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Micro RNA 192 Gene Expression and Pathogenesis of Nephrotic Syndrome in Egyptian Children

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Abstract

Idiopathic Nephrotic syndrome (INS) is a condition in which the glomeruli of the kidney leak protein from the blood into the urine. This leads to hypoproteinaemia and generalized edema. Children with NS have an increased risk of bacterial infection and are often accompanied with malnutrition due to the loss of a large number of plasma proteins. NS pathogenesis and management remain unclear and the need for novel molecular mechanisms is necessary. The recent discovery of microRNAs (miRNAs) and their cellular functions provide an opportunity to fill these critical gaps. Because miRNAs possibly modulate the actions of key factors involved in nephrotic syndrome such as transforming growth factor-ß (TGF-β), they could be novel diagnostic biomarker of NS. This study examined association of serum miRNA-192 as a potential diagnostic biomarker of NS and its correlation with the pathogenesis of nephrotic syndrome including dyslipidemia, proteinuria and potential fibrosis. This study included 50 child NS cases and 50 age-matched healthy children as controls. MiRNA-192 expression levels were measured using quantitative real-time polymerase chain reaction (qRT-PCR) and TGF-ß serum levels were assessed by enzyme-linked immunosorbent assay (ELISA). Correlations among miRNA192, TGF-ß and severity of NS (dyslipidemia and proteinuria) were investigated. Our results revealed significantly lower miRNA-192 expression levels in NS patients than in the control group ($P < 0.001$). Serum TGF-ß levels were significantly increased in NS cases as compared to control group ($P < 0.001$). Furthermore, serum miRNA-192 expression levels were significantly negatively correlated with serum TGF-ß concentrations, dyslipidemia and proteinuria, whereas serum TGF-ß concentrations were significantly positively correlated with proteinuria. Collectively, levels of both serum miRNA-192 and TGF-ß may explain their role in pathogenesis of NS, and they are promising diagnostic biomarkers.

Keywords: Nephrotic syndrome, miRNA-192, TGF- ß, qRT-PCR, ELISA, dyslipidemia, proteinuria.

1. Introduction

Idiopathic Nephrotic syndrome (INS) is the most common chronic kidney disease among children which is characterized by triad of proteinuria, hypoalbuminaemia, and edema (Damien *et al.*, 2018). Its incidence is from 2 to 7 cases/100,000 children/year, and its global prevalence is of 16 cases/100,000 children aged below 16 years (Deschênes and Leclerc, 2010). Typically, the histological classifications corresponding with idiopathic childhood NS are described either as minimal change disease (MCD) or focal segmental glomerulosclerosis (FSGS) the quintessential podocyte diseases (A report of the International Study of Kidney Disease in Children, 1978). Glucocorticoids (GC) are the primary therapy of INS. However, not all children, who initially appear to have similar histological and clinical features of NS, respond to steroid therapy; they are classified into as steroid sensitive (SS) or steroid resistant (SR), and these differences in steroid response can be attributed to genetic factors (Rheault and Gbadegesin. 2016).

MicroRNAs (miRNAs) are endogenous, short non-coding RNA molecules that play a critical role in modulating many cellular and physiological activities (Schena *et al*., 2014). The miRNAs are relatively stable, which makes them ideal diagnostic & prognostic biomarkers for many diseases. Although plenty of miRNAs are widely expressed in many tissues, some miRNAs are found to be highly organ-specific (Liang *et al*., 2007). In the kidney, miRNAs are indispensable to development and homeostasis (Schena *et al*., 2014). Yinfeng et al. suggested possible roles and miRNAs expression in acute kidney injury and disease pathogenesis (Yinfeng *et al*., 2017). Another study was performed by Tesch proposed aberrant miRNAs expression level in renal fibrosis (Tesch, 2010). Additionally, according to their hypotheses, miRNAs lead to renal fibrosis by causing changes in TGF-β, extracellular matrix and epithelial-mesenchymal transition (Conway and Hughes, 2012 and Kato et al., 2012). TGF-β is a fibro genic cytokine that contributes to renal tissue fibrosis (Reeves and Andreoli, 2000). TGF-β causes proliferation in extracellular matrix, stimulating myofibroblastosis (LAN, 2011). The number of TGF-β receptors increases with renal damage, activating

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the TGF-β/Smad3 pathway and initiating fibrosis (Meng *et al.*, 2013). Recent studies have shown that miRNA-192 target sites are located within TGF-β mRNA; therefore, miRNA-192 can significantly inhibit the translation process after TGF-β transcription (Kato *et al.*, 2011). Therefore, we aimed to investigate possible role of miRNA-192 expression levels as a novel diagnostic biomarker and its correlation with the serum level of Transforming TGF-ß as a pro-inflammatory fibrotic cytokine and severity of NS.

