Atrial Natriuretic Peptide Deterioration Diabetic Nephropathy via Stimulating Secretion of Cytokines

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Research article

Keywords: atrial natriuretic peptide, type 2, diabetic nephropathy, albuminuria, cytokines

DOI: https://doi.org/10.21203/rs.3.rs-117465/v1

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Abstract

Background: Atrial natriuretic peptide (ANP) refers to a cardiovascular and metabolic hormone that has been identified recently for chronic kidney disease (CKD) without diabetes. Cytokines (e.g., interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α)) as well as adiponectin (ADP)) contribute to development of diabetes of type 2 (T2DM). The aim here was at investigating ANP and some cytokines extents for a systematic analysis of relationship level in T2DM nephropathy patients.

Methods: Plasma samples from T2DM patients (n=81) in accordance with albuminuria were split into normoalbuminuria (n=37), microalbuminuria (n=23) and macroalbuminuria (n=21), as collected after the consent was received. Based on enzyme-associated immunosorbent test, ANP, TNF-α, IL-6 and ADP in plasma underwent the quantification, and the data received the analysis for determining the correlation between ANP and cytokines among 3 cohorts.

Results: The present study reported that macroalbuminuria patients exhibited higher plasma levels of ANP, TNF-α, IL-6, ADP, serum creatinine (Cr), blood urea nitrogen (BUN) and duration of diabetes mellitus (DM) than the cases subjected to normoalbuminuria and microalbuminuria. Plasma ANP level was significantly associated with TNF-α, IL-6 and ADP, independently of duration of DM and BUN.

Conclusion: Elevated serum ANP levels are likely to impact the metabolism of adipose tissue and inflammation which involved in the pathogenesis and progression of DN.

Background

Diabetic nephropathy (DN) refers to one considerable microvascularly-related diabetes complication and takes up at least 50% of end-stage renal disease (ESRD) [1, 2]. Diagnosis of DN as soon as possible is conducive to preventing diabetic kidney diseases from progressing.

By analyzing fundamental cytokines, targets or markers may be revealed to identify and treat T2DM patients, particularly those predictive of DN. Atrial natriuretic peptide (ANP) refers to a member of family of cardiac- and vascular derived hormones, primarily secreted from atrial or ventricular involved in the control of increased volume or elevated blood pressure [3]. ANP has been recently identified as act on blood vessel and adrenal gland, exhibiting natriuretic and vasodilating properties and critically controlling volume homeostasis and blood pressures [4, 5]. Several analyses are carried out, demonstrating the elevated serum levels of natriuretic peptide in cases developing chronic kidney disease (CKD) under the complication of renal functions under impairment. In the mentioned reports, the mentioned peptides seem to be marking elements of renal function reduction or CKD progressing [6, 7]. ANP also participates in adipose metabolic pathways [8]. Adipose tissue is known as an important endocrine tissue that secretes numerous biologically active proteins and many cytokines by adipocytes and other adipose tissue stromal cells, respectively [9]. Adiponectin (ADP) refers to a specific protein under the exclusive secretion of adipose tissues and appears to act as a hormone which could down regulate inflammatory responses in vitro. Likewise, decreased ADP shows an accompany with T2DM and the relevant macrovascular complication, as well as insulin resistance [10, 11]. However, recent studies have pointed out that the levels of urinary or serum ADP is both elevated in primary nephrotic syndrome and DN patients [12, 13]. ANP may regulate adipocyte metabolism including ADP through lipolysis, lipid oxidation, and
adipocyte browning or directly influence on macrophage [14–16]. Several inflammation cytokines (e.g., interleukin-6 (IL-6) and tumor necrosis element-α (TNF-α)) refer to risks-related elements for the damages of glomerular and vascular endothelial cells, attempting to improve urinary albumin in T2DM cases.

The effect exerted by ANP on T2DM albuminuria patients was analyzed. Moreover, the concentrations of ADP along with TNF-α and IL-6 received the measuring process to elucidate the interaction, if any, of the mentioned cytokines and ANP in patients stratified by albuminuria.

