Original Article

# Retinol Binding Protein-4 and Procollagen III N-Terminal Peptide as Indicators of Nephropathy in Type 2 Diabetic Patients



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## ABSTRACT

**Objective:** To investigate the correlation between the parameter levels (Retinol Binding Protein-4 and Procollagen III N-Terminal Peptide) and multiple risk factors including resistant to insulin, HbA1c, overweight, obesity, eGFR and Urine albumin to creatinine ratio with linking these results with those of individuals in good health.

**Study Design:** Case control study

**Place and Duration of Study:** This study was conducted at the College of Medicine, Al-Nahrain University, Baghdad-Iraq from 1st March 2024 to 31st October 2024.

**Methods:** One hundred and thirty five 135 individuals were enrolled. The study population consisted of 45 healthy persons serving as a control group, 40 persons type-2 diabetes mellitus with normoalbuminuria, and 50 persons with T2DM nephropathy presenting microalbuminuria and macroalbuminuria.

**Results:** Both procollagen III N-terminal peptide and retinol binding protein- 4 levels were markedly increased in diabetic nephropathy. The levels in the blood of retinol binding protein-4 among patients along with type-2 diabetic nephropathy was markedly elevated compared to healthy individuals and type 2 diabetes patients without nephropathy. ($p < 0.001$). Moreover, those with type-2 diabetes that shows nephropathy demonstrated a significantly elevated serum procollagen III N-terminal peptide value compared to those without nephropathy, individuals with control subjects ($p < 0.001$).

**Conclusion:** Procollagen III N-terminal peptide and Retinol binding protein- 4 levels in diabetic nephropathy increase in proportion to the disease's progression. Moreover, individuals with elevated Retinol binding protein-4 levels and type-2 diabetes are at a higher risk of suffering from nephropathy previous in their illness course

**Key Words:** Retinol binding protein-4 (RBP-4), Procollagen III amino terminal peptide (PIIINP), Urine albumin to creatinine ratio (UACR), Type 2 diabetes mellitus, Diabetic nephropathy

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## INTRODUCTION

Type two diabetes mellitus is a long-term, multisystem condition and a considerable global worldwide health risk that regularly decreases the value of life. Diabetes-associated kidney disease (DKD) is characterized as a specific category of ongoing kidney damage resulting from type-2 diabetes, and these two terms are utilized simultaneously through this article.

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An integrated approach encompassing education, self-management, changes in lifestyle, medical intervention, cardiovascular disease early detection, and emotional assistance has become essential for reducing the rate of and development of diabetes-related kidney disease (DKD).[1] Hyperglycemia and hypertension are major factors contributing to the beginning of diabetic kidney damage ; therefore, optimizing the regulation of glucose levels and lowering blood pressure levels are essential for preventing the initial development of DKD (primary prevention) and/or mitigating its progression and associated complications (secondary prevention).[2]

Retinol binding protein 4, considered to be the key retinol transporter in bloodstream, appears primarily in the liver cells and is found in comparatively lower concentrations in the fat cells and muscular myocytes, RBP-4 enhances the migration of retinol from hepatic cells to peripheral target organs.[3] The correlation between heart disease, metabolic syndrome, diabetes, specifically type-2 and sensitivity to insulin, elevated RBP-4 levels, as well as inflammation.[4] A major need in this research area is the creation of quantitative

indicators for insulin resistance and complication of nephropathy, including biochemical biomarkers. Recent research indicates that circulating PIIINP may be linked to inflammations[5], the risk of cardiovascular mortality[6] and patients with hypertension.[7] The important component of collagen synthesis is PIIINP, particularly the aminoterminal peptides, it is partially cleaved by a specific enzyme procollagen proteinase during collagen maturation. Consequently, it is released into the bloodstream during the decomposition of collagen fibers[8] and inflammatory conditions, including pulmonary fibrosis, acromegaly, and rheumatoid arthritis.[9] Many research indicates that PIIINP levels elevate in persistent liver illnesses, pulmonary disorders, and heart conditions such as heart disease, stroke, and coronary artery disease[10] and are additionally linked with skeletal muscle repair and growth, associated with collagen production, which is critical for muscle regeneration and remodeling following injury or prolonged physical activity. Therapies using both growth hormone and testosterone could stimulate collagen synthesis, resulting in elevated levels of PIIINP.[11]

