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ORIGINAL ARTICLE

Plasma miRNA as a Predictive Biomarker of Diabetic Nephropathy

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ABSTRACT

Keyword: miRNA-155 5P; albuminuria; diabetes mellitus; PCR.

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Background: The most reliable method for diagnosing diabetic nephropathy (DN) is renal biopsy, which carries a significant risk of bleeding problems and is a highly intrusive and costly surgery. Thus, DN requires sensitive and trustworthy biomarkers. Owing to their stability in plasma and their involvement in various biological processes, miRNAs may be helpful in the early diagnosis of diabetic nephropathy. **Objectives:** This research sought to explore the expression patterns and clinical relevance of plasma miRNA 155-5p in the early diagnosis and advancement of DN. **Subjects and methods:** Ninety participants were divided into 3 equal groups according to their urine ALB level to: (healthy controls, Diabetic patients (normoalbuminuria) and Diabetic patients (microalbuminuria). **Results:** Our findings revealed significant downregulation of miR-155 5p expression between the diabetic (microalbuminuria) group and the other groups and between the diabetic (normoalbuminuria) group and the healthy control group and a significant positive correlation between miR-155 5p expression and the eGFR and a strong significant negative correlation between miR-155 5p expression and the urinary protein exertion rate. **Conclusions:** As miRNAs participate in different biological processes and are stable in plasma, they may be useful for the early detection of DN.

INTRODUCTION

End-stage renal disease (ESRD) is mostly caused by diabetic nephropathy (DN), one of the most harmful long-term microvascular effects of diabetes [(1).] Traditionally, the severity of DN is assessed by urinary albumin excretion measurements, such as the albumin-to-creatinine ratio (ACR).

While persistent macroalbuminuria is thought to be a predictor of end-stage renal disease (ESRD), microalbuminuria is usually thought of as a sign of DN. Recent research on the sensitivity and specificity of urine ALB excretion for DN, however, has produced conflicting results. Therefore, in order to assess the onset and progression of DN for early intervention, a trustworthy instrument is required [(2).] A common class of unbranched polymeric molecules known as ribonucleic acids (RNAs) act as intermediaries in the process of transferring amino acids to ribosomes via transfer

RNA (tRNA), decoding genetic information from DNA to ribosomes via messenger RNA (mRNA), and transferring ribosomal RNA (rRNA) during protein synthesis. MicroRNAs (miRNAs) are a novel family of noncoding RNA molecules that were discovered in 1993. Through their modulation of mRNA stability and translational efficiency, these small molecules play crucial roles in the regulation of the cell growth cycle, differentiation, and survival. In the past 20 years, the function of miRNAs in maladaptive repair and a variety of illnesses has been clarified. Furthermore, miRNAs have been investigated as possible targets for therapy as well as for the diagnosis and prognosis of disease [(3).] Endogenous noncoding single-stranded RNAs with 21–25 nucleotides are called microRNAs. MicroRNAs have the ability to destroy messenger RNAs and stop proteins from translating. Furthermore, they significantly regulate the development and course of kidney disease. The microRNA miR-155-5p dramatically improves renal tubular selectivity in patients with diabetic nephropathy [(4).] MicroRNA 155-5p plays a critical function in the development of DN and is significantly overexpressed in the renal tubules of patients with the disease [(5).]

SUBJECTS AND METHODS

Study design: This case control study was carried out at medical biochemistry department, Aswan University and the participant cases & controls was selected from the attendee at nephrology department and internal medicine outpatient clinic, Aswan University Hospital.

Ethical consideration: This study was approved by the ethical committee of the faculty of medicine, Aswan University (**EC Ref NO :**Asw.Uni./616/3/22), and all the participants provided written informed consent prior to participating in the study.

Eligible participants:

Study subjects:

- Group A: Sixty diabetic patients were subdivided into two subgroups according to urinary ALB levels :

A1: Patients with normal albuminuria <30 mg

A2: Patients with microalbuminuria (30:300) mg

✓ Exclusion criteria: those who had ketoacidosis, any autoimmune disease, known thyroid diseases, trauma, hepatitis C virus, hepatitis B virus(HBV) or human immunodeficiency virus (HIV) (+ve) infection , pregnancy, malignancy, patients with macroalbuminurea and patients who refused to participate.

- Group B: Thirty healthy control subjects, recruited from staff or relatives of the patients matched for both age and sex served as the control group.

