 4. IL-17 in placenta in different groups. IL-17 = interleukin 17.

3.9. Nephrin expression and podocyte apoptosis following IL-17 treatment

The expression of nephrin in podocytes was significantly higher in the control group than in groups treated with different concentrations of IL-17 (10, 40, or 80 ng/mL) ($P < .05$, Table 8). However, the apoptosis rate in podocytes was significantly lower

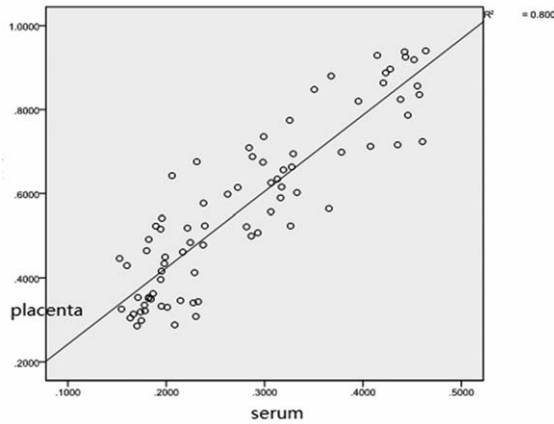


Figure 5. Correlation of miRNA-155 between serum and placenta. miRNA-155 = microRNA-155.

in the control group than in the IL-17-treated groups ($P < .05$, Table 8, Fig. 6). A multiple comparison method was used to analyze the nephrin expression and apoptosis rate in podocytes, and significant differences were found among the 4 groups ($P < .05$, Table 8). The nephrin expression and apoptosis rate also showed significant differences among the 4 groups ($P < .05$, Table 8).

3.10. Nephrin expression and apoptosis rate in podocytes at different times after IL-17 treatment

Nephrin expression in podocytes was significantly higher in the control group than in the IL-17-treated groups (40 ng/mL 8 hours, 40 ng/mL 12 hours, and 40 ng/mL 24 hours) ($P < .05$, Table 9). However, the apoptosis rate in podocytes was significantly lower in the control group than in the different time point groups ($P < .05$, Table 9, Fig. 7). A multiple

Table 6

Correlation between IL-17 expression and other indexes in serum.

Index (n=80)	r	P value
IL-17 mRNA in placenta ($2^{-\Delta\Delta Ct}$)	0.823	.000
miRNA-155 in serum ($2^{-\Delta\Delta Ct}$)	0.723	<.001
miRNA-155 in placenta ($2^{-\Delta\Delta Ct}$)	0.762	<.001
24-hour urine protein	0.781	<.001
Podocyte in urine	0.756	<.001
Nephrin in urine	0.735	<.001

IL-17 = interleukin 17, miRNA-155 = microRNA-155.

Table 7

Nephrin and podocyte apoptosis expression in different transfected groups.

Group	Nephrin/actin	Apoptosis rate, %
miRNA-155 mimic negative control group (n=3)	1.015 ± 0.07602	(11.73 ± 0.964)
miRNA-155 mimic group (n=3)	0.4872 ± 0.05119*	(25.81 ± 2.786)*

miRNA-155 = microRNA-155.

* $P < .05$, means compared with the miRNA-155 mimic negative control group.

Table 8

Nephrin and apoptosis rate in podocyte in different concentration.

Group	Nephrin/actin	Apoptosis rate, %
Control group (n=3)	1.0030 ± 0.06642	(9.805 ± 1.348)
10 ng/mL IL-17 (n=3)	0.7193 ± 0.03231*	(27.83 ± 2.738)*
40 ng/mL IL-17 (n=3)	0.5103 ± 0.04139*	(35.01 ± 3.758)*
80 ng/mL IL-17 (n=3)	0.1833 ± 0.01831*	(49.72 ± 5.100)*

IL-17 = interleukin 17.