2. Subject and method

2.1. Subjects

The current study was conducted in the Nephrology Unit Pediatrics Department and Medical Biochemistry Department; Zagazig University. The protocol was approved by the ethics committee of Zagazig University. Written informed consent was obtained from all children parents. Fifty patients with steroid sensitive NS with an age range between 5 and 9 years (28 males and 22 females) were diagnosed clinically and proven by laboratory results. These patients had been followed up at the Nephrology unit of the Department of Pediatrics, Zagazig University Hospital. The control group consisted of 50 age- and sex-matched apparently healthy children selected from the general pediatric outpatient department. All samples were withdrawn during the period from March 2018 to June 2018. Demographic data of the patients were recorded from the medical history and electronic files. The inclusion criteria were both male and female children, age at start of study greater than 5 years, and normal kidney function in the control group. The exclusion criteria were a family history of premature cardiovascular diseases.

2.2. Blood Sampling

A 5 mL blood sample was withdrawn under aseptic conditions from each patient and healthy children and left for 30 min for spontaneous clotting at room temperature before being centrifuged at 3,000 rpm. Serum samples were immediately stored at −70 °C for determination of TGF-ß levels and RNA extraction.

2.3. Measurement of serum TGF-ß level:

Serum samples of all subjects were aliquoted and levels of TGF-ß were measured by an enzyme-linked immunosorbent assay (ELISA) kit provided from R&D Systems, Inc., Minneapolis, USA (Cat No. DB100B) and applied according to manufacturer instructions. The minimum detectable dose (MDD) of TGF-β ranged from 1.7-15.4 pg/mL.

2.4. Extraction of serum miRNA-192:

Serum samples of all subjects were aliquoted and all procedures occurred on ice bar and repeated freeze/thaw cycles were avoided to prevent RNA damage.

Total RNA including miRNAs was extracted using RNA extraction kit (Qiagen Inc., Foster city, California, USA) following the manufacturer's instructions (Cat. No.217004). Briefly, 500 µl QIAzol lysis reagent was added to 100 µl serum samples and the mixture was incubated for 5 min at room temperature. Then, addition of (100 µl) chloroform was performed and tube was vigorously shaken by hands for 15 s and incubated at room temperature for 5 mins. After centrifugation at 12,000 × g for 15 min at 4 °C, the upper aqueous phase of supernatant was transferred to a fresh tube and 700µl of 100% ethanol were added. 700 µl of this mixture was transferred into RNeasy mini spin column and centrifuged at 8000 × g for 1 min at room temperature. After the mixture had completely passed the column, this step was repeated until all of the mixture had completely passed the column to the mini column. Next, the column was washed two times with 500 µl RNA wash buffer (RWT buffer) and centrifuged for 15 s at 8,000× g at room temperature. Then, 500 µl buffer RPE was added to the column and centrifuged at 8000×g at room temperature for 15 s. Another 500 µl buffer RPE was added to the column and centrifuged at 8000×g at room temperature for 2 min. For RNA elution, the column was transferred to a new 1.5 mL collection tube and 40 µl RNase-free water was directly pipetted onto the column and centrifuged for 1 min at 8000 × g. Finally, the quantity and purity of serum RNA were confirmed by the value of optical density (OD) at 260 and 280 nm using a spectrometer, with acceptable RNA purity ranging from 1.8 to 2.1.