All the included participants were subjected to the following :

- Data on medical history, along with details about sex, age, height, weight, disease duration, blood pressure, and glycemic control, were gathered from completed questionnaires provided by patients.

- 10 ml of venous blood drained from the antecubital vein divided into 2 tubes (plain tubes & EDTA tubes) 5ml each.
    - One milliliter of the EDTA sample was used to measure hemoglobin A1c (HbA1c).

- The other Nine milliliter of each sample was centrifuged at 3000 rpm/1000 x g for 3 minutes to get plasma & serum and then were stored at -80°C until batch analysis.

✓ In plasma: used for plasma detection of miRNA -155-5p via real time PCR.

✓ In serum: tube was used to evaluate kidney function.

➢Urine sample: A well-mixed (5mL) (1st morning) urine sample will be used for determination of microalbuminuria.

Statistical analysis: Data analysis was performed via SPSS (Statistical Package for Social Science) software version 21.0 (SPSS Inc., Chicago, IL).

RESULTS

Ninety participants were split into three equal groups according on their urine ALB levels for the study: healthy controls, diabetic patients (normoalbuminuria) and diabetic patients (microalbuminuraia). The studied patients were admitted to the nephrology department & internal medicine outpatient clinic at Aswan University Hospital, Egypt, and controls were matched with respect to sex, socioeconomic state and educational level recruited from outpatient clinics, hospital personnel and volunteers.

Table (1) demonstrates the demographic information of the three study groups' members. The mean age was 51.92 ± 11.71 years ranged from 26 - 70 years. Most patients were females (60%) and males (40%).

Table (2) shows the results of the laboratory investigations for the whole study group. The mean HbA1c was 7.34 ± 2.13 % and ranged from 4.3 to 11.84 % ,the mean eAG was 163.83 ± 61.15 mg/dl and ranged from 77 to 292 mg/dl, the mean urea level was 35.17 ± 19.85 mg/dl and ranged from 16.1 to 120 mg/dl ,the mean creatinine was 1.13 ± 0.67 mg/dl and ranged from 0.4 to 4.5 mg/dl ,the mean eGFR was 78.71 ± 27.87135 mL/min/1.73 m^2 and ranged from 11 to 135 135 mL/min/1.73 m^2 and the mean MAU level was 57.31 ± 99.25mg and ranged from 2 to 650 mg.

Table (3) presents the gene expression data for the whole study group;the mean gene expression was 4.34 ± 1.1 and ranged from 2.21 to 5.69.

Table (4) shows The gene expression in the three groups, there was a notable decline in gene expression among the diabetic group (microalbuminuria) and the other groups and between the diabetic group (normoalbuminuraia) and the healthy control group (P value <0.05).

Table (5) shows the correlation between miRNA-155 5P expression and other laboratory investigations. There were moderate statistically significant negative correlations between miRNA -155 5P expression and HbA1c, eAG and urea, whereas there was a moderate statistically significant positive correlation with the eGFR. However, there were strong statistically significant negative correlations between gene expression and creatinine and MAU with P Values <0.001 for all the parameters.

Table (6) According to the ROC curve for the ability of miRNA-155 5P expression to predict diabetic nephropathy cases, it could significantly predict diabetic nephropathy cases when it was ≤3.4501, with a sensitivity of 100% and a specificity of 100% , with a P value <0.001.

Table 1: Demographic data for the whole study group.

		Mean ± SD N (%)	Median (IQR)	Range
Age		**51.92 ± 11.71**	**53 (45 - 62)**	**(26 - 70)**
Sex	**Male**	**36 (40%)**		
	Female	**54 (60%)**		

Table 2: Laboratory investigations of the whole study group.

	Mean ± SD	Median (IQR)	Range
HbA1c %	**7.34 ± 2.13**	**6.96 (5.6 - 8.8)**	**(4.3 - 11.84)**
eAG mg/dl	**163.83 ± 61.15**	**153 (114 - 206)**	**(77 - 292)**
Urea mg/dl	**35.17 ± 19.85**	**28.75 (21 - 42)**	**(16.1 - 120)**
Creatinine mg/dl	**1.13 ± 0.67**	**0.9 (0.76 - 1.18)**	**(0.4 - 4.5)**
eGFR mL/min/1.73 m2	**78.71 ± 27.87**	**88 (62 - 97)**	**(11 - 135)**
MAU mg	**57.31 ± 99.25**	**15 (7.2 - 58)**	**(2 - 650)**

Table 3: expression of miRNA-155 5P in the whole study group.