* $P < .05$, means compared with control group.

comparison method was used to analyze nephrin expression and apoptosis rate in podocytes, and significant differences were found among the 4 groups ($P < .05$). Results of the nephrin

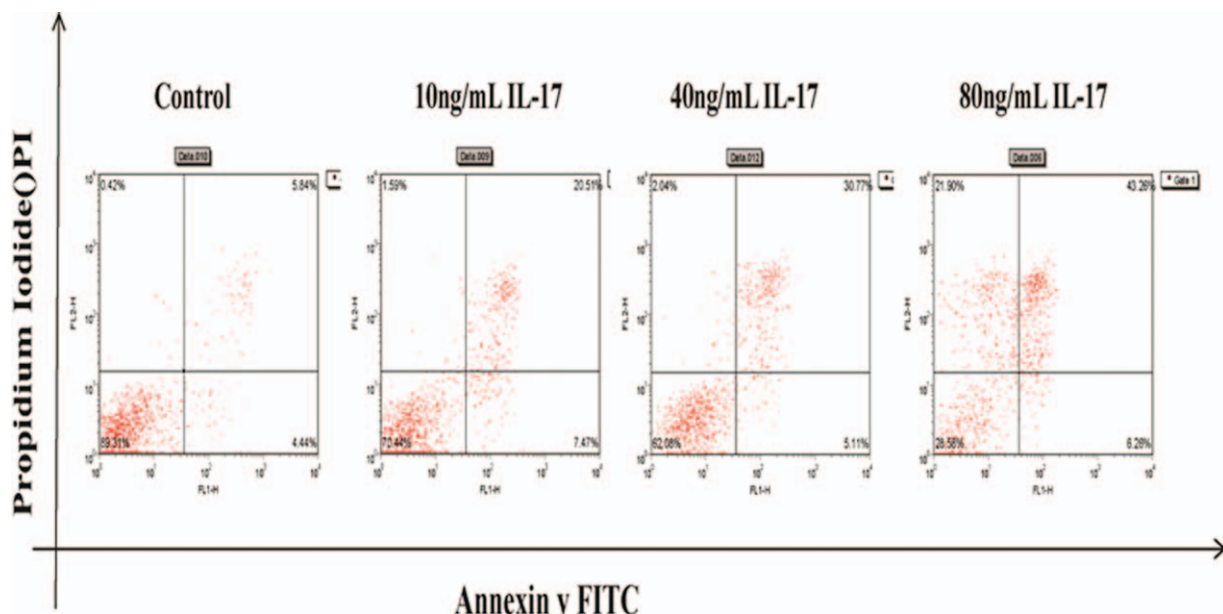


Figure 6. Apoptosis rate at different concentration.

