Nuclear Factor Kappa B (NF-κB) in Patients with Nephrotic Syndrome and Acute Renal Failure

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Abstract

Background: In renal tissue injury, activation of the transcription factor NF-κB has a central role in the induction of proinflammatory genes expression, which are involved in the development of progressive renal inflammatory disease.

Aim of Work: The aim of our study was to evaluate the in situ expression of activated transcription factors NF-κB in renal biopsy sections of patients with nephrotic syndrome and acute renal failure and to study its role as a prognostic marker in proteinuric renal disease and acute renal failure by correlating its level with follow-up investigations of glomerulonephritis after 3 months of treatment. The study included 30 patients with acute renal failure and nephrotic syndrome recruited from nephrology clinic in Kasr El-Aini Hospital and King Fahd unit during year 2007 till 2010. The patients were divided in 3 groups: Group I included 10 patients with idiopathic nephrotic syndrome due to various glomerulonephropathies, group II included 10 patients presenting with nephrotic syndrome due to focal segmental glomerulosclerosis (FSGS), group III included 10 patients presenting with acute renal failure (ARF).

Results: By comparing NF-κB in different groups it was highest in group 3 including patients with acute renal failure. Results showed no significant difference in relation to the two pathological entities that were found, acute interstitial nephritis (mean: 1102.7 density/mm², ±SD 548.4) and acute tubular necrosis (ATN) (mean 1222.5, ±SD 432.0). On evaluation of NF-κB levels in patients with nephrotic syndrome resulted in, that patients with membranoproliferative (MPGN) had the highest levels, mean 795 density/mm² SD±643.5, followed by the mesangioproliferative (MesPGN) patients, mean 598.7, SD±166.5. The lowest levels were found in patients with membranous nephropathy, mean 387.5 and SD±101.1. Taking the patients as one group before treatment, NF-κB showed significant positive correlation with, mean BP, the serum creatinine level (r=0.531, p=<0.01) and inverse correlation with proteinuria (r=-0.415, p=<0.05). Logistic Regression analysis was done to evaluate the NF-κB as a predictor of outcome showing that NF-κB isn’t predictor for outcome in any group of the three.

Conclusion: The study shows that NF-κB levels are higher in patients with severe degrees of tubulointerstitial injury. Hence, it can be utilized as a marker of disease severity within this regard. Our results have also showed that, higher levels of NF-κB were detected MPGN more than other disease forms with the membranous nephropathy having the lowest levels. Our results didn’t prove NF-κB as a prognostic tool in glomerulonephritis and acute injury probably due to small numbers of patients.

Key Words: Nuclear factor kappa B (NF-κB) – Nephrotic syndrome – Acute renal failure.

Introduction

THE NF-κB family of transcription factors regulates the induction and resolution of inflammation. NF-κB is found in almost all animal cell types and is involved in cellular responses to stimuli such as stress, cytokines, free radicals, ultraviolet irradiation, oxidized LDL, and bacterial or viral antigens [1].

NF-κB plays important roles in the immune system; incorrect regulation of NF-κB has been linked to cancer, inflammatory and autoimmune diseases, septic shock, viral infection, and improper immune development [2].

In renal tissue injury, activation of the transcription factor NF-κB has a central role in the induction of proinflammatory genes expression, which are involved in the development of progressive renal inflammatory disease [3].

Two main pathways, classical and alternative, control the nuclear translocation of NF-κB. Classical NF-κB activation is usually a rapid and transient response to a wide range of stimuli whose main effector is RelA/p50. The alternative NF-κB pathway is a more delayed response to a smaller range of stimuli resulting in DNA binding of RelB/p52 complexes. Additional complexity in
this system involves the posttranslational modification of NF-κB proteins and an ever-increasing range of co-activators, co-repressors, and NF-κB complex proteins. Collectively, NF-κB regulates the expression of numerous genes that play a key role in the inflammatory response during human and experimental kidney injury. Multiple stimuli activate NF-κB through the classical pathway in somatic renal cells, and noncanonical pathway activation by Tumour necrosis factor like weak inducer of apoptosis (TWEAK) occurs in acute kidney injury. Under most test conditions, specific NF-κB inhibitors tend to reduce inflammation in experimental kidney injury but not always [4].