2.5. Reverse transcription and quantification of serum miRNA-192:

The miScript II reverse transcription kit (Qiagen Inc., USA, and Cat. No. 218161) was used for polyadenylation and reverse transcription of miRNA to complementary DNA (cDNA). After mixing with template RNA (100 ng), 5X miScript buffer, miScript reverse transcriptase mix and RNase-free water in a final volume of 20 µl, the mixture was centrifuged briefly and incubated for 60 min. at 37°C. To inactivate the reverse transcriptase mix, the samples were incubated for 5 min. at 95°C and then placed on ice for further PCR analysis. The diluted cDNA templates were either stored at –20 °C or used to proceed further to quantitative real-time polymerase chain reaction (qRT-PCR).

2.6. The expression level of serum miRNA-192:

The resultant cDNA was subjected to qRT-PCR to evaluate expression of serum of miRNA-192 using miScript SYBR® Green PCR Kit with miScript Primer assays (Qiagen Inc., USA). This kit includes QuantiTect SYBR Green PCR Master Mix and the Primer (reverse primer used to detect miRNA-192) was provided from miScript Primer Assay kit that specifically recognizes the targeted miRNA-192(Cat. No. MS00003689). The expression levels of miRNA were normalized to RNU6, which was selected as internal control (Cat. No. MS000033740). The PCR conditions were as follows: 95°C for 15 min, followed by 40 cycles at 94 °C for 15 s, 55 °C for 30 s and 70 °C for 34 s. The cycle threshold (CT) is defined as the number of cycles required for the fluorescent signal to cross the threshold in real-time PCR. The expression of miRNAs was reported as the ΔCt value, which was calculated by subtracting the CT values of miRNA U6 from the CT values of the miRNA-192. Because there is an inverse correlation between ΔCt and miRNA expression levels, lower ΔCt values are associated with increased miRNA expression. The relative gene expression was calculated as ΔΔCt. The amplitude of change of the expression miRNA observed in patients in relation to control group was analyzed by the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

2.7. Statistical Analysis:

All data were collected, tabulated and statistically analyzed using SPSS 20.0 for windows (SPSS Inc., Chicago, IL). Quantitative data were expressed as the mean ± standard deviation (SD), and percentages when appropriate. Student's t-test was used to compare two groups of normally distributed data. Receiver operating characteristic (ROC) curve analysis was used to identify the optimal sensitivity and specificity of miRNA-192 and TGF-ß. The correlation coefficients were calculated using Spearman correlation. Multiple logistic regression analysis was conducted to test possible association between miRNA-192 and TGF-ß as well as other parameters and to also evaluate whether the biomarker miRNA-192 and TGF-ß could be used as predictors for nephrotic syndrome. P-value < 0.05 was considered statistically significant.

3. Results

3.1. Clinico-demographic

characteristics, biochemical laboratory and miRNA-192 expression profiles among nephrotic patients and healthy controls

Data represented in Table 1 indicated that sex distribution was comparable between the patient and control groups. Body mass index (BMI) and systolic blood pressure (SBP) was significantly greater in the patient group than the control group (Table 1). Serum total cholesterol(TC), serum triglycerides (TGs) and serum low density lipoprotein- cholesterol (LDL-C) were higher significantly in cases of patients than in healthy children (P <0.001) while serum levels of high density lipoprotein-cholesterol were significantly lower in patients than in healthy controls(p<0.001). Urinary protein was significantly higher in the patient group (P < 0.001 for each) while serum total protein was significantly lower among patients' group than the control group (P < 0.001 for each) (Table 1).

In this study, serum miRNA-192 expression was measured using qRT-PCR and normalized to RNU6 as reference control. The expression level of miRNA 192 was significantly lower in nephrotic children in comparison with healthy controls (P < 0.001 for each) (Table 1). Also, serum TGF-ß level was significantly higher among patients' children compared to control group (P <0.001).

Table 1. Clinico-demographic characteristics, biochemical laboratory and miRNA- 192 expression profiles in nephrotic patients and healthy controls

