

Original Article

Urinary Transforming Growth Factor Beta-1 in Children With Nephrotic Syndrome and End-Stage Renal Disease

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ABSTRACT:

Background: Nephrotic syndrome is one of the most important renal disorders in childhood and it may end with chronic renal failure. Transforming growth factor-beta1 (TGF- β 1) is a multi functional cytokine that plays an important role in glomerulosclerosis that leads to resistance to steroid therapy and chronic renal failure.

Objectives: This study aimed at evaluating the role of estimation of TGF- β 1 in urine of nephrotic children as a parameter of progression of the disease and a predictor of response to steroid therapy.

Methods: Forty-four children attending the Nephrology Unit of Ain Shams University Children's Hospital were enrolled in the study, 30 with idiopathic nephrotic syndrome and 14 with end-stage renal disease under regular hemodialysis and were compared to 30 normal; age and sex matched controls. The nephrotic children were divided according to their response to steroid therapy into 13 dependent and 10 resistant cases. In addition to 7 newly diagnosed nephrotic children who were sampled at presentation and after one month of steroid therapy. All patients were subjected to history taking and complete clinical examination. Laboratory investigations included, serum albumin and creatinine, random urinary protein and creatinine and urine protein to creatinine (uPr/Cr) ratio was calculated. In addition to determination of TGF- β 1 in the random urine sample by ELISA technique and its expression as a ratio to urinary creatinine.

Results: uTGF- β 1/Cr was highly significantly elevated in all nephrotic groups when compared to controls and a significant difference was found between steroid dependent and steroid resistant groups (14.46 ± 5.13 and 48.4 ± 23.13 pg/mg Cr respectively). In the newly diagnosed group the mean uTGF- β 1/Cr ratio at presentation was significantly higher than after one month and both were higher than the control (57.28 ± 66.18 , 25.66 ± 29.6 and 4.76 ± 3.3 pg/mg Cr respectively). The lowest uTGF- β 1/Cr values were among patients who turned to be steroid responsive and the highest values were among steroid resistant patients. A significant positive correlation was found between uTGF- β 1/Cr ratio and uPr/Cr ratio in all studied nephrotic groups, while no correlation could be elicited between uTGF- β 1/Cr and age, sex, blood, pressure, duration of illness or dose of steroid. In the end stage renal disease (ESRD) group, the highest mean value of uTGF- β 1/Cr ratio among the studied group was found (219.86 ± 19.79 pg/mg Cr), but it could not be correlated with age, sex, duration of dialysis, type of dialyzer membrane, type of dialysate, blood pressure, serum albumin or serum creatinine. Patients with uTGF- β 1/Cr ratio lower than a cut-off value of 11.9 pg/mg Cr (mean \pm 2.5 SD) have a higher chance to be steroid responsive or dependent while those with values above the cut-off are mostly steroid resistant.

Conclusions: uTGF- β 1 is a good diagnostic and prognostic marker in patients with nephrotic syndrome. It is a simple and easy marker for prediction of response to steroid therapy in nephrotic patients, in addition to its value in monitoring progression of glomerulosclerosis among nephrotic patients that ends in chronic renal insufficiency.

INTRODUCTION

Childhood nephrotic syndrome (NS) is characterized by proteinuria, hypoalbuminemia, hypercholesterolemia and edema. An

increased glomerular permeability resulting in proteinuria is the primary renal abnormality in NS while hypoalbuminemia, edema and hypercholesterolemia are believed to be

secondary pathologic events⁽¹⁾.

TGF- β is a multifunction cytokine, discovered about 20 years ago. Five distinct isoforms of TGF- β have been described, and three of these, TGF- β 1, TGF- β 2 and TGF- β 3 are found in all mammalian tissues⁽²⁾ and are structurally closely related to one another⁽³⁾. In the kidney TGF- β 1 is the most highly expressed being present both in tubular epithelial cells and in the glomerulus⁽⁴⁾. The gene for each isoform is located on a different chromosome⁽²⁾.

Transforming growth factor beta one (TGF- β 1) is a multifunctional cytokine that plays an important role in embryonic development and in regulating repair and regeneration after tissue injury. It is involved in angiogenesis, regulation of inflammation, control of cell proliferation and cell adhesion protease⁽⁵⁾. TGF- β 1 functions in an autocrine or paracrine fashion to elicit profound effects on cell growth and extra-cellular matrix (ECM) accumulation in many cell types⁽⁶⁾. TGF- β 1 is a pleiotropic cytokine involved in a tremendous biological process including ECM remodeling, embryo development, wound healing, bone formation, immune suppression and carcinogenesis⁽⁷⁾.