	Mean ± SD	Median (IQR)	Range
miRNA155-5P expression	**4.34 ± 1.1**	**4.64 (3.26 - 5.27)**	**(2.21 - 5.69)**

Table 4: The gene expression in the three groups.

	Group			Test of significance		
	Healthy controls	Diabetic (normoalbuminuraia)	Diabetic (Micro albuminuraia)	Controls Vs. Diabetic (normoalbuminuria)	Controls Vs. Diabetic (Micro albuminuria)	Diabetic (normoalbuminuraia) Vs. Diabetic (Micro albuminuraia)
	Mean ± SD	Mean ± SD	Mean ± SD	P value (Sig.)	P value (Sig.)	P value (Sig.)
miRNA 155-5P expression*	5.43 ± 0.18	4.65 ± 0.4	2.95 ± 0.4	<0.001 (S)	<0.001 (S)	<0.001 (S)

Table 5: Correlations between miRNA -155-5P expression and laboratory investigations.

miRNA 155- 5P expression	HbA1c	eAG	Urea	Creatinine	eGFR	MAU
Pearson Correlation	-0.477	-0.477	-0.541	-0.608	0.526	-0.767 (S)
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table 6: ROC curve for miRNA 155- 5P expression to predict diabetic nephropathy patients.

AUC	95% CI	Sig.	Cut off value	Sensitivity	Specificity	+PV	-PV
1.00	0.960 to 1.00	<0.001	≤3.4501	100	100	100	100

DISCUSSION

The expression profile and clinical relevance of plasma miRNAs during DN were investigated in this study. Diabetic patients with microalbuminuria and normoalbuminuria had their plasma miRNA levels compared to healthy controls. Compared to controls and diabetes patients with normoalbuminuria, the plasma levels of miR-155-5p are significantly lower in patients with microalbuminuria. These findings support recent findings [(6)] and show that these miRNAs' plasma levels are lowered in the early phases of diabetic nephropathy. But according to Zhou et al. (2021), hyperglycemia raised the expression of miR-155-5p both in vitro and in vivo, which is comparable to the observed rise in renal miR-155 expression in DN in people [(7).] In addition, a study on a smaller patient group found that patients with diabetic nephropathy had higher levels of miR-155-5p in their glomeruli and renal tubules than did healthy individuals. Patients with diabetic nephropathy in two trials had higher serum levels of miR-155. [(8).] There have been reports of reduced expression of miR-155 in patient serum and peripheral blood mononuclear cells (PBMCs) as compared to the control group [(9).]

However, several studies have shown that glomerular mesangial cells (GMCs) in humans and rats have significantly higher expression of miR-155 when high-glucose is present. Remarkably, cells lacking TLR4 show blunted upregulation of miR-155, suggesting that inflammation is a key regulator of miR-155 transcription. This viewpoint is supported by the fact that proinflammatory cytokines cause human GMCs to express miR-155 more frequently [(7).] Furthermore, study of clinical indicators, such as GFR, also showed increased expression of miR-155, and assessment of miR-155 5p expression in the serum and urine of nephrolithiasis patients showed considerably higher expression compared to healthy persons.Reduced renal filtration is linked to it [(10).]
Additionally, a study of patients with IgA nephropathy found that the higher levels of miR-155 in their urine and kidney biopsies were associated with the severity of their disease [(11).] Interestingly, our findings showed a strong significant negative association between miR155-5p expression and the urine protein excretion rate ($r = -0.767$ P value < 0.001) and a substantial positive correlation between miR-155-5p expression and the GFR ($r = 0.526$ and P value < 0.001). These findings completely concur with those of Wang et al. [(6),] who hypothesized that the onset of renal impairment would be linked to a decline in the miR155-5p level.
According to a recent study, urine vitamin D binding protein (VDBP) and serum miR 155 5p are strongly expressed in DN patients, and there is a positive correlation ($P<0.05$) between urine VDBP, serum miR 155 5p, mAlb, Cys C, and 24-hour urine protein [(12).] There was a significantly negative association between creatinine and relative miR-155-5p expression, as measured by correlation with the outcomes of other laboratory tests. These results align with those of Huang et al. [(8).] who found that miR-155 is a reliable indicator of kidney damage.
Additionally, a substantial inverse relationship was seen between estimated average glucose and relative miR-155-5p expression. On the other hand, new research indicates that high HbA1c levels may result from aberrant glucose metabolism and insulin resistance, which may be influenced by the enhanced expression of miR-155-5p [(13).] In vitro and in vivo, hyperglycemia was observed to

enhance the expression of miR-155-5p, mimicking the findings of heightened renal miR-155 expression in individuals with diabetic nephropathy [7].