# METHODS

This case-control study was comprised 135 individual and conducted at College of Medicine, Al-Nahrain University, Baghdad-Iraq from 1st March 2024 to 31st October 2024. The study population consisted of 45 healthy persons serving as a control group, 40 persons type-2 diabetes mellitus with normoalbuminuria and 50 persons with T2DM nephropathy presenting microalbuminuria and macroalbuminuria. In the present investigation, involved overall 95 patients diagnosed with type-2 diabetes mellitus, with and without nephropathy, and individuals who did not have diabetes mellitus were included. The renal disease in the end stage (ESRD), heart disease, cancer, thyroid, pregnancy, disorders, and liver disease. Furthermore, patients diagnosed with diabetes with type 1 mellitus have been accepted for the study excluded.

Everyone in the group underwent surveys that gathered demographic and baseline information, encompassing their sex, age, medical history, smoking status, hypertension, BMI, height, and tests for blood, all of which were then submitted for audit. The evaluation is advised for both the patients and the experimental cohort, and consent has been obtained. The venous fasting samples of blood, about between six and eight milliliters, were drawn from each subject in the morning during a 12-hour fast using a syringe that was disposable and promptly stored at –20 °C. All of the blood samples were divided into two distinct portions. Initially, two ml of human blood were obtained in tubes containing EDTA for HbA1c measure. Furthermore, six ml of whole blood specimens were obtained in a gel tube and permitted to remain stationary for 20 minutes at ambient temperature. After coagulation, isolate the clot using centrifugal force at 2,000-3,000 revolutions per minute for 20 minutes. Urine samples were promptly collected from both patients and controls and will be packed into sterile containers. The current investigation incorporates assays to quantify the levels of albuminuria and creatinine in urine. The study participants were assessed using the urine albumin-to-creatinine ratio (ACR), a physiological indicator for kidney disease, classified as being normal (<30 mg/g), microalbuminuria (30 to 299 mg/g), and macroalbuminuria (≥300 mg/g). Serum levels of glucose, urea, creatinine, and lipid parameters were immediately evaluated using the Cobas Roche 311. Serum levels of insulin and indicators of disease, especially RBP-4 (SunLong Biotech, China) and PIIIN-P (SunLong Biotech, China), were determined in serum samples kept at -20°C using ELISA technique.

The statistical assessment was carried out using SPSS-26.0. To assess the degree of association between two numerical variables, the Pearson correlation coefficient was calculated. The statistically significant level was determined at an appropriate p-value threshold of $p < 0.05$. The diagnostic importance of the indicators was determined by calculating the area under curve the Receiving operating characteristic curve studies.[12]

# RESULTS

The study population's characteristics showed in table 1. Table 2 provides an illustration of the clinical features of the research population. Subjects were divided into a total of three categories: normoglycemic, type-2 diabetes mellitus with albumin in the urine and type-2 diabetes mellitus without albuminuria. There were no statistically significant variations in age were seen across the research groups in relation to those in the control group. Individuals with type-2 diabetes mellitus, either with or without albuminuria, have increased levels of the body's mass index, FBS, HbA1c, HOMA-IR, LM, PIIINP, and UACR, as demonstrated in Tables 2, 3 and 4 and Figures 1 and 2. Decreased levels of eGFR, a significantly significant difference was observed in RBP-4, PIIINP, FBS, and HbA1C, among the four groups ($p < 0.001$)

The correlation between medical and laboratory elements along with serum levels of each indicator RBP-4 and PIIINP. The next stage of this research involves evaluating the association between blood levels of PIIINP, RBP-4 and other parameters across all demographic categories by Pearson correlation coefficients analysis (Table 5).Serum PIIINP had a substantial positive connection with RBP-4 ($p=0.016$, $r=0.53$), FBS ($p=0.004$, $r=0.275$), HbA1C ($p=0.019$, $r=0.185$), HOMA ($p=0.0027$, $r=0.283$), and UACR ($p<0.001$, $r=0.551$). and shown a substantial negative connection with eGFR ($p = 0.009$, $r = -0.302$).Serum RBP-4 demonstrated a positive and

statistically significant correlation with age (p=0.037, r=0.31), duration of diabetes (p=0.011, r=0.47), and UACR (p<0.018, r=0.52). and shown a significant negative connection with eGFR (p< 0.012, r = -0.66).