A causal role of TGF- β 1 in the over-production of extracellular matrix by glomerular cells has been indicated by studies of experimental nephritis and human glomerulonephritis. Thus it appears that TGF- β 1 plays a central role in the process of mesangial expansion, glomerular sclerosis, and interstitial fibrosis⁽⁸⁾.

However, the dark side of TGF- β 1 effect is that it induces deposition of ECM at site of tissue injury can lead to scarring, and

fibrosis. Furthermore, the ability of TGF- β 1 to induce its own production may be the key to development of the scarring and fibrosis into chronic, progressive conditions that will in time obliterate the tissue structure⁽⁹⁾.

Most important is the recognized ability of TGF- β 1 to initiate and then terminate the repair process. Failure to terminate the action of TGF- β 1 glomerulus would set the stage for progressive glomerulosclerosis⁽²⁾.

The expression of TGF- β 1 is enhanced in renal disease and available evidence suggests that its activity in promoting the synthesis of ECM play a crucial role in fibrotic deposition and the decline in renal function, TGF- β 1 is however also expressed in response to renal injury and may play an important role in normal repair process, it appears that renal diseases may result from inappropriate regulation of TGF β ⁽¹⁰⁾.

TGF- β 1 activity has been successfully suppressed in vivo, in the kidney, in the skin, and central nervous system injury by administering anti TGF- β 1 antibodies capable of preventing the binding of TGF- β 1 to its receptor and blocking the action of TGF- β 1 dramatically decreased the excessive deposition of ECM but didn't interfere with normal healing of tissues⁽¹¹⁾.

AIM OF THE WORK

The study aims at evaluating the role of TGF- β 1 assay in urine of nephrotic children as a parameter of progression of the disease and predictor of response to steroid therapy, in addition to the study of its behaviour in children with ESRD.

SUBJECTS AND METHODS

The study comprised 44 patients

selected from the Pediatric Nephrology Unit of Ain Shams University Children's Hospital. They were compared to 30 healthy children as a control group.

Patients were classified according to their response to corticosteroids as defined by Barratt and Clark (1999)⁽¹²⁾ into the following groups.

Group I:

Included seven newly diagnosed NS cases. They were four males and three females whose age ranged from 1.25 to 7 years (mean 5.53 ± 3.16 years). They were sampled twice, the first was at their first presentation and before receiving any definitive therapy, while the second sample after entering in remission or ending one month of full dose steroid therapy.

Group II:

Included 13 patients with steroid dependent NS. They were 10 males and three females. Their ages ranged from 1.5 to 12 years (mean 6.84 ± 3.53 years).

Group III:

Included 10 patients with steroid resistant NS comprising seven males and three females. Their ages ranged from 6 to 12 years (mean 8.7 ± 5.7 years). They were receiving either cyclosporine A (CsA) or levamisole in addition to prednisone.

Nephrotic children who showed elevated serum creatinine or signs of chronic illness especially rheumatic and hepatic diseases were excluded from the study. Diabetic children either primary or steroid induced were also excluded.

Group IV:

Included 14 patients with ESRD on regular hemodialysis. They were six males and eight females. Their ages ranged from 7

to 12 years (mean 10.92 ± 1.59 years). Ten of them were receiving erythropoietin therapy and nine were dialysed using a polysulfone membrane, while eight were dialysed using a bicarbonate dialysate.

Control Group:

The control group comprised 30 healthy children after excluding clinically the possibility of renal disease and by performing complete urine analysis. They were 17 males and 13 females and their age ranged between 2 and 12 years (mean 7.2 ± 2.78 years).

Children in the study were subjected to:

1. Thorough clinical evaluation.
2. Laboratory tests:
 - a. Complete urine analysis.
 - b. Random urinary creatinine (uCr) using modified rate Jaffé method.
 - c. Random urinary protein (uPr) using colourimetric method.
Random urinary protein/creatinine (uPr/Cr) ratio was calculated.
 - d. Determination of urinary transforming growth factor beta one (uTGF- β 1) in the random urine sample by ELISA technique (MEDGENIX TGF- β 1 EASIA kit, Biosource Europe S.A., Zoning Industriel B-1400 Nivelles, Belgium).
Urinary TGF β -1/urinary creatinine ratio (uTGF- β 1/Cr) was calculated to avoid the cumbersome 24 hours urine collection.
 - e. Serum albumin (S.Alb) and serum creatinine (S.Cr) levels were measured.