**Methods**

According to the Department of Endocrinology of Nanjing First Hospital, Nanjing Medical University, China, the present study was carried out from May to August 2016. 81 T2DM cases subjected to different stages of DN were involved according to the guidelines for chronic kidney disease[17]: 21 with macroalbuminuria (Macro-MA cohort), 23 with microalbuminuria (Micro-MA cohort) and 37 with normoalbuminuria (Nor-MA cohort). Macroalbuminuria, microalbuminuria and Normoalbuminuria received the definition of urine albumin excretion amount (UAE) > 300 mg/24 h, 30–300 mg/24 h and < 30 mg/24 h [17], separately.

The exclusion standards consist of: (1) cases exhibiting a history of overt cerebrovascular accident (myocardial infarction, angina pectoris and stroke); (2) cases subjected to severe non-regulated high blood pressure gaining the definition of systolic pressure of blood > 160 mmHg/ diastolic blood pressure > 100 mmHg; (3) cases subjected to severe dyslipidemia (total cholesterol > 400 mg/d; (4) cases subjected to urine tract infection and any other acute inflammation or infection; (5) cases subjected to kidney diseases except DN and using anticonvulsant drugs, anti-inflammatory drugs or nephrotoxic drug users; (6) cases exhibiting diagnosed liver failure, malignancy under the previous diagnosis; (7) pregnant women, lactation; (8) positivity for islet cell autoantibodies indicating possibility of type 1 diabetes mellitus; (9) patients treated with thiazolidinediones which are known to partly increase plasma ADP level.

Physical examinations were performed as measurement of blood pressure, height, weight and other anthropometric parameters. Samples of venous blood received the drawing process in all patients when the 8 h fasting and treating process were completed in a separate manner. Biochemical and hematological properties received the determination with routine technologies with one analyzing element automation. Blood received the collection from 4 ml EDTA containing element and the centrifugation in 30 min based on 3000 rpm for 10 min. Serum samples underwent the subsequent storing process inside aliquots with no preservatives at −80 °C for 3 months on average. Such process stopped in advance to cytokines study. Plasma levels of ANP, ADP, IL-6 and TNF-α received the measuring process based on human enzyme-associated immune sorbent testing process (ELISA) tools with commercial availability (ANP: catalogue number bsk00431, Bioss, Burlington, ON, Canada; ADP: catalogue number bsk00199, Bioss, Burlington, ON, Canada; IL-6: catalogue number bsk00040, Bioss, Burlington, ON, Canada; TNF-α: catalogue number bsk00162, Bioss, Burlington, ON, Canada). The detection limit for ANP, ADP, IL-6 and TNF-α was 10 ng/L, 30 ug/L, 2 ng/L and 8 pg/L, separately. The intra-test and intertest change coefficients took up 5% and 10%, separately.

Body mass index (BMI) was calculated as weight (kg)/square of height.

**Statistical Analysis**
Based on Windows 20.0 statistical software, data received the analysis based on SPSS (Statistical Package for Social Sciences). In terms of continuous normal data, Mean ± SD values were reported; otherwise, median (interquartile range) received the report. For assessing the correlation of Macro-MA and Nor-MA cohorts based on categorical variables, the authors performed Chi-square testing processes. This study conducted one ANOVA testing for comparing differences of mean between 3 cohorts. In addition, the authors employed the Kruskal-Wallis testing for comparing diversifications of mean of three cohorts based on normal distributing process. Correlations were tested by conducting regression analysis. For determining the relationship between numerical variables for normally distributed cohorts, Pearson correlation analysis was used, and Spearman test was used for abnormally distributed cohorts. Logistic regression analysis was conducted to assess the associations between ANP level and the other parameters assessed. A two-tailed $p$ values < 0.05 were considered exhibiting significance.

