# Evaluation of Urinary Annexin V as a Prognostic Marker in Children with Nephrotic Syndrome

**Ahmed H. Hassan, Magid A. Abdel Fattah Ibrahim, Mona M. Zaki, Zeinab M. Shahat**

*Departments of Pediatrics and Clinical Pathology, Ain Shams University, Cairo, Egypt.*

## ABSTRACT

**Background/Aim:** Nephrotic syndrome include various pathological types including minimal change nephropathy, focal glomerulosclerosis, membranous nephropathy and membranoproliferative nephropathy. From a therapeutic perspective, nephrotic syndrome may be classified as steroid sensitive and steroid resistant. A kidney biopsy is not indicated for first presentation of idiopathic nephrotic syndrome unless the history, physical findings, or laboratory results indicate the possibility of secondary nephrotic syndrome or primary nephrotic syndrome other than minimal change, in patients younger than 1 year, when genetic forms of congenital nephrotic syndrome are more common, and in patients older than 8 years. Renal biopsy like most invasive medical procedures is not without risk as related vascular injuries are frequent. Apoptosis is increased in children with steroid resistant nephrotic syndrome. Urinary annexin V excretion may be an indicator of apoptosis and renal injury and hence, a predictor in children with steroid resistant nephrotic syndrome and prognostic marker in children with nephrotic syndrome. The study aims to evaluate urinary annexin V as a marker for resistance and prognosis in children with nephrotic syndrome. **Methodology:** Thirty children with nephrotic syndrome at one centre were included in a cross sectional study (15 with steroid responsive group 1 and 15 with steroid resistant nephrosis group 2, in remission). Twenty newly diagnosed nephrotic patients (group3) were included in a prospective study. Control group with age and sex match. Urinary annexin V by ELISA, urinary protein creatinine ratio, lipid profile and renal functions were done for all patients initially and at follow up for newly diagnosed ones (divided into steroid responsive, group 3 a, and resistant, group 3 b, in remission). **Results:** highly significant difference between group 1 and control, group 2 and control, group1 and group2, group 3 initially and control, group3 initially and in follow up, group 3 a and group 3 b, group 3 b and control as regards urinary annexin V, urinary protein/creatinine ratio and serum albumin. Highly significant difference between group 3 a and control in urinary annexin V level and no difference in urinary protein/creatinine ratio and serum albumin. Highly significant correlation between urinary annexin V and urinary protein/creatinine ratio in all studied groups, as well as no significance difference between urinary annexin V and serum albumin in all studied groups. Using ROC curve, a cut off value of urinary annexin V level of 3.15 (sensitivity of 0.778 and specificity of 0.364) and cut off value of urinary protein/creatinine ratio of 8.5 (sensitivity of 0.778 and specificity of 0.364) in analyzing the parameters of group 3 initially and in follow up group 3 a and b. **Conclusion:** urinary annexin V is a non invasive marker of steroid resistance but not of high sensitivity.

## BACKGROUND

Nephrotic syndrome, or nephrosis, is defined by the presence of nephrotic-range proteinuria, edema, hyperlipidemia, and hypoalbuminemia. It could be idiopathic or secondary to systemic diseases that affect other organs in addition to the kidneys, such as diabetes, amyloidosis, and lupus erythematosus. Nephrotic syndrome include various pathological types including minimal change nephropathy, focal glomerulosclerosis, membranous nephropathy and membranoproliferative nephropathy. From a therapeutic perspective, nephrotic syndrome may be classified as steroid sensitive and steroid resistant. A kidney biopsy is not indicated for first presentation of idiopathic nephrotic syndrome in the child 1-8 years of age unless the history, physical findings, or laboratory results indicate the possibility of secondary nephrotic syndrome or primary nephrotic syndrome other than minimal change. Kidney biopsy is indicated in patients younger than 1 year, when genetic forms of congenital nephrotic syndrome are more common, and in patients older than 8 years, when chronic glomerular diseases such as focal segmental glomerulosclerosis have a higher incidence, but renal biopsy like most invasive medical procedures is not without risk as related vascular injuries are frequent.