**Table No.1: Details about the socioeconomic background of the research group**

| Variables | Control (n=45) | T2DMW.A.(n=40) | T2DM.Mi (n=25) | T2DM.Ma (n=25) | p –value |
|---|---|---|---|---|---|
| Ages (years) (Range) | 51.63±5.46 (44 – 65) | 53.09±7.18 (48-65) | 56.31±6.38 (52-67) | 57.98±3.66 (48-70) | 0.255 |
| BMI ($k/m^{2)}$ (Range) | 24.4±1.93 (18.8-26.1) | 27.6±3.84 (20.5-28.6) | 30.6±2.86 (25-33) | 34.28±3.8 (30-37) | <0.01 |
| Duration years (Range) | -------- | -------- | 14.2±3.91 (8.0-21) | 17.18±3.70 (11.0-23) | <0.05 |
| **Hypertension** | | | | | |
| Yes | 45 (100%) | 34 (75.5%) | 7 (28%) | 2 (8%) | <0.001 |
| No | - | 11 (24.5%) | 18 (72%) | 23(92%) | |

**Table No.2: Comparison of the research groups' biochemical parameters**

| Variable | Group 1 (n=45) | Group 2 (n=40) | Group-3 (n=50) | P value |
|---|---|---|---|---|
| FBS (mg/dl) | 96.9±6.98 | 200±50.16 | 259±41.43 | <0.01 |
| HbA1C (%) | 5.18±0.28 | 7.7±1.42 | 9.22±1.01 | <0.001 |
| TC (mg/dl) | 169.00±11.5 | 205.4±25.5 | 208.70±28.0 | <0.001 |
| TG (mg/dl) | 114.00±11.1 | 162.6±32.96 | 191.2±41.84 | <0.001 |
| HDL (mg/dl) | 49.70±8.03 | 41.00±7.05 | 33.16±4.00 | <0.01 |
| LDL (mg/dl) | 91.07±7.00 | 109.30±18.81 | 124.1±13.39 | <0.01 |
| VLDL (mg/dl) | 22.79±2.23 | 31.75±7.22 | 38.24±8.39 | <0.01 |
| HOMA-IR (µU/ml) | 2.03±0.27 | 8.80±2.36 | 15.77±3.38 | <0.001 |
| eGFR ml/min/1.73m2 | 122.0±28.71 | 102.90±18.72 | 48.81±10.96 | <0.001 |
| UACR (mg/g) | 15.24±7.524 | 19.37±5.163 | 355.6±34.06 | <0.001 |

n: the quantity of encounters; data presented as mean & standard deviation; One-way ANOVA; Post hoc Tukey's test; Highly significant at P < 0.001 or 0.01; significant at P ≤ 0.05

**Table No. 3: The result for procollagen III N-terminal peptide in all study groups**

| Indicator/Groups | G1 (n=45) | | G2 (=40) | | G3 (50) | |
|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % |
| **PIIINP (pg/ml) FR (%)** | | | | | | |
| Below N (<13) | - | - | - | - | - | - |
| Normal (13-800) | 43 | 95.5 | 29 | 72.5 | 14 | 28.0 |
| Above N (>800) | 2 | 5.5 | 11 | 27.5 | 16 | 72.0 |
| Mean ± SD | 360.3±57.5 | | 721.6±80.0 | | 860.9±47.67 | |
| p-value | G1/G2: <0.01 | | G1/G3:<0.001 | | G2/G3: p<0.05 | |

n: the number of experiences; data provided as mean and standard - deviation; One-way ANOVA; Post hoc Tukey's test; very significant at P < 0.001 or 0.01; significant at P ≤ 0.05

