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MIR-30A AS A NONINVASIVE DIAGNOSTIC AND PROGNOSTIC BIOMARKER IN PEDIATRIC NEPHROTIC SYNDROME

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Nephrotic syndrome (NS), a common pediatric kidney disorder, is characterized by severe proteinuria, leading to hypoalbuminemia, hyperlipidemia, edema, and various life-threatening complications. Early detection is critical for reducing disease progression and mitigating associated risks, highlighting the need to identify noninvasive biomarkers that can improve diagnostic accuracy. MicroRNAs (miRNAs), particularly miR-30a, have emerged as promising biomarkers for kidney diseases due to their involvement in key pathological processes such as inflammation and fibrosis. This study aimed to assess the diagnostic and prognostic utility of miR-30a in pediatric NS by evaluating its expression across different clinical subtypes. This study was conducted on 100 children from those enrolled at Assiut University Hospital. Participants were divided into five groups: Group 1 (Control), Group 2 (Steroid Sensitive), Group 3 (Steroid Resistant), Group 4 (Immunosuppressive Resistant), and Group 5 (Refractory NS), with 20 participants in each group. The expression level of miR-30a was quantified using quantitative real-time PCR (qRT-PCR). The results demonstrated significantly elevated miR-30a levels in NS patients compared to controls. Receiver operating characteristic (ROC) curve analysis demonstrated a high diagnostic accuracy of miR-30a in distinguishing NS patients from healthy controls, with an AUC of 0.920 (95% CI: 0.894–0.946; P < 0.001), an accuracy of 94.5%, a sensitivity of 88.6%, and a specificity of 100.0%. It also showed strong discriminatory power among the various NS subtypes. These findings suggest that miR-30a may serve as a promising diagnostic and prognostic biomarker, providing valuable insights for treatment stratification in pediatric NS.

Keywords: *Nephrotic Syndrome; Pediatric kidney disorder; Proteinuria; MicroRNAs; miR-30a*

INTRODUCTION

Nephrotic syndrome (NS) is a common pediatric kidney syndrome that has been defined by high protein leakage into urine as a result of a damaged glomerular filtration barrier and increased permeability. This condition often results in hypoalbuminemia, with subsequent hyperlipidemia, edema, and various life-threatening infections [1].

The etiology of nephrotic syndrome (NS) can be classified as either primary or secondary. Primary NS results from intrinsic kidney disease, whereas secondary NS develops as a consequence of systemic conditions [2]. Clinically, NS is classified based on histopathological glomerular changes and disease severity into minimal change disease (MCD), focal segmental glomerulosclerosis (FSGS), and membranous glomerulonephritis (MGN) [3]. The incidence of NS in childhood varies among populations depending on origin or ethnicity, with 2 to 7 cases per 100,000 individuals under the age of 16 [4].

For years, glucocorticoids have been utilized as the primary treatment for patients with NS. However, 75% to 95% of children achieve complete remission when treated with oral corticosteroids[5], but about 60% of them experience frequently relapsing nephrotic

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syndrome (FRNS) or develop steroid-dependent nephrotic syndrome (SDNS), necessitating an extended duration of steroid therapy or the use of immunosuppressant therapy [6].

Up to 40% of children with NS died due to various infections such as sepsis, cellulitis, and peritonitis, as well as potentially life-threatening complications, including thromboembolic complications such as Thromboembolism (TE) associated with NS, which can be attributed to delayed identification and treatment, leading to significant morbidity and mortality [7].

Strategies to minimize relapses after remission remain insufficient. Therefore, early diagnosis and timely treatment of FRNS/SDNS can lower relapse rates, potentially reducing child mortality from these conditions [8].

MicroRNAs (miRNAs) are short, noncoding RNA molecules (~22 nucleotides) that regulate gene expression by degrading mRNA or inhibiting its translation. They play key roles in biological processes such as cell growth, proliferation, differentiation, and apoptosis. Dysregulated miRNA expression has been associated with various pathological conditions, including cancer, inflammation, metabolic disorders, and kidney diseases [9] such as polycystic kidney disease, lupus nephritis, diabetic nephropathy, IgA nephropathy, glomerulonephritis, and chronic kidney disease (CKD) [10].

Several miRNAs such as miR-10, miR-20, miR-26, miR-30, miR-93, miR-106, miR-145, miR-192, miR-194, miR-200, and miR-1286, have been shown to contribute to various disease progression by regulating inflammation, apoptosis, fibrosis, and angiogenesis. Their stability in urine and plasma, as well as tissue, makes them candidate biomarkers and therapeutic targets for diagnostic purposes [11–13].