C. Statistical Methods:

Data were analyzed with Statistica Software Package v.5 (Statsoft, Tulsa, OK, USA). All numeric data were expressed as

mean \pm standard deviation (SD). Data were analyzed using student t test, paired t test and ANOVA test to compare mean values of different variables. Pearson r correlation coefficient was used to determine the relationship between different quantitative variables. For all tests a probability of less than 0.05 was considered as significant.

RESULTS

The mean uPr/Cr ratio was significantly higher in the steroid resistant than in the steroid dependent group.

The mean uTGF- β 1/Cr ratio was significantly higher in the steroid dependent, steroid resistant and ESRD groups when compared with that of the control (14.46 ± 5.13 , 48.4 ± 23.13 , 219.86 ± 119.47 and 4.76 ± 3.3 pg/mg Cr respectively) (Table 1).

In all studied patients groups, the highest mean uTGF- β 1/Cr ratio was found in the ESRD group and a significant difference in the mean u TGF- β 1/Cr ratio was found between each two studied groups (Figure 1).

In the newly diagnosed group, the mean uTGF- β 1/Cr ratio dropped significantly after one month of therapy but still its mean values both before and after treatment (57.28 ± 66.18 and 25.66 ± 29.6 pg/mg Cr respectively) were significantly higher than the control.

A cut off value of uTGF- β 1/Cr ratio was calculated from the normal control group (= mean \pm 2.5 SD = 11.90 pg/mg Cr and applied to the newly diagnosed patients. Before treatment, only one patient (14%) out of the seven patients had a value below the cut off value and he entered into

spontaneous remission. After treatment, two patients out of six (33.34%) responded to steroid treatment and both had a value below the cut off value and the other four patients (66.66%) were steroid resistant and had a higher values, except for one patient who received Endoxan therapy. In the group of steroid dependent cases, uTGF- β 1/Cr was higher than the cut-off value in eight out of 13 patients (61%), while in the steroid resistant group all patients had high uTGF- β 1/Cr values.

In the ESRD group, uTGF- β 1/Cr ratio was not affected by the type of hemodialyzer membrane, dialysate fluid or erythropoietin therapy. In addition, sex did not influence uTGF- β 1/Cr ratio in any of the studied groups.

A significant positive correlation was found between uTGF- β 1/Cr ratio and uPr/Cr ratio in the steroid dependent. ($r = 0.90$, $p < 0.05$), steroid resistant ($r = 0.79$, $p < 0.05$), newly diagnosed both at presentation ($r = 0.92$, $p < 0.05$) and after one month of treatment ($r = 0.97$, $p < 0.05$).

DISCUSSION

Nephrotic syndrome is the clinical presentation of various pathologic processes⁽¹³⁾. Accordingly its fate cannot be predicted accurately in the course of the disease. Various prognostic indices were implemented in a trial to anticipate the possible outcome of syndrome. Indeed, all still do not give us a 100% index about the possible fate⁽¹²⁾.

Glomerulosclerosis, which is now believed to be a central factor in the pathogenesis of progressive renal insufficiency, typically occurs after an

Table 1: Comparison between mean values of uTGFβ1/Cr ratio in the studied groups.

uTGF- β1/Cr	Group II (steroid- dependent)	Group III (steroid-resistant)	Group IV (ESRD)	Control
Mean ± SD (pg/mg Cr)	14.46 ± 5.13	48.4 ± 23.13	219.86 ± 119.5	4.76 ± 3.3
Group II		< 0.05	< 0.05	< 0.001
Group III			< 0.05	< 0.001
Group IV				< 0.001