**Results**

According to Table 1, the individuals in 3 cohorts were well matched for age and gender composition. SBP was significantly higher in Macro-MA cohort than other 2 cohorts, while DBP was comparable between 3 cohorts. No differences were identified in alanine transaminase (ALT), aspartate transaminase (AST), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), Na, K and uric acid (UA) between 3 cohorts. Naturally, glycosylated hemoglobin (HbA1c) levels of all study patients were above normal values, whereas HbA1c was lower in Macro-MA cohort than other two cohorts ($p < 0.05$). Moreover, glucose in Macro-MA cohort was 6.35 (5.09, 8.08) mmol/L, which was distinctly lower than other two cohorts. Macroalbuminuria patients showed remarkably higher duration of DM, Cl, serum creatinine, urea, but low plasma albumin levels ($p < 0.05$ in all comparisons).
| Variable       | Nor-MA       | Micro-MA     | Macro-MA     | \( P \) value |
|----------------|--------------|--------------|--------------|---------------|
| N              | 37           | 23           | 21           | -             |
| Men (%)        | 23(62.2)     | 11(47.82)    | 11(52.38)    | 0.533\(^a\)   |
| Age (years)    | 61.32(11.69) | 66.17(13.41) | 66.10(8.68)  | 0.181\(^b\)   |
| BMI (kg/m\(^2\)) | 23.24(20.85,26.86) | 24.24(21.97,26.67) | 24.78(22.95,27.24) | 0.709\(^b\) |
| Duration (years) | 3.40(3.49)   | 10.13(6.61)  | 16.52(7.90)**| < 0.001\(^a\) |
| SBP (mmHg)     | 127(13)      | 133(17)      | 139(17)**    | 0.025\(^a\)   |
| DBP (mmHg)     | 78(7)        | 77(8)        | 79(8)        | 0.522\(^a\)   |
| UAE (mg/24 h)  | 11.94(7.44,21.09) | 96.90(54.78,168.66) | 1155.77(854.69,1575.00)** | < 0.001\(^a\) |
| HbA1c (%)      | 8.50(7.00,10.55) | 9.10(7.00,10.20)  | 7.10(6.15,8.20)* | 0.018\(^b\)   |
| ALT (IU/L)     | 23.16(11.78) | 30.09(40.82) | 19.19(9.14)  | 0.299\(^a\)   |
| AST (IU/L)     | 27.57(19.13) | 30.83(26.49) | 20.82(13.53) | 0.256\(^a\)   |
| TC (mmol/L)    | 4.78(3.80,5.35) | 4.45(3.22,5.53) | 5.26(3.84,6.14) | 0.286\(^b\) |
| TG (mmol/L)    | 1.88(1.66)   | 2.18(2.05)   | 2.71(2.43)   | 0.317\(^a\)   |
| HDL-C (mmol/L) | 1.15(0.98,1.35) | 1.05(0.92,1.36) | 1.05(0.87,1.24) | 0.421\(^b\) |
| LDL-C (mmol/L) | 2.54(1.91,3.03) | 2.27(1.37,2.84) | 2.95(1.80,3.50) | 0.229\(^b\) |
| BUN (mmol/L)   | 6.02(1.26)   | 7.28(3.84)   | 11.26(6.41)**| < 0.001\(^a\) |

Continuous variables were expressed as mean (standard deviation) and non-normally distributed variables were expressed as median (interquartile range). An ANOVA test was used to to compare differences of mean between 3 groups (\(^a\)). The Kruskal-Wallis test was used to compare differences of mean between 3 groups with normal distribution (\(^b\)).

\*\( P < 0.05 \) compared with control group, \**\( P < 0.01 \) compared with control group.

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; UAE, urinary albumin excretion; HbA1c, glycosylated hemoglobin; ALT, alanine transaminase; AST, aspartate transaminase; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; BUN, blood urea nitrogen; Cr, plasma creatinine; TC, total cholesterol; TG, triglycerides; UA, uric acid; ALB, plasma albumin.
As revealed from the results here, the serum ANP level in Macro-MA cohort was significantly higher than the other two cohorts (11.25 ± 4.05 ng/L and 13.58 ± 6.43 ng/L in cases subjected to normoalbuminuria and microalbuminuria, respectively). Interestingly, compared to cases subjected to normoalbuminuria, macroalbuminuria patients had apparently higher serum ADP ($p < 0.001$). Furthermore, the levels of TNF-α, IL-6 also increased as nephropathy progressed, with median serum TNF-α levels of 13.49 pg/mL in the normoalbuminuric cohort, 15.22 pg/mL in the Micro-MA cohort, and 18.28 pg/mL in the Macro-MA cohort. The median serum levels of IL-6 was 2.13 ng/L in the normoalbuminuric cohort, 2.20 ng/L in the Micro-MA cohort, and 3.21 ng/L in the Macro-MA cohort (Table 2).
Table 2