Parameters	Patients (N=50)	Controls(N=50)	P-value
Age (range)	7.4±1.1 (5-9)	7.36±1.0 (5-9)	> 0.9
Gender, n (%)			
Male	28 (56)	28 (56)	> 0.9
Female	22 (44)	22 (44)	
BMI (Kg/m^2)	31.7 ± 4	25.4 ± 1.6	<0.001*
SBP (mmHg)	117.4 ± 4.4	108 ± 12	<0.001*
DBP (mmHg)	75.4 ± 8.4	79.4 ± 4.2	0.003
Total cholesterol (mg/dL)	404±55	126.1±2.3	<0.001*
Triglycerides(mg/dL)	463.4±93	120±17.8	<0.001*
HDL-C(mg/dL)	29.7±5.4	62.6±6.6	<0.001*
LDL-C(mg/dL)	379.4±56	63.5±6.7	<0.001*
Serum Total Protein (g/dL)	4.1 ± 0.9	7.0 ± 0.5	<0.001*
Urinary Protein (g/dL)	1.45 ± 0.2	0.17 ± 0.07	<0.001*
Serum TGF-ß (pg/mL)	167.5 ± 7.9	50.1 ± 4.4	<0.001*
miRNA 192 expression levels (fmol/L)	47.9 ± 1.7	263.5 ± 12.6	<0.001*

BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, TGF-ß: transforming growth factor- beta.

(*) Considered significant statistically (P-value < 0.05).

3.2. ROC curve analysis: receiver operating characteristics (ROC) was used to determine the threshold (cutoff) value for optimal sensitivity and specificity at which the biomarker could be used to differentiate between nephrotic cases and healthy controls.

ROC analysis (Figure.1) revealed that miRNA-192 could differentiate nephrotic patients from healthy controls with an AUC of 0.978 for miRNA-192 (95% CI: 0.940 – 1.016, P < 0.001). The optimal sensitivity and specificity to differentiate nephrotic children from controls were (94.0% and 98.0% at a cutoff expression value <149). This finding suggests that miRNA-192 could be a potential diagnostic biomarker in childhood nephrotic syndrome.

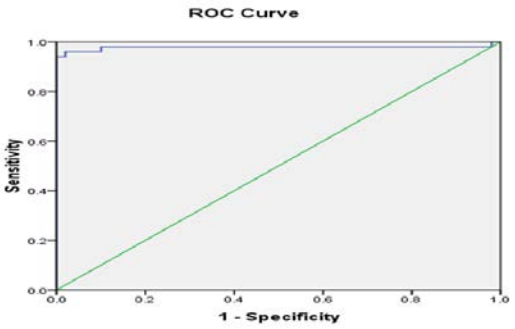

Figure 1. Receiver operating characteristic (ROC) curve analysis for miRNA-192 discriminating between nephrotic patients and healthy controls.

Moreover, ROC analysis (Figure.2) showed that TGF-ß could differentiate nephrotic patients from healthy controls with an AUC of 0.970 (95% CI: 0.940 – 0.999, P < 0.001). At cutoff value >160, TGF-ß provided sensitivity of 80.0% and specificity of 98.0% for acting as a novel diagnostic marker for nephrotic syndrome.

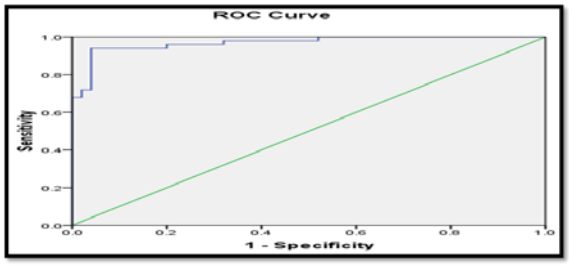

Figure 2. Receiver operating characteristic (ROC) curve analysis for TGF-ß discriminating between nephrotic patients and healthy controls.

3.3. Correlation of miRNA-192 expression level with serum level of TGF-ß and Urinary protein among nephrotic patients:

The relation between the quantitative expression levels of miRNA-192 with serum TGF-ß level and Urinary protein among the nephrotic patients was clarified using Spearman correlation analysis. As shown in Figures (3 and 4), miRNA-192 expression levels were negatively correlated with both TGF-ß (r = - 0.88, P < 0.001) and urinary protein (r=-0.88, P<0.001).