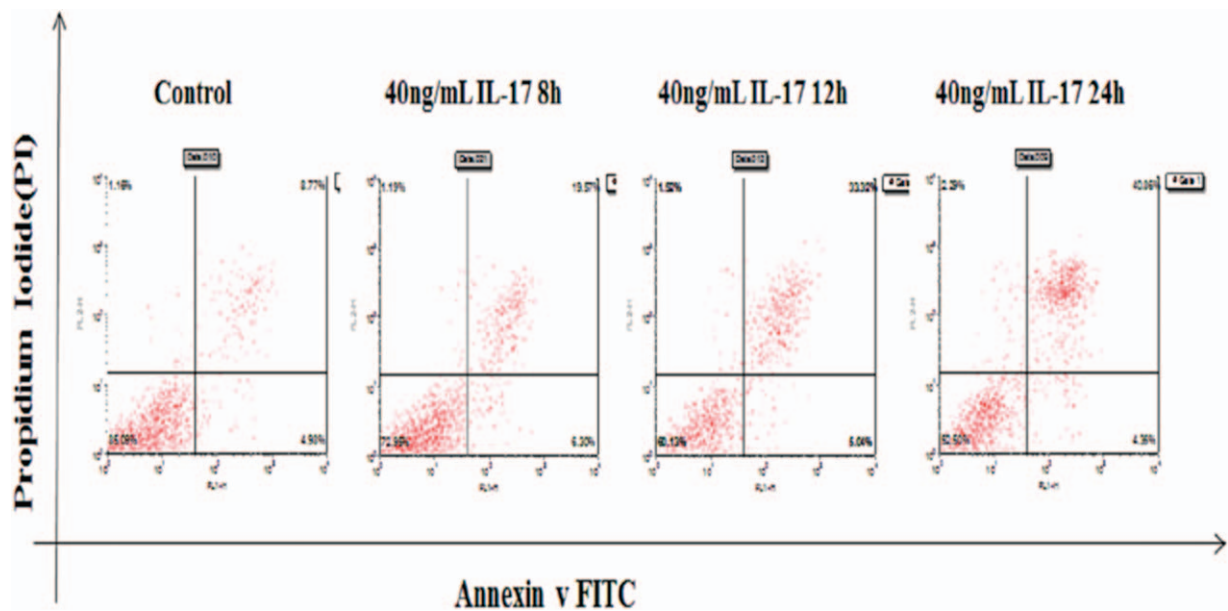


Figure 7. Apoptosis rate at different time points.

expression apoptosis rate analysis also showed significant differences between 4 groups ($P < .05$, Table 9).

4. Discussion

Recent studies provide strong evidence that proteinuria in PE is associated with cytokine and T-cell disorders and results in glomerular podocyte dysfunction.^[26] Furthermore, an increase in IL-13 production by CD3+, CD4+, and CD8+ T cells was shown to mediate steroid-sensitive nephrotic syndrome during relapse.^[27]

It has been reported that podocyturia occurs in pregnant women with PE from the end of the second trimester until delivery time.^[2] The results of the present study suggest that podocyturia identifies a subclinical disease among pregnant women who subsequently develop the clinical characteristics of PE. As such, podocyturia may serve as an early indicator and diagnostic test for PE.^[28] This is supported by data from the present study. In addition, we previously demonstrated a redistribution and reduction in nephrin proteins in human podocytes treated with IL-17.^[29] Exposure of nephrin molecules to IL-17 caused them move and accumulate internally toward the cytoplasmic actin filaments, suggesting that the observed redistribution and reduction in nephrin proteins could be involved in the pathogenesis of PE. These results are similar to previous findings that nephrin in podocytes is also affected by diabetic conditions, causing hyperpermeability at early stages.^[30]

Table 9
Nephrin and apoptosis rate in podocyte at different time points.

Group	Nephrin/actin	Apoptosis rate, %
Control group (n=3)	1.0030 ± 0.06642	(12.38 ± 1.637)
40 ng/mL IL-17 8 h (n=3)	0.5010 ± 0.04102*	(25.53 ± 2.384)*
40 ng/mL IL-17 12 h (n=3)	0.2921 ± 0.03478*	(36.59 ± 2.600)*
40 ng/mL IL-17 24 h (n=3)	0.1801 ± 0.02421*	(45.72 ± 3.241)*

IL-17 = interleukin 17.

* $P < .05$, means compared with the control group.

MicroRNAs are increasingly recognized as critical regulators of tissue-specific patterns of gene expression. For example, CD4+ T cells lacking miR-155 exhibit a bias toward Th2 differentiation, indicating that the absence of an individual miR can alter CD4+ T-cell differentiation. We have shown that miR-155 is induced upon T-cell activation and promotes Th1 differentiation when overexpressed in the activated CD4+ T cells. Antagonism of miR-155 leads to induction of interferon (IFN)-gamma receptor alpha-chain (IFN-gammaRalpha), and a functional miR-155 target site has been identified within the 3' untranslated region of IFN-gammaRalpha.^[31] These results identify IFN-gammaRalpha as a second miR-155 target in T cells and suggest that miR-155 contributes to Th1 differentiation in CD4+ T cells by inhibiting IFN-gamma signaling.^[32]

We recently demonstrated that IL-17 induces apoptosis in cultured rat podocytes.^[33] In IL-17-induced podocyte, apoptosis was detected using electron microscopy as well as a TUNEL assay. Nephrin expression was reduced at 8 and 12 hours. We presume that podocyte apoptosis contributes to the occurrence of proteinuria. Interestingly, a highly significant negative correlation was found between nephrin expression and apoptotic podocytes.^[34] Our data show that IL-17 induced nephrin derangement or even its decrement, which may play a role in podocyte apoptosis induction.

5. Conclusion

A positive correlation was reported between the expression of miR-155 and IL-17 in the serum and placenta, and the number of podocytes in the urine in PE. Over-expression of miR-155 caused an increase in IL-17 production, which induced podocyte apoptosis in a dose- and time-dependent manner.

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