Plasma cytokines levels of patients according to the albuminuria categories

| Variable | Nor-MA   | Micro-MA  | Macro-MA  |
|----------|----------|-----------|-----------|
| ANP (ng/L) | 11.25(4.05) | 13.58(6.43) | 19.20(13.43)** |
| TNF-α (pg/mL) | 13.49(4.67) | 15.22(7.57) | 18.28(13.50)* |
| ADP (ug/L) | 4.96(2.52) | 5.55(2.74) | 7.96(6.16)** |
| IL-6 (ng/L) | 2.13(1.18) | 2.20(1.35) | 3.21(2.95)* |

*P < 0.05 compared with control group, **P < 0.01 compared with control group.

ANP, atrial natriuretic peptide; ADP, adiponection; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α.

The authors assessed the mentioned variables based on multiple variates-based analyzing processes, Table 3 lists the relevant results. Specific to the entire subject group, Pearson's or Spearman linear correlating process displayed a noticeable positive relationship of ANP and UAE (r = 0.235, p = 0.035), BUN (r = 0.235, p = 0.034), as well as plasma ADP (r = 0.772, p < 0.001), (Fig. 1), IL-6 (r = 0.816, p < 0.001), (Fig. 2) and TNF-α (r = 0.876, p < 0.001), (Fig. 3). An important correlation was noted between ANP and duration of DM based on simple regression study on research subjects. The authors reported noticeably negative relationship of ANP and plasma albumin (r = -0.267, p = 0.016). This study reported not any noticeable correlations of ANP and other variables covering BP, lipid profiles, glucose, liver enzyme and urea acid (Table 3). In univariate analysis, all the parameters listed in Table 4 received the judging process as displaying noticeable associations to DN developing process. In Model 1, because duration of diabetes was an independent risk element, we excluded it from the analysis, identifying all the parameters shown in this model as significant elements for DN development. In Model 2, the authors covered duration of diabetes, results showed that both UAE and BUN turned out to be not significant; plasma ANP and DM duration were correlated. For this reason, the authors considered duration of DM a powerful cofounder in the relationship between ANP and DN development. Furthermore, ADP, TNF-α and IL-6 still correlated independently with ANP (p < 0.05) after adjustment for duration of diabetes and BUN.
Table 3
Univariate linear regression analysis between serum ANP and the clinical parameters

| Variable      | r     | P       |
|---------------|-------|---------|
| Age           | 0.034 | 0.760a  |
| BMI           | 0.109 | 0.334b  |
| Duration      | 0.285 | 0.010a  |
| SBP           | 0.066 | 0.559a  |
| DBP           | 0.087 | 0.442a  |
| HbA1c         | -0.003| 0.978b  |
| UAE           | 0.235 | 0.035b  |
| TC            | 0.072 | 0.552b  |
| TG            | 0.106 | 0.346a  |
| HDL-C         | -0.023| 0.838b  |
| LDL-C         | 0.034 | 0.764b  |
| BUN           | 0.235 | 0.034a  |
| Cr            | 0.198 | 0.076a  |
| UA            | 0.123 | 0.274b  |
| ALB           | -0.267| 0.016b  |
| ADP           | 0.772 | <0.001a |
| IL-6          | 0.816 | <0.001a |
| TNF-α         | 0.876 | <0.001a |

*a* Pearson test  
*b* Spearman test  

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; UAE, urinary albumin excretion; HbA1c, glycosylated hemoglobin; ALT, alanine transaminase; AST, aspartate transaminase; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; Cr, plasma creatinine; BUN, blood urea nitrogen; TC, total cholesterol; TG, triglycerides; UA, uric acid; ALB, plasma albumin; ANP, atrial natriuretic peptide; ADP, adiponectin; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α.
Table 4

Multiple regression model for the determinants of serum ANP

| Independent variable | Model 1 |       |       |       | Model 2 |       |       |       |
|----------------------|---------|-------|-------|-------|---------|-------|-------|-------|
|                      | β       | P     | β     | P     |         | β     | P     |       |
| Duration (years)     | -       | -     | 0.049 | 0.044 |         |       |       |       |
| UAE (mg/24 h)        | 0.150   | 0.033 | 0.059 | 0.045 |         |       |       |       |
| TNF-α (pg/mL)        | 0.528   | < 0.001 | 0.519 | < 0.001 |       |       |       |       |
| ADP (ug/L)           | 0.233   | 0.001 | 0.224 | 0.001 |         |       |       |       |
| IL-6 (ng/L)          | 0.255   | 0.006 | 0.233 | 0.005 |         |       |       |       |
| BUN (mmol/L)         | 0.127   | 0.032 | 0.041 | 0.592 |         |       |       |       |

UAE, urinary albumin excretion; ANP, atrial natriuretic peptide; ADP, adiponection; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; BUN, blood urea nitrogen.