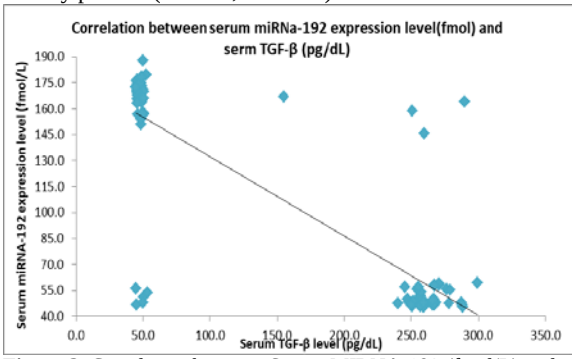

Figure 3. Correlation between Serum MIRNA-192 (fmol/L) and serum levels of TGF-β (pg/mL)

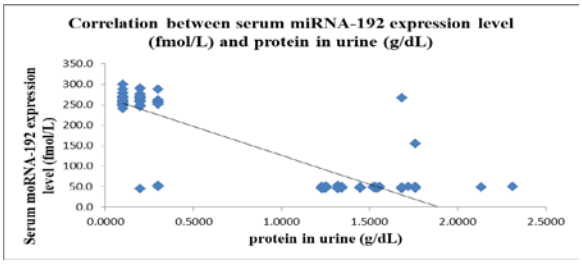

Figure 4. Correlation between Serum miRNA-192 (fmol/L) and Urinary protein (g/dL)

3.4. Correlation of miRNA-192 expression level with serum level of lipid indices among nephrotic patients:

The quantitative expression levels of miRNA-192 showed significant negative correlations with serum LDL-c (r = -0.88, P-value <0.001), TGs (-0.84, P-value <0.001) and TC (-0.87, P-value <0.001) level among the nephrotic patients. As shown in Figure (5, 6and 7)

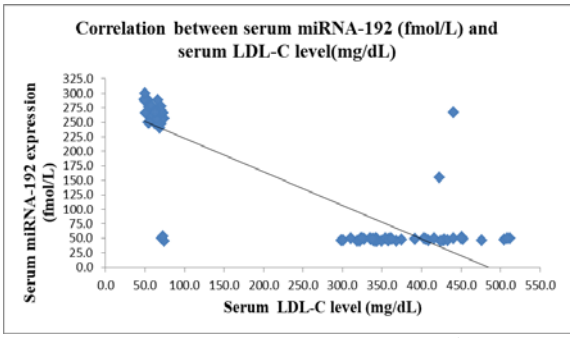

Figure 5. Correlation between Serum MIRNA-192 (fmol/L) and Low density lipoprotein – Cholesterol (LDL-C) (mg/dL)

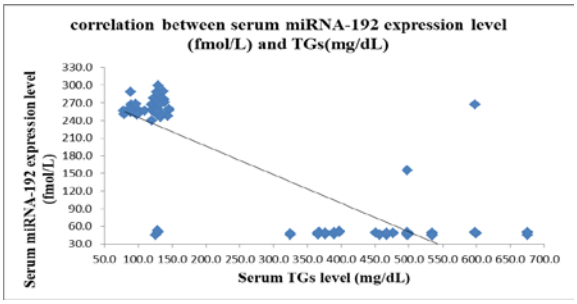

Figure 6. Correlation between Serum MIRNA-192 (fmol/L) and Triglycerides (TGs) (mg/dL)

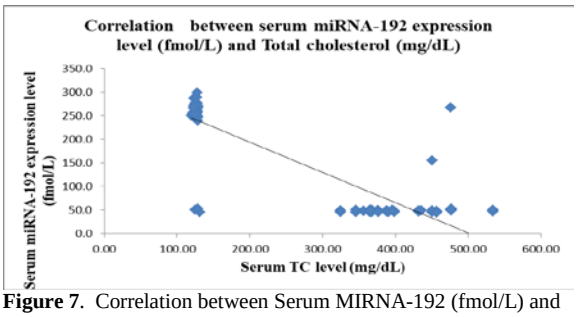

Figure 7. Correlation between Serum MIRNA-192 (fmol/L) and Total Cholesterol (TC) (mg/dL)

3.5. Correlation TGF-ß with urinary level of protein among nephrotic patients:

As shown in Figure (8), the serum level of TGF-ß showed a highly significant positive correlation with urinary protein level among the nephrotic patients (r = 0.86, P<0.001).