Recent advancements in genetic research have also highlighted the significance of gene polymorphisms in pathogenesis, susceptibility, and progression of various kidney disorders. Polymorphisms in genes related to immune response, inflammation, and fibrosis have been associated with increased risk of nephrotic syndrome, progression to CKD, and differential treatment responses among affected individuals [14]. These genetic variations can influence glomerular structure, podocyte integrity, and drug metabolism, thereby contributing to disease heterogeneity and resistance to standard therapies. Incorporating genetic profiling alongside molecular biomarkers such as microRNAs may enhance diagnostic accuracy and guide personalized treatment strategies in pediatric nephrology [15].

miR-30a-5p, a member of the miR-30 family, has been associated with various kidney diseases and may play a significant role in renal pathophysiology. Its upregulation in kidney diseases suggests a potential involvement in disease progression and renal fibrosis, possibly through the modulation of inflammatory response mechanisms in kidney cells [16,17]. Growing evidence suggests that miR-30a-5p could serve as a biomarker for kidney dysfunction, as its expression levels correlate with renal function parameters [18]. However, further studies are needed to clarify its exact role in the diagnosis and prediction of steroid treatment response in childhood idiopathic NS.

The present work aimed to assess the role of miR-30a as an appropriate biomarker in childhood NS and to explore its potential prognostic value in differentiating between NS subtypes.

MATERIALS AND METHODS

This study was carried out in the laboratory of the Clinical Pathology Department, Assiut University Hospital, Faculty of Medicine, Assiut University, Egypt, between August 2023 and April 2024. The study included 100 children (32 females and 68 males), 20 healthy volunteer children as a control group, and 80 children diagnosed with NS; their ages ranged from 2 to 18 years old. Cases were selected from patients who were admitted to the Pediatric Hospital at Assiut University. This study was ethically approved by Assiut University, Faculty of Medicine. Written informed consent was obtained from the parents or legal guardians of all participants.

Inclusion criteria

Children diagnosed with nephrotic syndrome who were admitted to a pediatric hospital and ranged in age from 2 to 18 were included in this study.

Exclusion criteria

Children with other renal illnesses, congenital or infantile nephrotic syndrome, and

children with abnormal kidney function tests were excluded from this work.

Experimental design

The participants included in this study were classified into five groups: **Group 1** (Control): Included 20 healthy children, aged between 6 and 13 years (11 males and 9 females). **Group 2** (Steroid sensitive): Included 20 children with steroid-sensitive NS, aged between 8 Ms. and 16 years (16 males and 4 females). **Group 3** (Steroid resistant): Included 20 children with steroid-resistant NS, aged between 2 and 18 years (13 males and 7 females). **Group 4** (Immunosuppressive resistant): Included 20 children with Immunosuppressive resistant NS, aged between 3 and 18 years (14 males and 6 females). **Group 5** (Refractory NS): Included 20 children with refractory NS, aged between 2 and 13 years (14 males and 6 females).

Biochemical analysis

A blood sample (3 ml) was collected from each subject in the morning after an overnight fast and divided into two parts; 2 mL blood was used for the separation of serum, which was used for measurement of serum albumin, and the lipid profile was conducted using Cobas-C311 (Roche Diagnostics, Hitachi, Tokyo, Japan).

Protein concentration in urine was analyzed by the Cobas-C311 (Roche Diagnostics, Hitachi, Tokyo, Japan) autoanalyzer. 24-h urine samples were collected from 7 a.m. to 7 a.m. the following day after emptying the bladder in the morning. The total 24-hour urinary protein was calculated according to the formula:

$$\text{Total 24-h urinary protein (g)} = \frac{\text{Urine protein concentration (g/L)} \times \text{Total urine volume collected (L)}}{100}$$

Determination of plasma miRNA-30a by (qRT-PCR)

1 mL of blood was collected on EDTA EDTA-coated tube and stored at -80ºC for determination of miRNA-30a level using real-time PCR (Applied Biosystems, USA) with commercial kits (Cat # 217004, miRNeasy Mini Kit, QIAGEN, Germany) following the manufacturer's protocol. Complementary DNA (cDNA) was synthesized through reverse transcription using the miScript II RT kit (Cat. # 218160, QIAGEN, Germany). The detection of miR-30a was carried out using SYBR Green qPCR Master Mix (Cat. # 218073, QIAGEN, Germany) on a 7500 Fast Real-Time PCR system (Applied Biosystems, USA). The qRT-PCR protocol included the following steps: an initial activation phase at 95°C for 5 minutes to activate HotStarTaq DNA polymerase, followed by 40 amplification cycles. Each cycle consisted of DNA denaturation at 94°C for 15 seconds, annealing at 55°C for 30 seconds, and extension at 55°C for 5 minutes. Fluorescence measurements were recorded at each cycle to monitor the amplification process. Quantification of mature miRNAs using qRT-PCR Real Real-time PCR was carried out using miScript Universal Primer Assay for miR-30a (Cat. No MS00031486; Qiagen– Germany). SNORD- 80-1 small nuclear RNA (Cat. No MS00033712; Qiagen– Germany) was used as an endogenous control for data normalization.