Discussion

To our knowledge, DN has been commonly confirmed by clinical symptoms. A large percentage of T2DM is unconventionally assessed with a renal biopsy and even though pathological diagnosis. T2DM cases subjected to albuminuria fail to diagnose to DN in clinical promptly. Moreover, progressive renal lesions in DN are considered difficult to reverse. Therefore, it is imperative to gain insights into albuminuria, and the subsequent analysis and exploration of new targets or predictive markers for DN. Given the information acquired by the authors, the present study initially demonstrates that serum inflammatory cytokines display an independent association with ANP in DN. As our excluded criteria, we ruled out cases subjected to any acute inflammation or infection, renal insufficiency, serious cardiac insufficiency and treated with thiazolidinediones. Therefore, we have tried to eliminate confounding elements which probably interfere with the association between ANP and inflammation cytokines.

The level of ANP was augmented in Macro-MA cohort opposed to Nor-MA cohort ($p < 0.001$). Moreover, the level of inflammatory cytokines was elevated obviously in Macro-MA cohort, which was accordance with several recent studies [18–20]. Plasma ANP extents were reported here displaying positive relationships to UAE, BUN, IL-6, TNF-α and ADP as well as negatively correlated with plasma albumin by simple regression. When the influence exerted by other variables based on partially-related correlation study, the existing relationships of ANP and duration of DM, IL-6, TNF-α and ADP level remained significantly, but not the relationship between ANP and BUN or UAE. To be specific, ADP, IL-6 and TNF-α are likely to be particularly important since the relationship of the ANP plasma extents and DN developing process was determined by inflammatory response.

ANP is a cardiac hormone provides a negative feedback to elevated blood pressure and fluid volume [3]. Robert et al. [21] proved ANP may well improve intravascular protein’s glomerular permeability in the glomerulus though infusion of synthetic human ANP in cases subjected to nephrotic syndrome. ANP dilate afferent arteriolar and constrict efferent arteriolar producing an increase in the glomerular capillary pressure and lead to a rise in fractional excretion of proteins [22]. For the foregoing reasons, ANP may induce microalbuminuria.
According to Desai et al. [23], the higher the plasma level of ANP was, renal function will be deteriorated more rapidly. We did not expect ANP was elevated with DN development, whereas there are several possible explanations for such finding. First, reduction of renal function with disease course may be required to be considered as capable of elevating fluid volume and also because left atrial pressure induces augments secretions of ANP. Second, the one major metabolic pathways of ANP involved binding to clearance receptors (NPR-C) [24]. As revealed from one recent study, the number of NPR-C on platelets in elderly subjects decreased, which could be associated with a reduction the synthesis of this receptor or a delayed recycle to the cell surface [25]. Third, ANP was knocked down by the neutral endopeptidase (NEP), the enzyme capable of being expressed in kidney. NEP expression in renal tissue showed the down-regulation among chronic renal failure patients. The mentioned eventually result in decreased clearance of ANP from circulation. Thus, ANP may participate in DN development. Nevertheless, precise mechanisms remain unclear.