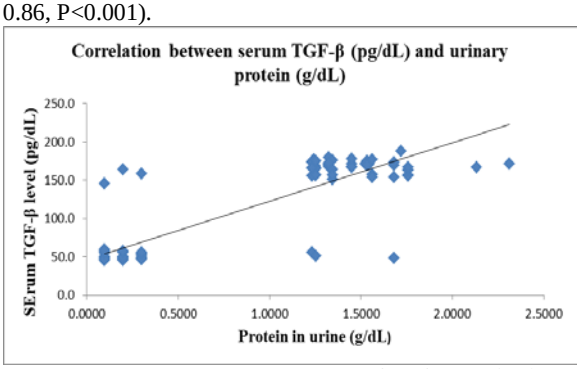

Figure 8. Correlation between Serum TGF-β (pg/mL) and Urinary protein (g/dL)

3.6. Analysis of the power of miRNA-192 and TGF-ß to predict nephrotic syndrome pathogenesis:

Multiple Logistic regression analysis was performed to evaluate whether miRNA-192 & TGF-ß could predict the NS pathogenesis in nephrotic patients. We found that

miRNA-192 was an independent predictor for significant dyslipidemia (-2.88±0.79), P-value <0.001 and TGF-ß serum level (-.051±0.17), P-value<0.001while it did not show any significant regression with the other parameters (P-value >0.05) as shown in Table 2.

Table 2. Multiple logistic regressions Analysis for miRNA- 192 and other parameters among Nephrotic syndrome patients

Parameters	B ± SE	P-value
Age (years)	4.1±4.7	>0.05
BMI(Kg/m^2)	-2.0±1.69	>0.05
Blood Pressure(mmHg)	0.068±0.54	>0.05
Protein in Serum(g/dL)	-4.3±7.0	>0.05
Dyslipidemia(mg/dL)	-2.88±0.79	<0.001*
TGF-β (pg/mL)	-0.51±0.17	<0.001*

(*) Considered significant statistically (P-value < 0.05).

Furthermore, TGF-ß was found to be an independent predictor for significant serum miRNA-192 level (-0.17 ±0.05, P-value <0.001) and proteinuria (34.6±13.7, P-value = 0.01), while it did not show any significance regression to any other parameters as shown in Table 3.

Table 3. Multiple logistic regressions Analysis for TGF-ß and other parameters in Nephrotic syndrome

Parameters	B ± SE	P-value
Age (years)	3.85±2.68	>0.05
BMI(Kg/m^2)	1.03±0.97	>0.05
Blood Pressure(mmHg)	0.15±0.31	>0.05
Protein in Serum (g/dL)	-2.7±4.0	>0.05
Protein in Urine (g/dL)	34.6±13.7	0.01*
Dyslipidemia(mg/dL)	0.6±0.48	>0.05
miRNA-192(fmol/L)	-0.17±0.05	<0.001*

4. Discussion

Despite several advances in INS pathophysiology**,** MCD and FSGS still remain complex diseases for which new therapies are needed. Clinical evolution is still Our study also revealed a significant negative correlation between serum levels of miRNA-192 and dyslipidemia, which reflects the possibility to metabolic syndromes arising from insulin resistance accompanying with abnormal adipose deposition, causing alternation disturbances in biological activities in disease pathology including glucose homeostasis, vascular signaling affecting cardiovascular risks and obesity. Kato and his colleagues matched our hypothesis; their study examined the involvement of miRNA- 192 in the regulation of biological events related to the pathogenesis of diabetes (Kato et al., 2007). Also, another study in mice model explored the role of miRNA-192 during the development of obesity associated with dyslipidemia, glucose intolerance (Castaño et al., 2018). Shah et al., correlated miRNA-192 expression level with waist circumference and visceral fat quantity in humans as well as an increase in TG/HDL ratio (Shah et al., 2017). In line with our findings, Hamdia and her colleagues suggested that miRNA-192 expression levels accompanied with dyslipidemia and obesity are risk factors related to the prevalence of renal, cardiovascular, ocular and nervous system complications among diabetic patients (Hamdia et al., 2013).