$2^{-\Delta\Delta Ct}$ method was used to calculate the relative expression of RNAs. The results were expressed as Fold Change (FC), considering the normal value and assumed to equal 1.

Kidney Histopathological Assessment

Kidney tissue specimens were taken and immediately fixed in 10% neutral formalin solution for 24 h, then embedded in paraffin wax according to standard procedure. Five micrometer-thick sections were cut by the microtome and stained with Hematoxylin and Eosin (H&E) stains [19]. The stained tissue sections were examined under a light microscope (Olympus, Shinjuku, Japan) for the identification of MCNS, FSGS, and MN.

Statistical analysis

Statistical Package for Social Science (SPSS), version 26.0 was used for data analysis. Quantitative data represented as mean ± SD. One-way Analysis of Variance (ANOVA) was used to compare mean differences between groups, followed by Dunnett's post hoc analysis between the control and patient groups if ANOVA was significant. Receiver operating characteristic (ROC) curve analysis was performed to analyze the diagnostic value of miR-30a to discriminate between those with NS. The optimal cutoff value. Cutoff points were calculated by obtaining the best Youden index

(sensitivity + specificity − 1). ($P < 0.05$) is considered statistically significant.

RESULTS AND DISCUSSION

Our findings indicate that there are no statistically significant differences in age and gender between the groups under study **(Table 1)**.

According to our findings, a marked difference in the lipid profile (cholesterol, HDL, and LDL) was found. The NS groups exhibited significantly higher cholesterol and LDL, and significantly lower HDL compared to controls. However, the triglyceride levels showed no notable difference **(Table 2)**.

Table 1: Demographic data in the different study groups.

Variables	**Controls (n=20)**	**SSNS (n= 20)**	**SRNS (n =20)**	**IRNS (n =20)**	**RNS (n= 20)**	***P***
Age (years)						
Range	6.0-13.0	8 Ms.- 16.00	2.0-18.0	3.0-18.0	2.0-13.0	P =0.483
Median	7.50	6.50	7.50	8.00	6.25	
Gender						
Males	11 (55%)	16 (80%)	13 (65%)	14 (70%)	14 (70%)	P =0.552
Females	9 (45%)	4 (20%)	7 (35%)	6 (30%)	6 (30%)	

SSNS: Steroid-Sensitive Nephrotic Syndrome, RNS: Steroid-Resistant Nephrotic Syndrome, IRNS: Immunosuppressive Resistant Nephrotic Syndrome, RNS: Refractory Nephrotic Syndrome.

Table 2: Comparison of lipid profile and protein levels between the studied group.