**Table No.4: Retinol binding protein-4 outcome for every investigation groups**

| Indicator/Groups | G1 (n=45) | | G2 (=40) | | G3 (50) | |
|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % |
| RBP-4 (ng/ml) FR(%) | | | | | | |
| Below-Normal (<0.1) | 4 | 8.9 | | | | |
| Normal (0.1-8) | 40 | 88.9 | 32 | 80.0 | 13 | 26.0 |
| Above - Normal (>8) | 1 | 2.2 | 8 | 20.0 | 37 | 74.0 |
| Mean ± SD | 6.20±0.04 | | 7.99±0.28 | | 10.08±0.33 | |
| p-value | G1/G2: <0.05 | | G1/G3: <0.01 | | G2/G3<0.05 | |

n: the quantity of encounters; data presented as mean & standard deviation; One-way ANOVA; Post hoc Tukey's test; Highly significant at p< 0.001 or 0.01; significant at P ≤ 0.05

**Table No. 5: The relationship between PIIINP, RBP-4, and the laboratory and clinical indicators**

| Parameters | PIIINP | | RBP-4 | |
|---|---|---|---|---|
| | P | r | P | r |
| Age (years) | 0.848 | 0.015 | 0.037 | 0.310 |
| Duration of disease | | | 0.011 | 0.47 |
| PIIINP | | | 0.016 | 0.530 |
| RBP-4 | 0.016 | 0.530 | | |
| FBS (mg/dl) | 0.004 | 0.275 | 0.6 | 0.10 |
| HbA1C (%) | 0.019 | 0.185 | 0.02 | 0.17 |
| HOMA-IR (µU/ml) | 0.0027 | 0.283 | 0.0027 | 0.283 |
| eGFR ml/min/1.73m2 | 0.0097 | -0.302 | 0.009 | -0.302 |
| UACR (mg/g) | <0.001 | 0.551 | 0.018 | 0.52 |

Significant difference P < 0.05

**Table No.6: Shows the ROC and cutoff values for RBP-4 and PIIINP in the groups under study**

| Marker | AUC | SE | 95%CI | Cutoff | Sensitivity | Specificity |
|---|---|---|---|---|---|---|
| RBP-4 | 0.998 | 0.002 | 0.955 to 1.000 | >3.29 | 100.00 | 97.70 |
| PIIINP | 0.970 | 0.023 | 0.910 to 0.994 | >587.00 | 100.00 | 97.52 |