We also found a significant negative correlation between miRNA-192 expression level and proteinuria reflecting changes in glomerular protein filtration and/or defects in tubular reabsorption that cause the appearance of proteins in the urine. The presence of proteinuria emphasizes alternation in the intercellular junction and cytoskeletal structure of the foot processes of podocyte, and the cell showed a simplified, effaced phenotype (Kriz et al., 2012). Damage to these cells may manifest vacuolization, fusion of foot processes, and focal detachment of epithelial cells from the underlying basement membrane. These changes appear to be the consequence mainly of persistent abnormalities in intra glomerular capillary hemodynamics (Rennke and Klein, 1989). Another study by Putta et al. found that the suppression of miRNA-192 in diabetic mice attenuated proteinuria and induced renal fibrosis (Putta et al., 2012). In contrast to our results, Xiaoyi et al. found a positive significant correlation between the serum expression level of miRNA-192 and urinary protein level in patients with FSGS and MCD (Xiaoyi et al., 2013). Also, a recent study reported that miRNA-192 was significantly higher in overt proteinuria than in normoalbuminuria and microalbuminuria groups (Hung-Yu et al., 2016). Further studies are needed to highlight this relation and influence the diagnosis and management of nephrotic syndrome.

TGF-β is a potent and versatile cytokine. When activated, it exerts both physiological and pathologic functions including immune functions, inflammation, wound healing and organ fibrosis (Karim et al., 2019). TGF-β is ubiquitously expressed and all cells appear to respond TGF-β (Jenkins, 2008). TGF- β induces trans-differentiation of extracellular matrix components (Humphreys et al., 2010). TGF- β exerts its profibrotic activity through stimulation of fibroblast proliferation, extracellular matrix synthesis (e.g., collagen types I, III, and IV, proteoglycans, laminin, and fibronectin), and epithelial-to-mesenchyme transition (EMT). Induced expression of ECM remodeling genes, increasing in the apoptosis rate, and EMT lead to tubulointerstitial fibrosis and glomerulosclerosis (Lamouille et al., 2014). In our study, there was a significant increase of serum TGF-β levels in nephrotic children as compared to healthy controls. ROC analysis confirmed that TGF- β may be useful as potential diagnostic biomarkers in childhood nephrotic syndrome (sensitivity: 80.0% and specificity 98.0% at a cutoff value > 160). In accordance with our data, Xiaoyi, et al. reported that TGF- β level was significantly increased Diabetic Nephropathy Patients (Xiaoyi, et al., 2016). This result was expected by another study which identified TGF- β as being upregulated during the course of progressive renal injury (Bottinger, 2007). Similarly, inhibition of TGF- β has been shown to ameliorate renal injury (Border and Noble, 1997). Our findings also showed a highly significant positive correlation between serum TGF- β and proteinuria. Goumenos believed that TGF-β was not only involved in ECM accumulation, fibrosis, and progressive renal impairment, but also played a role in changes to the glomerular filtration barrier and induction of proteinuria. There is substantial evidence to support this observation. Urinary TGF- β correlates with the degree of proteinuria (Goumenos et al., 2002).

Also, a previous study demonstrated that activation of TGF-β pathway contributes to the progression, invasion and poor prognosis of renal cancer patients (Kato et al., 2007).