Variables	**Controls (n=20)**	**SSNS (n= 20)**	**SRNS (n =20)**	**IRNS (n =20)**	**RNS (n= 20)**	***P***
Cholesterol (mg/dL)						
(Range) Median	(67.0-150.0) 140.0	(179.0-705) 385.5	(120.0-700) 380.0	(101.0-635) 380.0	(107-700) 354.0	$P<0.001$
P		p_1<0.001***	p_2<0.001***	p_3<0.001***	p_4<0.001***	
Triglyceride (mg/dL)						
Range	90.0-140	91.0-150	95.0-142	93.0-150.0	92.0-149	
Mean ± SD	122.5±24.39	130.35±14.5	120.60±21.14	121.45±20.69	124.35±19.43	P =0.796
P		p_1=0.227	p_2=0.762	p_3=0.864	p_4=0.780	
LDL (mg/dL)						
Range	19.4-115.4	35.0-128.0	27.0-127.4	19.4-123.1	14.4-128.0	
Mean ± SD	56.44±31.34	88.18±34.49	89.14±28.67	79.30±38.07	87.45±36.09	$P<0.01$
P		p_1=0.004**	p_2=0.003**	p_3=0.036*	p_4=0.005**	
HDL (mg/dL)						
Range	36.8-56.1	36.8-51.2	37.6-59.9	36.8-56.1	36.8-51.2	
Mean ± SD	45.82±6.21	43.02±5.11	44.93±7.66	41.89±5.62	41.61±3.67	$P<0.05$
P		p_1=0.131	p_2=0.631	p_3=0.035*	p_4=0.024*	
Albumin (mg/dL)						
(Range) Median	(2.50-5.0) 4.40	(0.50-4.50) 1.85	(0.60-4.09) 1.85	(1.30-4.20) 1.70	(1.00-4.90) 1.90	$P<0.01$
P		p_1=0.045*	p_2<0.001***	p_3=0.008**	p_4=0.009**	
T. protein (mg/dL)						
(Range) Median	(3.80-13.0) 8.0	(1.40-9.90) 6.8	(4.0-9.1) 6.6	(3.0-9.10) 6.5	(2.0-8.90) 5.10	$P<0.01$
P		p_1=0.023*	p_2=0.024*	p_3=0.003**	p_4<0.001***	
Protein in urine(mg/dL)						
(Range) Median	(26.1-55.0) 30.0	(65.0-430) 144.6	(56.2-375.4) 181.5	(69.2-429.0) 190.7	(57.9-397.9) 180.7	$P<0.01$
P		p_1<0.001***	<0.001***	p_3<0.001***	p_4<0.001***	
Protein 24 hrs. in urine (mg/24h)						
(Range) Median	(39.6-140.0) 77.50	(160-5148) 313.72	(160-5466) 507.50	(192.5-5148) 440.30	(155-4555) 1364.1	$P<0.001$
P		p_1<0.001***	p_2<0.001***	p_3<0.001***	<0.001***	

p_1: SSNS Vs Controls, p_2: SRNS Vs Controls, p_3: IRNS Vs Controls, p_4: RNS Vs Controls.
SSNS: Steroid-Sensitive Nephrotic Syndrome, RNS: Steroid-Resistant Nephrotic Syndrome, IRNS: Immunosuppressive Resistant Nephrotic Syndrome, RNS: Refractory Nephrotic Syndrome.

Our results also showed that the NS groups had considerably lower serum albumin and total protein levels than controls ($p < 0.01$). In contrast, urinary protein and 4-hour protein were significantly higher in the NS groups than in the controls ($p<0.01$, $p<0.001$, respectively) **(Table 2)**.

Our results showed that NS groups exhibited significantly elevated levels of miRNA-30a than controls ($p < 0.05$). Furthermore, children diagnosed with steroid resistant, immunosuppressive resistant, and refractory NS have exhibited significantly higher plasma miRNA-30a level in comparison with those of steroid sensitive NS. However, no significant differences were observed in miRNA-30a levels when comparing the steroid-resistant, immunosuppressive-resistant, and refractory NS groups with each other, as shown in **(Table 3)**.

Our results revealed a statistically significant lower miRNA-30a level in MCNS compared to MN, and in FSGS in comparison to MN ($p <0.05$). However, the levels of miRNA-30a in MCNS and FSGS did not differ significantly ($p =0.191$) **(Table 4)**.

From our results, miRNA-30a demonstrated strong potential to predict NS patients from healthy individuals. At a cutoff value of 2.97, miRNA-30a effectively distinguished NS patients from healthy individuals, yielding an AUC of 0.920 (95% CI: 0.894–0.946; $P < 0.001$), with an accuracy of 94.5%, sensitivity of 88.6%, and specificity of 100.0% **(Fig. 1a)**. Additionally, at a cutoff of 16.72, miRNA-30a could differentiate SRNS from SSNS, achieving an AUC of 0.801 (95% CI: 0.755–0.847; $P < 0.001$), with an accuracy of 85.0%, sensitivity of 75.0%, and specificity of 95.0% **(Fig. 1b)**. Moreover, miRNA-30a at a cutoff of 16.73 demonstrated the ability to distinguish IRNS from SSNS, with an AUC of 0.800 (95% CI: 0.755–0.845; $P < 0.001$), accuracy of 87.0%, sensitivity of 78.9%, and specificity of 95.0% **(Fig. 1c)**. At the same cutoff, miRNA-30a also showed strong discriminatory power in identifying RNS compared to SSNS, with an AUC of 0.900 (95% CI: 0.847–0.953; $P < 0.001$), accuracy of 92.5%, sensitivity of 90.0%, and specificity of 95.0% **(Fig. 1d)**. Finally, miRNA-30a successfully differentiated between treatment-resistant NS subtypes (SRNS, IRNS, and RNS) and SSNS, with an AUC of 0.834 (95% CI: 0.745–0.920; $P < 0.001$), accuracy of 88.5%, sensitivity of 81.4%, and specificity of 95.0% at a cutoff value of 16.72 **(Fig. 1e)**

Table 3: Comparison of miRNA-30a levels between the studied group.