This controversy about miR-155-5p expression may be due to variations in the origin of samples in different studies and also variations in miR-155-5p expression in different tissues. In the present study, we used plasma samples, not urine or kidney tissue samples.

CONCLUSION

MiR-155 5p could be an informative biomarker for the early detection progression of diabetes and nephropathy. In addition to a significant positive correlation between miR-155 5p expression and eGFR, a strong significant negative correlation between miR-155 5p expression and urinary protein exertion rate, and a moderate negative correlation between miR-155 5P expression and HbA1c, there was a significant downregulation of miR-155 5p expression between the diabetic group (microalbuminuria) and the other groups, as well as between the diabetic group (normoalbuminuria) and the healthy control group.

REFERENCES

1. Sagoo, M. K., &Gnudi, L. (2020). Diabetic nephropathy: an overview. Diabetic Nephropathy: Methods and Protocols, 3-7.
2. Wang, J., Wang, G., Liang, Y., & Zhou, X. (2019). Expression Profiling and Clinical Significance of Plasma MicroRNAs in Diabetic Nephropathy. J Diabetes Res., 2019, 5204394.
3. Shaffi, S. K., Galas, D., Etheridge, A., & Argyropoulos, C. (2018). Role of microRNAs in renal parenchymal diseases—a new dimension. International Journal of Molecular Sciences, 19(6), 1797.
4. Wang, Y., Zheng, Z. ji, Jia, Y. jie, et al. (2018) 'Role of p53/miR-155-5p/sirt1 loop in renal tubular

injury of diabetic kidney disease', *Journal of Translational Medicine*. BioMed Central .; 16(1):1–9.
5. Baker MA, et al.(2017) Tissue-specific MicroRNA expression patterns in four types of kidney disease. J Am Soc Nephrol. ;28(10):2985–92.
6. Wang, J., Wang, G., Liang, Y., & Zhou, X. (2019). Expression Profiling and Clinical Significance of Plasma MicroRNAs in Diabetic Nephropathy. J Diabetes Res., 2019, 5204394.
7. Zhou, Y., Ma, X.-Y., Han, J.-Y., Yang, M., et al. (2021). Metformin regulates inflammation and fibrosis in diabetic kidney disease through TNC/TLR4/NF-kappaB/miR-155-5p inflammatory loop. World J. Diabetes, 12, 19–46
8. Huang, Y., Liu, Y., Li, L., Su, B., et al. (2014). Involvement of inflammation-related miR-155 and miR-146a in diabetic nephropathy: Implications for glomerular endothelial injury. *BMC Nephrology*, 15(1), 142. doi:10.1186/1471-2369-15-142
9. Mazloom H, Alizadeh S, Pasalar P, Esfahani EN, Meshkani R. Downregulated microRNA-155 expression in peripheral blood mononuclear cells of type 2 diabetic patients is not correlated with increased inflammatory cytokine production. Cytokine 2015; 76(2):403-8 (PMID: 26188366).

10. Hu YY, Dong WD, Xu YF, et al. Elevated levels of miR-155 in blood and urine from patients with nephrolithiasis. Biomed Res Int 2014;2014:295651 (PMID: 25197634).
11. Wang, G., Kwan, B. C.-H., Lai, F. M.-M., et al. (2011). Elevated Levels of miR-146a and miR-155 in Kidney Biopsy and Urine from Patients with IgA Nephropathy. Dis. Mark., 30, 171–179.
12. Bai, X., Luo, Q., Tan, K., & Guo, L. (2020). Diagnostic value of VDBP and miR-155-5p in diabetic nephropathy and the correlation with urinary microalbumin. Experimental and Therapeutic Medicine, 20, 86. https://doi.org/10.3892/etm.2020.9214.
13. Elhag, D. A., & Al Khodor, S. (2023). Exploring the potential of microRNA as a diagnostic tool for gestational diabetes. *Journal of Translational Medicine*, 21(1), 392. doi:10.1186/s12967-023-04269