ANP is capable of regulating lipolysis and lipid mobilization in humans. Moreover, it might regulate adipose tissue inflammation by regulating the secretion of inflammation cytokines [26, 27]. The elevation levels of TNF-α, IL-6 and ADP here showed strong positively correlations with ANP and the degree of albuminuria, abiding by existing studies [18, 19]. As revealed from one existing study, adipocytes probably expressed all components of the ANP-signaling pathway [16]. ANP inducted secretion of IL-6 and TNF-α via the guanylate cyclase-coupled A receptor (NPR-A) with mRNA coding expressed on macrophages [28]. Activation of the NPR-A receptor stimulates a rise in cGMP levels, activating protein kinase G; as a result, lipolysis is stimulated to IL-6 and TNF-α productions. ADP, a “conducive” adipokine withdraw inflammation, is reduced in cases subjected to diabetes, and high ADP levels were associated with a lower risk of developing T2DM [29–31]. Nerveless, ADP concentrations are higher in cases subjected to increasing albuminuria especially in Macro-MA cohort and positive predict process of this disease [32]. ADP is known to activate via the AMP-activated protein kinase, so dose ANP in adipocytes [33]. The similar effect was also indicated among healthy people, demonstrating that ANP raises ADP in dose-dependent manner [34]. The phenomenon complies with the possible mechanism that ANP directly preserving ADP counteracted ANP-induced lipolysis. One existing study also reported in a small clinical study that decompensated heart failure patients having undergone therapeutic ANP infusions had increased plasma levels of total and high molecular weight ADP [35]. As reported by recent studies, during DN development, increased production and excretion of inflammation cytokines result in aggravated glomerular hypertrophy and disappeared podocyte foot, which are activated and contribute to deteriorate kidney impairment [36]. As a result, ANP may promote progression of DN by stimulating secretion of inflammation cytokines. Further investigation ANP receptor antagonist or enzymes inhibitors can significantly improve and postpone the progress of DN.

Notably, HbA1c level was more decreased in Macro-MA than the other 2 cohorts. Some of the mentioned reasons could be explained that the patients who with longer duration of DM and with diabetic complications have stronger consciousness in blood glucose management. Besides, as suggested from a previous study, ANP may impact etiology of diabetes primarily due to inhibiting effect on glucagon secretion [37]. Moreover, Suzanne et al.[38] conducted the isolation of pancreatic islets in adult mice and showed that ANP enhanced insulin secretion under the stimulation of glucose and triggered β-cell growth.

This study has some limitations. First, the cross-sectionally-related designing analysis on baseline data of a cohort analysis and the restricted amount belonging to each cohort are likely to have causal relationship, and
more randomization-based and further analyses containing larger samples require in-depth explorations. Second, we cannot completely exclude the possibility that ANP influenced on clearance of renal or hepatic cytokines. Finally, only plasma cytokines have been measured, but at what stages ANP and other cytokines levels in urine were not investigated, probably help interpret the result here.

Conclusion

To sum up, increased circulating extents of plasma ANP, displaying relationships to duration of DM, could precede type 2 diabetic kidney disease developing process with no noticeable cardiovascularly-located disease, revealing one likely effect exerted by such bioactive peptide in monitoring DN developing process at early phase. ANP may exert a potential endocrine function though adipose tissue to regulate inflammatory cytokines amounts participating in pathogenesis and progression of DN.

Abbreviations

ANP, Atrial natriuretic peptide; CKD, chronic kidney disease; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; ADP, adiponectin; T2DM, type 2 diabetes; Cr, serum creatinine; BUN, blood urea nitrogen; DM, duration of diabetes mellitus; CKD, chronic kidney disease; UAE, urine albumin excretion; ALT, alanine transaminase; AST, aspartate transaminase; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; UA, uric acid; HbA1c, glycosylated hemoglobin.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Nanjing First Hospital, Nanjing Medical University with the approval number 2016-Research-28. Due to the retrospective nature of the study, the requirement to obtain informed consent was waived.

Consent to publish

Not applicable.

Availability of data and materials

Our data will not be shared due to it was involved in individual privacy and needed further study.

Competing interests

The authors declare that they have no competing interests

Funding
No funding was obtained for this study.

Authors' Contributions

CL, Qi L and Qian L designed experiments. CL, Qi L and XF performed experiments. CL clinically managed the patient, conducted literature review and wrote the manuscript. Qi Li helped with the acquisition and interpretation of data and with manuscript revisions. Ashley Shane Vadamootoo helped to polish the language. CL and Qi L analyzed the data. Qian L and JZ guaranteed this work and provide academic guidance. All authors read and approved the final manuscript.

Acknowledgements

We would like to thank the members of the Central Laboratory of Nanjing First Hospital Affiliated to Nanjing Medical University and the Department of Endocrinology for sharing ideas. The authors thank all the patients and staff who participated in this study.

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