	Controls (n=20)	**SSNS (n= 20)**	**SRNS (n =20)**	**IRNS (n =20)**	**RNS (n= 20)**	***P***
miRNA-30a (ng/µL)						
Range	(0.22-2.97)	(0.001-36.86)	(0.54-450.06)	(0.79-378.46)	(0.81-350.6)	$P < 0.001$
Median	1.16	8.55	26.44	27.93	27.64	
		p_1=0.024*;	p_2<0.001***	p_3<0.001***	p_4<0.001***	

p_1: SSNS Vs Controls, p_2: SRNS Vs Controls, p_3: IRNS Vs Controls, p_4: RNS Vs Controls.
SSNS: Steroid-Sensitive Nephrotic Syndrome, RNS: Steroid-Resistant Nephrotic Syndrome, IRNS: Immunosuppressive Resistant Nephrotic Syndrome, RNS: Refractory Nephrotic Syndrome**.**

Table 4: Comparison of miRNA-30a among patients with nephrotic syndrome according to biopsy results.

	MCNS	**FSGS**	**MN**	***P***
miRNA-30a (ng/µL)				
Range Median	(0.00-450.06) 9.98	(0.39-378.46) 23.16	(20.59-160.22) 43.5	$P = 0.025$
P	p_1=0.191, p_2=0.044*, p_3=0.007**			

p_1: MCNS Vs Group FSGS, p_2: FSGS Vs Group MN, p_3: MCNS Vs MN.