Interestingly, we observed a significant negative correlation between miRNA-192 expression levels and TGF-β in nephrotic children (r =- 0.88 and p <0.001). We hypothesized that the microRNA-192 which expression correlated with TGF-β levels might be linked with progression of NS and may lead to increased fibrosis resulting in disarrangements in renal functional parameters that induce angiotensin, proteinuria, and hypoxia. This theory is in line with another study performed by Wang and his colleagues that demonstrated the potential for enhanced airway renal fibrosis mainly through TGF-ß activity (Wang et al., 2010). In parallel with our findings, Krupa et al. explained the same relation as TGF- β 1 inhibited miR-192 expression in human proximal tubular cells (PTCs) and deficiency of miR-192 associates with renal fibrosis acceleration and GFR reduction in Diabetic Nephropathy. There are two transcription factors namely zinc finger E-box binding homeobox-1 (Zeb1) and Zeb2 that are located downstream of TGF-β signaling pathway can suppress E-cadherin and control renal fibrosis. Overexpression of miRNA-192 could inhibit the TGF-β1 signaling pathway and the expression of Zeb1 and Zeb2 and then prevented the kidney from fibrosis. So, it was reported that TGF-β1 inhibits the expression of miRNA-192 that targeted Zeb1/2 to activate TGF-β1 signaling pathway and accelerate renal fibrosis in DN (Krupa et al., 2010). Similarly, another study by Wang et al. in rat model reported that the expression of miRNA-192in rat PTCs, mesangial cells, and human podocytes was decreased by TGF-β (Wang et al. 2013). Recently, Xiaoyi et al. found that miR-192 was negatively correlated with TGF-β1 and Fibronectin, two parameters which represent the fibrosis extent of the kidney (Xiaoyi et al., 2016). However, there are different studies with opposite conclusions. Kato and colleagues found a significant expression of miRNA-192 increased in mesangial cells due to high glucose level, and it has a vital role in the kidney disease pathogenesis as it amplifies TGF-β1 signaling (Kato et al., 2012). The discrepancy in these studies may be due to the different samples species, cell types differences (including podocyte, renal tube cells, and mesangial cells), and experiment conditions.unpredictable. No cohort study has succeeded in bringing out prognostic factors. The discovery of the genetic role in the pathophysiology of nephrotic syndrome or disease progression is one of the hot spots in pediatric nephrology and has an important clinical impact (Suvanto *et al*., 2016). Several studies revealed the involvement of miRNA as a diagnostic parameter in NS (Wang *et al*., 2013 and Luo *et al*., 2013). Recent studies have shown that miRNA-192 target sites are located within TGF-β mRNA; therefore, miRNA-192 can significantly inhibit the translation process after TGF-β transcription (Kato *et al.*, 2011).

Therefore, we hypothesized that the different expression of miRNA-192 between nephrotic patients and healthy children leads to alterations in its target, Smad-interacting protein 1 located within TGF-β mRNA, in childhood nephrotic syndrome. This disturbance may lead to podocyte alterations with protein expression resulting proteinuria or localization defects, actin cytoskeleton remodeling, or intracellular signaling pathway activation and influencing metabolism leading to Hyperlipidemia. Furthermore, alterations lead to a disturbance of glomerular filtration barrier properties. Besides, miRNA-192 could be a novel diagnostic biomarker of NS patients which helps in rapid assessment of the disease and limiting its progression. Our study revealed that as compared with healthy children, the serum level of miRNA-192 expression level in nephrotic patients showed significance decrease. ROC analysis confirmed that miRNA-192 may be useful as potential diagnostic biomarkers in childhood nephrotic syndrome (sensitivity: 94.0% and specificity 98.0% at a cutoff value <149).

Most investigations have found that miR-192 expression increases in diabetic nephropathy (DN) models and in renal cells, but several other studies have reported that miR-192 expression decreases (Yang *et al.*, 2013). Our results are in accordance with the study that explored the potential diagnostic difference between FSGS and MCD on basis of miR-192 and approved that serum miR-192 had higher expression level in patients with FSGS than those with MCD but lower than healthy controls (Xiaoyi *et al.*, 2013). Similarly, Sayilar and his colleagues revealed that miR-192 expression was 1.5 fold lower in plasma and 1.8 fold higher in urine samples of stage five chronic kidney disease (CKD) patients compared to the control group, while it was much higher in both plasma and urine samples (3.8 and 3.3 fold, respectively) of stage three CKD patients (Sayilar *et al*., 2016). In contrast, Kato et al.'s rat model study showed increased miR-192 expression in diabetic rats (Kato *et al.*, 2007). A recent systematic literature survey found that miRNA-192 is increased in body fluids (including plasma and urine) of patients with diabetic nephropathy (Yang *et al.*, 2013). Similar to this study, further support of miRNA-192 involvement in the pathobiology of the kidney, it has been recently reported that sera of patients with early stages of DN had a higher expression of miR-192 compared to the late stages cases of clinical course of this disease process (Krupa *et al*., 2010). Also, a study established by Barutta et al. found that miRNA 192 expression levels is higher in patients with microalbuminuria DN (Barutta *et al.*, 2013). This discrepancy might be due to the different samples used between our study and others.

5. Conclusions

Our study revealed that serum miRNA-192 has a high predictive pathogenic value in NS prognosis and management as a promising severity biomarker through its negative correlations with proteinuria and TGF-β as well as dyslipidemia.

Although our findings did not match other experimental studies, further studies are needed.

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