In previous years, many non invasive markers developed to detect response to steroids rather than the need for biopsy, as these markers are excreted in urine in cases of renal injury as urinary annexin V. Annexins are a superfamily of calcium- and phospholipids-binding proteins which originally evoked interest as mediators of the anti-inflammatory actions of glucocorticoids. Annexin V has been reported to possess anticoagulant activity, inhibition of phospholipase A2 as well as regulation of membrane transport, proliferation and signal transduction. Annexin V binds to the phosphatidyl serine-exposing apoptotic cell and can inhibit the procoagulant and proinflammatory activities of the dying cell. Nephrotic syndrome is associated with and probably caused by, abnormalities in T lymphocyte function, which may be related to increased apoptosis of circulating T lymphocytes, increased T lymphocyte apoptosis, assessed by annexin V. Previous studies shown that apoptosis is increased in children with steroid resistant nephrotic syndrome particularly those with more severe pathology such as focal segmental glomerulosclerosis.

## OBJECTIVES

The study aims to evaluate urinary annexin V as a marker for resistance and prognosis in children with nephrotic syndrome.

## METHODS

A cross sectional and prospective study was conducted in Nephrology Clinic, Children Hospital, Ain Shams University during the period from November 2013 to April 2014.

**Patients:** Fifty children are included, 31 males and 19 females, ages ranged from 2 years to 13 years and classified into 3 groups:

**Group I:** This group included 15 children with steroid sensitive nephrotic syndrome in remission. It included 10 males and 5 females; their ages ranged from 2 to 13 years with a mean age 7.87±3.68 years.

**Group II:** This group included 15 children with steroid resistant nephrotic syndrome in remission. It included 9 males and 6 females; their ages ranged from 2 to 13 years with a mean age 8.53±3.02 years.

**Group III:** This group included 20 newly diagnosed cases of nephrotic syndrome are followed up from initial presentation for 6-8 weeks and are classified into SRNS and SSNS then are followed up until remission. This group and 11 cases categorized as SSNS included and 9 cases categorized as SRNS, 12 males and 8 females, their ages from 2 to 13years with a mean age is 7.45±3.39 years.

**Control group** 10 healthy children, with age and sex match to patient group 6 males and 4 females, their ages from 2 to 13 years with a mean age 8.4±2.91 years.

**Inclusion criteria:**

Patients with primary nephrotic syndrome.

**Exclusion criteria:**

Patients with renal impairment.

Patients with congenital nephrotic syndrome.

**Methods**

All subjects included in the study underwent the following:

Detailed history and clinical examination.

Laboratory work up including:

1) serum creatinine, serum blood urea nitrogen, total serum proteins and serum albumin.

2) Complete urine analysis and quantitative assessment of proteinuria by protein/creatinine ratio.

3) Assay of urinary annexin V level in urinary samples using sandwich-enzyme-linked immunosorbent assay (ELISA).

The above tests are done once in group I, II, IV (control group) and twice in group III (initial presentation and in remission).

## RESULTS

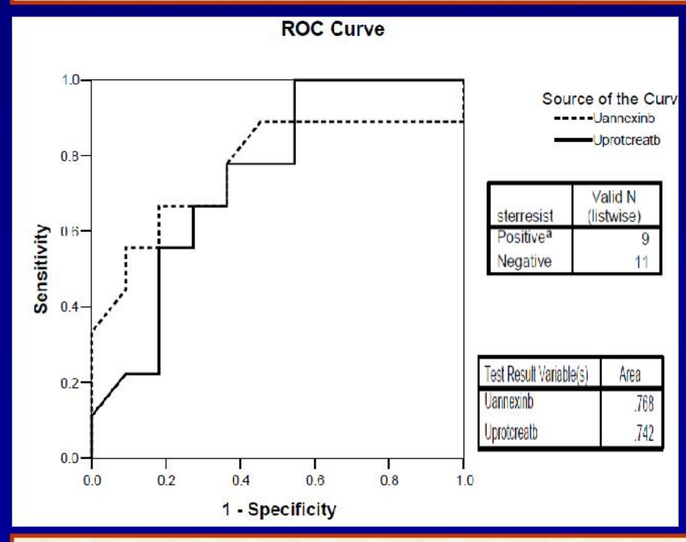


**Figure 1. ROC curve:** cut off value of urinary annexin V level of 3.15 (sensitivity of 0.778 and specificity of 0.364) and cut off value of urinary protein/creatinine ratio of 8.5 (sensitivity of 0.778 and specificity of 0.364)