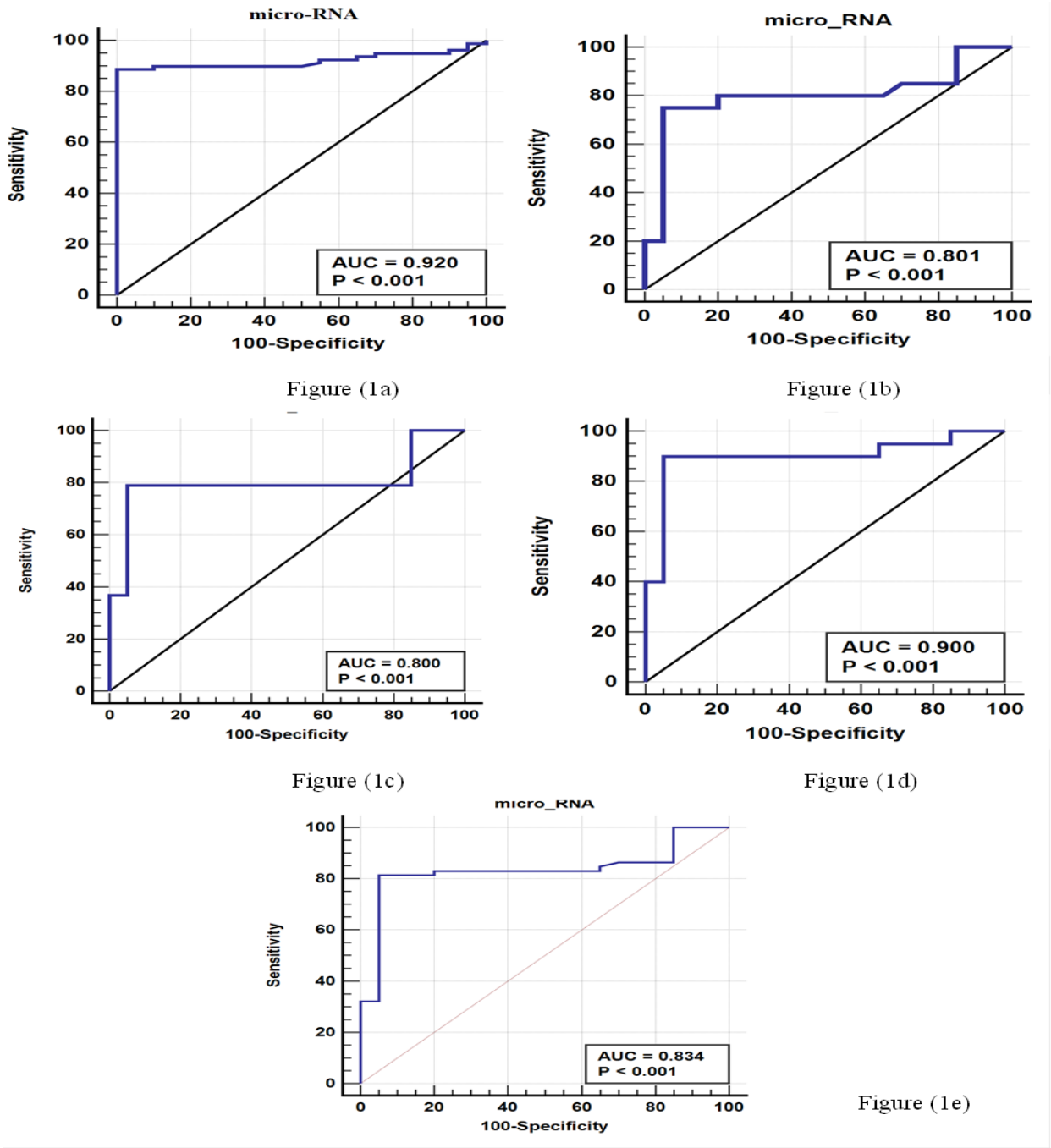

Fig. 1: ROC curve of miRNA-30a for diagnosing NS and its subgroups. (1a) Diagnosis of NS from controls: At a cutoff value of 2.97, miRNA-30a distinguished NS patients from controls, yielding an AUC of 0.920 (95% CI: 0.894–0.946; P < 0.001), with an accuracy of 94.5%, sensitivity of 88.6%, and specificity of 100.0%. (1b) Diagnosis of SRNS from SSNS: At a cutoff of 16.72, miRNA-30a differentiated SRNS from SSNS, achieving an AUC of 0.801 (95% CI: 0.755–0.847; P < 0.001), with an accuracy of 85.0%, sensitivity of 75.0%, and specificity of 95.0%. (1c) Diagnosis of IRNS from SSNS: miRNA-30a at a cutoff of 16.73 distinguished IRNS from SSNS, with an AUC of 0.800 (95% CI: 0.755–0.845; P < 0.001), accuracy of 87.0%, sensitivity of 78.9%, and specificity of 95.0%. (1d) Diagnosis of RNS from SSNS: At a cutoff of 16.73, miRNA-30a also identified RNS from SSNS, with an AUC of 0.900 (95% CI: 0.847–0.953; P < 0.001), accuracy of 92.5%, sensitivity of 90.0%, and specificity of 95.0%. (1e) Diagnosis of (SRNS/IRNS/RNS) from SSNS: miRNA-30a differentiated treatment-resistant NS subtypes (SRNS, IRNS, and RNS) from SSNS, with an AUC of 0.834 (95% CI: 0.745–0.920; P < 0.001), accuracy of 88.5%, sensitivity of 81.4%, and specificity of 95.0% at a cutoff value of 16.72.

Discussion

Nephrotic syndrome (NS) is the most common glomerular disease in children, accounting for almost 90% of all nephrotic syndrome cases. It can affect children of any age, with males demonstrating a higher incidence rate compared to females, with a reported ratio of 2:1in children[20].

Corticosteroids are the primary treatment for childhood nephrotic syndrome (NS). However, approximately 50% of children experience frequent relapses, known as refractory NS, or develop steroid resistance (SRNS)[21]. For children with SRNS, immunosuppressive drugs such as calcineurin inhibitors show potential as an alternative treatment. Despite this, up to 15% of children with SRNS fail to respond to immunosuppressive therapy [22]. Children with untreated NS face heightened risks of thromboembolic events, abnormalities in lipid profiles, and nutritional deficiencies. Additionally, 50% of affected children may progress to end-stage renal disease (ESRD) within 15 years [23].

Previous research suggests that early identification of NS, coupled with appropriate interventions, effectively reduces recurrence rates and life-threatening infections in the long term [24].

Common diagnostic indicators for childhood NS include serum albumin, lipids, and proteinuria; however, due to the disease's variability, these indicators might not be able to predict a patient's outcome [25]. Currently, renal biopsy is the gold standard for clinical diagnosis and monitoring of NS. However, in children, biopsies are generally not recommended unless necessary due to the associated risks and invasive nature of the procedure. In contrast, renal biopsy is indicated in cases of SRNS. Additionally, repeated monitoring can be technically challenging. Children may feel anxious about medical procedures due to their age, which could affect the outcomes of the diagnosis [26].