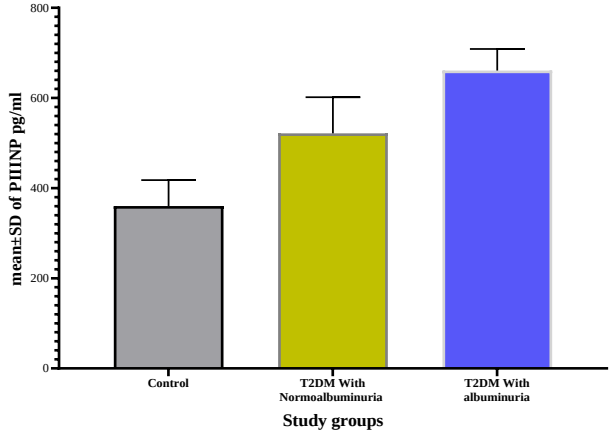


**Figure No. 1: Serum levels of procollagen III N-terminal peptide in each study groups**

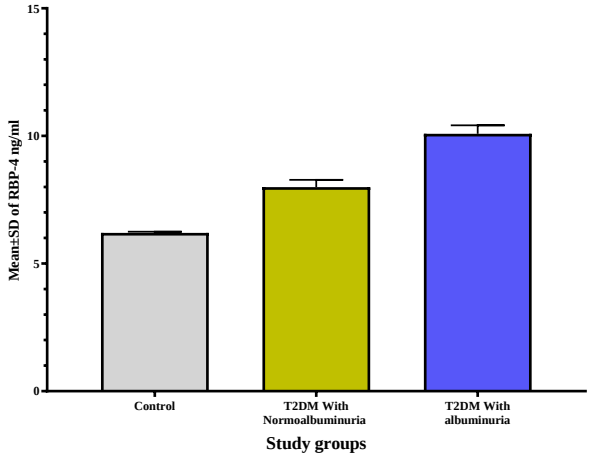


**Figure No. 2: Serum levels of Retinol binding protein-4 in each study groups**

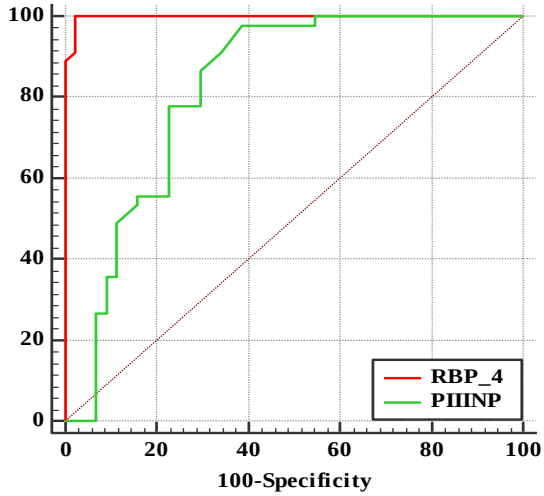


**Figure No. 3: ROC curve and cutoff values for RBP-4 and PIIINP in the study groups**

A receiver operating characteristic (ROC) curve analysis was performed due to the significant difference in RBP-4 and PIIINP amounts between individuals with Type 2 diabetes groups, both with and without albuminuria. The findings revealed excellent discriminatory power is demonstrated by RBP-4 AUC of 0.998, sensitivity of 100% and a specificity of 97.7%. The area under the curve 0.970 for PIIINP indicates high discriminating power. The cutoff is >587, with a specificity of 97.52% and a sensitivity of 100% (Fig. 3, Table 6)

# DISCUSSION

This case-control study revealed that the combination of RBP-4 levels and PIINP values can be utilized to

diagnose diabetic nephropathy, identifying RBP-4 + PIIINP as a unique independent marker for nephropathy. The principal conclusions of this study are as follows: Initially, ROC curve analysis revealed that the AUC of the RBP-4 group surpassed that of the individual PIIINP groups, indicating that the diagnostic accuracy for diabetic nephropathy utilizing combined RBP-4 and PIIINP data was superior to that of RBP-4 or PIIINP alone. In this Iraqi cohort, we identified an excellent dependent on concentration correlation between elevated plasma RBP-4 and PIIINP levels and a simulated complication of type 2-diabetes such as nephropathy. In the past few years, the adipokine RBP-4 was being investigated as a possible indicator of type 2-diabetic mellitus.[13] It has performed a series of tests to elucidate the relationship between RBP-4, T2DM, and diabetic nephropathy, particularly its influence on resistance to insulin and pancreatic β-cell functionality. The findings of these researches demonstrate that RBP-4 significantly impacts T2DM, with nephropathy identified as a potential biomarker for T2DM sequelae, particularly diabetic nephropathy. Nonetheless, debates have arisen regarding the association between RBP-4 levels and type 2 diabetes mellitus with nephropathy.

There exists a clinical requirement for detecting humans at risk of complications from type 2 diabetes mellitus using simple, accessible, and cost-effective techniques. This study investigated the association of the type III collagen production marker PIIINP with risk variables for diabetic nephropathy and its potential to predict future occurrences of the condition. A relationship was identified between higher plasma PIIINP levels in individuals with type 2 diabetes mellitus, both with and without nephropathy. Individuals with type-2 Diabetes (T2DM) frequently demonstrate resistance to insulin (IR), reduced tolerance for glucose, lipid disorders, and hypertension.[1] The results correspond with the present investigation, which demonstrated increased levels of triglyceride, total cholestrol, HOMA-IR, low density lipoprotein, and VLDL in both T2DM and diabetic nephropathy (DN) groups (Table 1). Hyperglycemia and high HbA1c amounts adversely affect profiles of lipid and heighten the development of type 2 diabetic and diabetic nephropathy.

Lipid profiles elevate the probability of developing type 2 diabetes mellitus and lipid disorders. Rise levels of free fatty acid (FFA) enhance triglycerides (TG) synthesis, which results in heightened production of apolipoprotein B, also known as (ApoB) and low-density lipoprotein. The hormone insulin generally facilitates the degradation of ApoB through the activation of phosphatidylinositol-3 kinase; however, this mechanism is compromised in insulin-resistant circumstances, thereby elucidating the increased triglyceride levels observed in such states.[14]

In this study, we identified an excellent dependent on concentration correlation between elevated plasma RBP-4 and PIIINP levels and a simulated complication of type 2-diabetes such as nephropathy. Our study is primarily constrained by rather tiny numbers of participants and the lack of the longitudinal follow-up. This study is, to our knowledge, the first to incorporate levels of PIIINP and UACR in patients with diabetic nephropathy. Higher PIIINP and RBP-4 concentration than in the control group, the diabetic nephropathy has changed both RBP-4 and PIIINP.