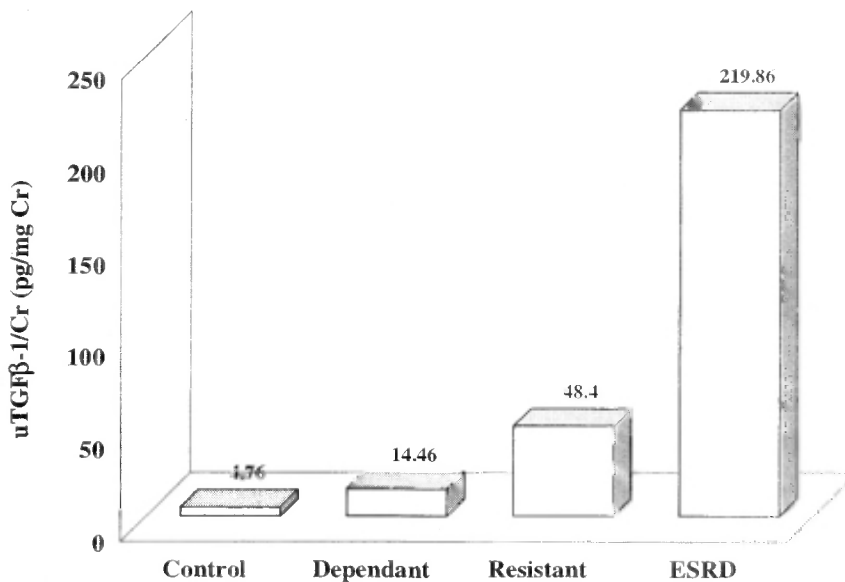


Figure 1: Mean uTGF-β1/Cr ratio in the studied groups

initial pathogenic insult to the resident glomerular cells. The autocrine and paracrine actions and interactions of glomerular cell cytokines increase matrix synthesis and decrease its breakdown, that perpetuate extra-cellular matrix deposition. As the ECM expands, a segmental collapse of the capillary bed occurs resulting in proteinuria, loss of filtration and finally ESRD⁽¹⁴⁾.

Accumulating evidence supports the fact that TGF-β1 is a major contributor to glomerulosclerosis and interstitial fibrosis, which are common features of progressive injury in glomerular disease⁽³⁾. Immunocytochemical and in situ hybridization studies of renal biopsy specimens indicate local TGF-β1 production in normal and in patients with various glomerular diseases by resident glomerular cells⁽¹⁵⁾. Direct

measurement of renal TGF- β 1 production is informative but repeated biopsies are invasive and therefore cause considerable hazards. As an alternative means of estimating the synthesis and secretion of TGF- β 1 in the kidney, TGF- β 1 levels are measured in urine which is an easy and less hazardous method⁽⁸⁾.

As far as we know, this study is the first to measure uTGF- β 1 in nephrotic children classified according to clinical course and response to steroid treatment, rather than according to the histopathological findings in the biopsies.

In the present study uTGF- β 1 was detected in nephrotic groups and it was significantly higher in the nephrotic children compared to the normal control denoting increased uTGF- β 1 excretion in the urine of nephrotic children. Also, uTGF- β 1 was not affected by the age, sex, duration of illness, blood pressure or dose of corticosteroids in nephrotic children. Thus uTGF- β 1 could be considered a good marker in case of NS as it is not affected by many clinical variables.

Galal (1998)⁽¹⁶⁾ found that Pr/Cr ratio measured in a random voided urine sample obtained during normal day time activities avoiding the first morning and evening spot samples, can under most clinical circumstances replace the measurement of protein excretion in 24 hr urine collection in grading of proteinuria, physiologic designated as Pr/Cr ratio < 0.1 and nephrotic proteinuria designated as Pr/Cr ratio > 1.0 and it is simple, time saving reliable and accurate method. Thus, this method was used in the present study as an indicator of proteinuria and showed a

significant positive correlation with uTGF- β 1/Cr in studied nephrotic patients whether dependent, resistant or newly diagnosed both before and after treatment. These correlations, together with a significant drop in Pr/Cr ratio and uTGF- β 1/Cr after one month of therapy justify to consider uTGF- β 1/Cr a parameter of activity in NS.

Comparing uTGF- β 1/Cr in each studied nephrotic group with control it was significantly higher in each group but the difference was more marked with steroid resistant. This result points to uTGF- β 1 as an additional diagnostic marker of NS particularly the steroid-resistant type.

In the steroid dependent group uTGF- β 1/Cr was higher than the cut-off value in eight out of 13 patients (61%), while in the steroid resistant group all patients had high uTGF- β 1/Cr values.

Tejani et al. (1999)⁽¹⁷⁾ found that in renal biopsies TGF- β 1 gene expression was detected in two out of 14 steroid sensitive cases, both of whose lesions later progressed to focal segmental glomerulosclerosis (FSGS) and also in biopsies of 33 out of 39 steroid resistant nephrotic children among these 18 out of 20 had FSGS.

The type IV collagen is one of ECM components whose production is regulated by TGF- β 1. As our results found higher values in TGF- β 1 in 100% of steroid resistant cases, Farid et al. (1999)⁽¹⁸⁾, studied urinary type IV collagen (u IV C) in NS and found a significant elevation of u IV C in steroid resistant cases, and they concluded that u IV C is a good diagnostic marker in steroid resistant patients at the beginning of disease, short of starting steroid therapy.

But contrary to our results, Farid et al. (1999)⁽¹⁸⁾, did not find a significant elevation of u IV C in steroid dependent or steroid sensitive patients compared to control or a significant decrease in u IV C after one month of steroid treatment in the newly diagnosed patients. These differences provide a good evidence for the effect of uTGF- β 1 in the course of NS, and add to the value of its assay in urine of nephrotic patients.