The increasing prevalence of NS and its lack of knowledge about metabolic pathways present significant challenges. Currently, there are no validated biomarkers capable of reliably predicting steroid resistance, leaving patients vulnerable to both disease progression and the adverse effects of prolonged glucocorticoid (GC) therapy [27]. Therefore, the search for efficient non-invasive biomarkers suitable for diagnosis, monitoring, and treatment remains a critical need. Numerous studies aimed to identify biomarkers capable of predicting clinical responses to treatment via elucidating specific biochemical pathways and molecular targets linked to clinical steroid resistance, which could help in designing more potent and less harmful tailored therapeutics for NS in the future [28].

MicroRNAs (miRNAs) are a subclass of non-coding RNAs (ncRNAs) that are employed as biomarkers for several human diseases, including renal cell carcinoma [29]. Earlier research has shown that miRNAs contribute to the advancement of several renal diseases, including MCNS, which is a major factor in NS progression. This suggests that they could be potential diagnostic markers and therapeutic targets for NS[30].

The present work aimed to evaluate the diagnostic value of plasma microRNA 30a as a non-invasive biomarker for the detection of NS progression.

Based on the outcomes of the current study, the serum cholesterol and LDL were significantly higher in the NS groups compared to controls. While serum HDL was significantly lower in the NS groups compared to controls. Our results were consistent with those reported by **Atal *et al.*** [31] and **Akinyosoye *et al*** [32], who reported that hyperlipidemia is an important feature of NS. NS could cause alterations in lipid levels, resulting in elevated lipid levels in NS patients that would persist even during remission in children with FRNS[33].

This study found that patients with NS have lower serum albumin, total protein levels and higher urinary protein levels compared to controls. These findings are consistent with previous research by **Hussein *et al.*** [34]**, Feng *et al.*** [26] and **Israt *et al.*** [35] which also reported marked hypoalbuminemia and increased proteinuria in NS patients. Proteinuria, a key factor in the pathogenesis and progression of NS, triggers inflammatory responses and oxidative stress, leading to kidney injury and systemic complications like hypoalbuminemia, edema, and hyperlipidemia [36,37].

The present study found that the children with NS exhibited significantly higher levels of miRNA-30a compared to controls. These results were augmented with a previous studies conducted by **Sreekumar *et al.*** [38], and **Luo *et al.*** [39] on Indian and Chinese children, respectively who demonstrated upregulation of

miRNA-30a in children with NS, suggesting a possible role for miR-30a in the disease's pathogenesis and progression.

In the same line, this work found that the children's steroid-resistant, immunosuppressive-resistant, and refractory NS exhibited significantly higher levels of miRNA-30a in comparison with those of steroid-sensitive NS. Our results came in accordance with those reported by **Teng *et al.*** [40], who reported that miRNA-30a overexpression in drug-resistant NS patients may indicate podocyte damage or the disease's pathophysiology. Experimental studies have shown that miR-30a-5p levels are diminished in podocytes of Dicer-knockout mice, leading to glomerular basement membrane damage. MiR-30a may protect podocytes by inhibiting Notch1 and P53, while its absence fosters damage [41,42]. **Chen *et al.*** [43] and **Luo *et al.*** [39] also supported our findings.

Regarding the miRNA-30a expression levels in NS children experienced with MCNS, FSGS, or MN. The current study revealed that miRNA-30a exhibited a higher level in NS children with MN and FSGS compared to those with MCNS. This was consistent with **Saadat *et al.***[44] who found a statistically significant decrease in miR-30a in FSGS compared to MN. Additionally, **Zhang *et al.***[45] found that the levels of urinary miR-30a-5p were higher in patients with active FSGS and MN.

Our results showed that miRNA-30a could effectively discriminate NS cases from controls, indicating its potential as a diagnostic biomarker for NS. In agreement with our findings, **Luo *et al.***[39] and **Feng *et al.*** [26] reported a high diagnostic accuracy of the plasma and urinary miR-30a n differentiating the NS cases from the control individuals. These findings support the potential of miR-30a -5p as biomarker for the diagnosis of NS.

Our results also showed that miRNA-30a could discriminate steroid resistant NS patients from those with steroid sensitive NS, indicating its potential as a diagnostic biomarker for SRNS. Similar to our finding, **Teng *et al.*** [40] found that miRNA-30a overexpressed significantly in SRNS cases, suggesting that miR-30a may help diagnose drug resistance and pathological type.