**Table (1):** Comparison between sensitive (group 1) and resistant (group 2) groups regarding the studied parameters

| | Sensitive (n=15) Group1 | | Resistant (n=15) Group2 | | P-value | | |
|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Group1 versus Group2 | Group1 versus control | Group2 versus control |
| Urinary annexin V (ng/mg creatinine) | 3.50 | 1.82 | 6.79 | 1.96 | 0.000 | 0.000 | 0.000 |
| Urinary protein/ creatinine ratio (mg/mg) | 0.85 | 0.96 | 1.26 | 0.39 | 0.006 | 0.020 | 0.007 |
| Total proteins(g/dl) | 5.66 | 0.57 | 5.35 | 0.54 | 0.134 | 0.165 | 0.004 |
| Serum albumin(g/dl) | 3.35 | 0.74 | 3.33 | 0.69 | 0.939 | 0.013 | 0.007 |
| Serum creatinine (mg/dl) | 0.33 | 0.15 | 0.29 | 0.11 | 0.411 | 0.528 | 0.872 |
| Serum urea (mg/dl) | 20.00 | 6.64 | 20.00 | 4.94 | 1.000 | 0.676 | 0.602 |
| Serum BUN(mg/dl) | 8.35 | 2.46 | 10.27 | 2.84 | 0.058 | 0.534 | 0.175 |

**Table (2):** Comparison between sensitive at follow up (group 3a) and resistant at follow up (group 3b) regarding the studied parameters

| | Sensitive at follow up (n=11) Group3a | | Resistant at follow up (n=9) Group3b | | P-value | | |
|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Group3a versus Group3b | Group3a versus control | Group3b versus control |
| Urinary annexin V(ng/mg creatinine) | 2.37 | 0.51 | 2.98 | 0.65 | 0.032 | 0.000 | 0.000 |
| Urinary protein/ creatinine ratio (mg/mg) | 0.11 | 0.09 | 1.55 | 1.14 | 0.001 | 0.457 | 0.001 |
| Total proteins(g/dl) | 5.68 | 0.39 | 4.99 | 0.33 | 0.001 | 0.102 | 0.000 |
| Serum albumin(g/dl) | 4.13 | 0.47 | 3.19 | 0.41 | 0.000 | 0.492 | 0.000 |
| Serum creatinine (mg/dl) | 0.27 | 0.08 | 0.30 | 0.07 | 0.430 | 0.445 | 1.000 |
| Serum urea (mg/dl) | 18.27 | 3.55 | 20.89 | 3.98 | 0.138 | 0.119 | 0.953 |
| Serum BUN(mg/dl) | 9.18 | 1.60 | 11.56 | 2.74 | 0.026 | 0.678 | 0.016 |

**Table (3):** Correlation of urinary annexin V with the studied parameters in all studied patients

| | Urinary annexin V (ng/mg creatinine) | |
|---|---|---|
| | r | p-value |
| Urinary protein/ creatinine ratio (mg/mg) | 0.363 | 0.001 |
| Total proteins(g/dl) | -0.261 | 0.019 |
| Serum albumin(g/dl) | -0.304 | 0.006 |
| Serum creatinine (mg/dl) | 0.039 | 0.729 |
| Serum urea (mg/dl) | -0.003 | 0.981 |
| Serum BUN(mg/dl) | 0.311 | 0.005 |

## FUNDING

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## CONCLUSIONS

**Urinary annexin V is highly elevated in cases with steroid resistant nephrotic syndrome and in new cases at initial presentation. It may be a prognostic biomarker for childhood nephrotic syndrome. It is positively correlated with urinary protein/ creatinine ratio. Urinary annexin V is a non invasive marker of steroid resistance but not of high sensitivity.**



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