To assess the prognostic value of uTGF- β 1, we calculated a cut off value from the control group and applied it to the newly diagnosed patients. Before start of therapy, we found one child with a value below the cut off level. He entered into spontaneous remission. The other six patients had a high uTGF- β 1 among these four became steroid resistant. After one month with steroid treatment, all steroid resistant patients had their uTGF- β 1 values decreased but still higher than the cut off value, except for one patient who received endoxan therapy. Monkanen et al. (1997)⁽¹⁹⁾ found that the immunosuppressive therapy significantly decreases uTGF- β 1 and that patients with persistent nephrotic syndrome and/or declining renal function had higher initial uTGF- β 1 excretion than those entering into partial or complete remission.

Thus, performing uTGF- β 1 after one month in patients resistant to corticosteroid treatment could be informative, simple and saves unnecessary renal biopsies. If it is above the cut off value renal biopsy can be done, otherwise steroid treatment can be continued, as there is more chance for the patient to be steroid responsive.

The most important complication in NS

is the progression to ESRD, and this progress is different between NS patients according to response to steroids, more than 3% of NS of steroid responder changed to resistant and more than 30% of the latter will progress to ESRD within 5 years⁽¹²⁾, while more than 50% of patients with steroid-resistant NS will progress to ESRD after a follow of up of 10 years⁽²⁰⁾. For this reason we studied the uTGF- β 1 in ESRD children under regular hemodialysis. As far as we know this is the first study estimating uTGF- β 1 in ESRD children. uTGF- β 1 showed the highest values among the different studied groups. Podracka et al. (1999)⁽²¹⁾ found that mean uTGF- β 1 was higher among patients with interstitial fibrosis compared to the group without fibrosis in different glomerular diseases. Young et al. (1997)⁽²²⁾ found that the degree of glomerulosclerosis in different renal biopsies from various glomerular diseases followed the TGF- β 1/betaactin mRNA ratio, this reflects the on going synthesis of TGF- β 1 in tissues.

Sutharithiran et al. (1998)⁽²³⁾ explained in their theory the effect of the level of TGF- β 1 in the progression of renal diseases. They correlated the TGF- β 1 levels and the rate of progression of renal diseases, and tested the postulate that high levels of TGF- β 1 in patients with diseases such as primary renal diseases or diabetes or hypertension, identifies a priori patients at risk for progression. They explained that the heightened risk of progressive renal failure is a consequence of increased TGF- β 1 protein production, and intrinsic renal diseases are envisioned as the initial injury that elicit an exaggerated TGF- β 1 response

and they found that high levels of TGF- β 1 lead to progression to ESRD while low or normal lead to slow or no progression to ESRD in patients with renal diseases, diabetes or hypertension.

It is clear that uTGF- β 1 is useful in monitoring the course of glomerular diseases. In the ESRD group, a non significant correlation was found between uTGF- β 1 and sex, age, serum albumin, serum creatinine years of dialysis, type of dialyzer membrane (polysulfone or cuprophane) or type of dialysate fluid (acetate or bicarbonate). This further proves that uTGF- β 1 is not affected by many factors that may affect other parameters. Sutharithiran et al. (1998)⁽²³⁾ also found no correlation between the above mentioned parameters and circulating TGF- β 1 in ESRD adults under regular hemodialysis.

We found that the mean uTGF- β 1 in patients not receiving erythropoietin therapy in ESRD group was higher than that of patients receiving erythropoietin but this difference was not statistically significant, may be because of the small number of

patients. Logofetove et al. (1998)⁽²⁴⁾ found a significant decrease in serum TGF- β 1 in patients with chronic renal failure after a single dose of recombinant human erythropoietin.

Contrary to the study of Sutharithiran et al. (1998)⁽²³⁾, we did not find any correlation between uTGF- β 1 and blood pressure whether systolic or diastolic, in the ESRD group, probably because they measured uTGF- β 1 in the serum of adult diabetic patients on regular HD and they concluded also that increased uTGF- β 1 may contribute independently to the pathogenesis of hypertension, or it may contributed to the vascular complications of increased blood pressure.

In conclusion, uTGF- β 1 is a good diagnostic and prognostic marker in patients with nephrotic syndrome. It is a simple and easy marker for prediction of response to steroid therapy in nephrotic patients, in addition to its value in monitoring progression of glomerulosclerosis among nephrotic patients that ends in chronic renal insufficiency.

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