Our results also revealed that miRNA-30a could effectively distinguish immunosuppressive resistant NS from steroid sensitive NS, indicating its potential as a diagnostic biomarker for immunosuppressive resistant NS. These results highlight the potential role of miR-30a in differentiating children with NS from healthy children, distinguishing between pathological subtypes and differentiating the onset from the remission phase of NS [39]. To our knowledge, this is the first study that investigated the role of miRNA-30a in the prediction of different NS types, suggesting miR-30a to be a potential diagnostic and prognostic biomarker for idiopathic pediatric nephrotic syndrome. As well as miR-30a would be a potential indicator for diagnosing the progression of NS and monitoring the treatment effect [26].

Conclusion

The present study concluded that plasma miR-30a levels are significantly elevated in nephrotic syndrome (NS) patients, their expression showing variations across different histopathological subtypes (MCNS, FSGS, MN), suggesting its potential as a prognostic biomarker for NS. miRNA-30a also demonstrated high diagnostic accuracy in distinguishing between NS cases and controls, and among NS types. These findings highlight miR-30a's value in distinguishing NS cases from healthy controls, differentiating resistant NS from steroid-sensitive NS, and serving as a biomarker for disease progression and treatment efficacy in pediatric NS.

Limitations

Despite the promising findings, this study has several limitations. First, the sample size was relatively small and limited to a single hospital, which may restrict the generalizability of the results. Therefore, large, multicenter, independent studies are needed to validate the diagnostic and prognostic value of miR-30a across diverse populations. Second, although plasma miR-30a levels were assessed, tissue-specific expression, particularly in renal biopsies, was not examined, which could provide deeper insight into the relationships between miR-30a expression and disease progression or treatment response and its pathophysiological role.

Ethical approval

The study protocol was reviewed and approved by the Institutional Review Board of the Faculty of Medicine in Assiut University,

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MiR-30a كمؤشر حيوي غير جراحي ومؤشر تشخيصي مبكر للمتلازمة الكلوية لدى الأطفال

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تعدّ المتلازمة الكلوية (NS) من أمراض الكلى الشائعة في مرحلة الطفولة، والتي تتسبب في فقدان البروتين عبر البول (البروتينوريا) مما يؤدي إلى نقص الألبومين في الدم، وفرط دهون الدم، والاستقساء، ومجموعة من المضاعفات الخطيرة التي قد تهدد الحياة. وعلى الرغم من أن علاج المتلازمة الكلوية يؤدي إلى التحسن فى معظم الحالات، إلا أن العديد من الأطفال يعانون من نوبات تكرارية أو يطورون اعتماداً على الستيرويدات. يُعتبر الاكتشاف المبكر للمتلازمة الكلوية أمراً بالغ الأهمية للتقليل من تقدم المرض والحد من المخاطر المرتبطة به، مما يعزز الحاجة إلى استكشاف مؤشرات حيوية غير جراحية جديده والتي قد تحسن دقة التشخيص. وقد ظهرت الحمض النووي الريبوزى المصغر(miRNAs) ، وخاصةmiR-30a ، كأحد المؤشرات الحيوية المحتملة لأمراض الكلى من خلال دورها في تنظيم عمليات مثل الالتهاب والتليف. هدفت هذه الدراسة إلى تقييم الفائدة التشخيصية والتنبؤية لـ miR-30a في المتلازمة الكلوية عند الأطفال، من خلال فحص تركيزه في الدم عبر الأنواع الفرعية للمتلازمة. أجريت الدراسة على ١٠٠ طفل في مستشفى جامعة أسيوط، حيث تم تقسيم المشاركين إلى خمس مجموعات: المجموعة ١ (المجموعة الضابطة)، المجموعة ٢ (مجموعة المستجيبين للستيرويدات)، المجموعة ٣ (مجموعة المقاومة للستيرويدات)، المجموعة ٤ (مجموعة المقاومة للعلاج المناعي)، والمجموعة ٥ (مجموعة المتلازمة الكلوية المقاومة)، وتضمنت كل مجموعة ٢٠ طفلاً. تم قياس مستوى تركيز miR-30a باستخدام تقنية qRT-PCR. أظهرت النتائج ارتفاعاً كبيراً في مستويات miR-30a لدى مرضى المتلازمة الكلوية مقارنةً بالمجموعة الضابطة ، كما أكدت تحليلات ROC curve دقته التشخيصية العالية في التمييز بين حالات المتلازمة الكلوية والمجموعة الضابطة، وكذلك بين الأنواع السريرية. تؤكد هذه النتائج على إمكانية استخدام miR-30a كمؤشر حيوي تشخيصي وتنبؤي، مما يوفر رؤى قيمة لتخصيص العلاج في حالات المتلازمة الكلوية لدى الأطفال.