To highlight the great relevance of this indicator in diabetes nephropathy, more cohort research on bigger groups among individuals with type-2 diabetes nephropathy and determination of the kidneys activity in subgroups of individuals with different type 2 diabetic stages is necessary.

# CONCLUSION

For immediately detection and follow-up of nephropathy across people who have type-2 diabetes, PIIINP and RBP-4 are useful biomarkers. Their complementing capacity to reflect renal fibrosis and tubular dysfunction respectively helps to control DN. Their extensive usage will be made possible by further investigation and clinical validation, hence possibly changing the field of diabetic nephropathy diagnosis and management.

**Author's Contribution:**

| | |
|---|---|
| Concept & Design or acquisition of analysis or interpretation of data: | Amanj Zrar Hasan, Mohammed I. Hamza |
| Drafting or Revising Critically: | Amanj Zrar Hasan, Mahmood Shakir Khudhair |
| Final Approval of version: | All the above authors |
| Agreement to accountable for all aspects of work: | All the above authors |

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# REFERENCES

1. DeFronzo RA, Reeves WB, Awad AS, Pathophysiology of diabetic kidney disease: impact of SGLT2 inhibitors. Nature Rev Nephrol 2021;17(5):319-34.
2. ElSayed NA. Diabetic kidney disease prevention care model development. Clin Diabetes 2024; 42(2):274-94.
3. Huang R, et al. Retinol-binding protein 4 activates STRA6, provoking pancreatic β-cell dysfunction in type 2 diabetes. Diabetes 2021;70(2):449-63.

4. Altaie AF. Correlation study of retinol binding protein4, vitamin a with liver function enzymes in iraqi fracture patients with and without DM2. Ibn AL-Haitham J Pure Appl Sci 2022;35(4):151-60.
5. Yi JY, et al. One-step detection of procollagen type III N-terminal peptide as a fibrosis biomarker using fluorescent immunosensor (Quenchbody). Analytica Chimica Acta 2024:9:342887.
6. Genovese F, et al. Plasma levels of PRO-C3, a type III collagen synthesis marker, are associated with arterial stiffness and increased risk of cardiovascular death. Atherosclerosis 2024;388: 117420.
7. Mohammed HF, Jaber SH, al-Zubaidi AJ. Precollagen type III N-terminal propeptide (PIIINP) a Biomarker Onset in Hypertensive Patients. Med Sci J Advan Res 2020;1(01):25-8.
8. Pellegrino R, et al. Muscle quality, physical performance, and comorbidity are predicted by circulating procollagen type III N-terminal peptide (P3NP): the InCHIANTI follow-up study. Geroscience 2024;46(1):1259-69.
9. Chu YH, et al. The intervention of curcumin on rodent models of hepatic fibrosis: A systematic review and meta-analysis. Plos one 2024;19(5): e0304176.
10. Lopez-Salazar MB, Gonzalez A, Diez-Martinez J. Circulating biomarkers of collagen metabolism in cardiac diseases. Circulation 2010;121(14): 1645-54.
11. Shin HE, Kim M, Won CW, Association between plasma procollagen type III N-terminal peptide (P3NP) levels and physical performance in elderly men: The Korean Frailty and Aging Cohort Study (KFACS). Experimental Gerontol 2021;154: 111523.
12. Bland M. An introduction to medical statistics. Oxford: Oxford University Press; 2015.p.412-8.
13. Fan J, Hu J, Retinol binding protein 4 and type 2 diabetes: from insulin resistance to pancreatic β-cell function. Endocrine 2024; 85(3): 1020-34.
14. Rooiqoh QF, Tamtomo DG, Cilmiaty R. Blood sugar levels and lipid profiles of T2DM among hypertension patients in Bambanglipuro Health Centre. ESHR 2022;4(1):29-35.