Activation of the transcription NF-κB has been shown to be involved in the development of human glomerulonephritis (GN), and the degree of glomerular expression of NF-κB correlated with the progression of glomerular injury [5].

We therefore addressed the idea that NF-κB could be an indicator of renal damage progression in human proteinuric nephropathies and acute renal failure.

The aim of our study was to evaluate the in situ expression of activated transcription factors NF-κB in renal biopsy sections of patients with nephrotic syndrome and acute renal failure and to study its role as prognostic marker in proteinuric renal disease and acute renal failure by correlating its level with follow-up investigations of glomerulonephritis after 3 months of treatment.

**Material and Methods**

This study included 30 patients with nephrotic syndrome and with acute renal failure recruited from nephrology clinic and king Fahd unit inpatient department. Their ages ranged between 14 years and 65 years. These patients were divided into three groups: Group I included 10 patients with idiopathic nephrotic syndrome due to various glomerulonephropathies, group II included 10 patients presenting with nephrotic syndrome due to FSGS, group III included 10 patients presenting with ARF. All patients were subjected to the following: Full history and clinical examination including measuring blood pressure and exclusion of possible causes of secondary nephrotic syndrome. Laboratory investigations including kidney function tests: Blood urea (normal range 10-50mg/dl), serum creatinine (normal range 0.5-1.5mg/dl), 24 hours urinary proteins (nephrotic value >3g/dl), CBC, fasting and 2 hours post prandial blood sugar, cholesterol, HDL, LDL, triglycerides, liver function test: AST, ALT, serum albumin, prothrombin time and concentration, hepatitis markers, auto immune profile: C3, C4, ANA and anti-ds DNA.

NF-κB was estimated in renal biopsies for all patients. RNA extraction from renal biopsies was performed using SVR total RNA isolation kit (Promega, Madison, WI, USA). Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR): A Primer used for detection of NF-κB was prepared using oligo-1000 DNA synthesizer (Beckman, California, USA), with the following sequences:

**Sense:** TACCATGCTGTTTTGATTAC 3’

**Reverse:** TCAAGCTACCAATGACTTTC 5’

Agarose Gel Electrophoresis: 10 μl of the PCR product were used, the agarose used was molecular biology grade (sigma) at a concentration of 1.5 X Tris-acetate-EDTA (TAE) buffer and stained with ethidium bromide. The resulting bands were visualized using UV-rays and compared to a standard PCR marker (Promega, Madison, WI, USA). Positive cases for NF-κB gave band 190bp (base pairs). Then the product was quantitated by using gel documentation system.

All patients were under tight control of BP targeting BP of 125/75. All patients with nephrotic syndrome received Renin angiotensin system (RAS) blockade and steroids from 1-2mg/kg/d, No other immunosuppressions were used at this phase (first three months).

Patients with acute renal failure (ARF) were treated accordingly, patients with acute interstitial nephritis received steroids and patients with ATN received fluids and conservative treatment. Laboratory and clinical follow-up was done after three months.

**Statistical analysis:**

Statistical analysis was done using IBM PC applying appropriate statistical methods i.e. Mean ±SD, r-value, ANOVA.

SPSS (statistical package social science) version 10.0 was used.

**Results**

This study comprised 30 patients, 43% of the males (13 patients) and 56.7% females (17 patients). The whole study group ages ranged between 14 years and 65 years with a mean of 34.67 ± 15.1 years. Their mean blood pressure (MBP) before treatment had a minimum of 80mmHg and a maximum of 130mmHg with a mean of 100.33 ± 13.88 mmHg. 20% were hypertensives (6 patients) and
80% were not. Their serum creatinine ranged from 0.4mg/dl to 17.89mg/dl with a mean of 4.65 ± 5.02mg/dl. The proteinuria ranged between 0.01g/24 hours and 10.2g/24 hours with a mean of 3.73±2.83g/24 hours.

The NF-κB for all groups ranged between 316 and 2102, with a mean of 760.9 ± 396.3 (density/mm²). Patients were divided into three groups: Group I (10 patients): Patients with nephrotic syndrome of various etiologies apart from FSGS. Group II (10 patients): Patients with nephrotic syndrome due to FSGS. Group III (10 patients): Patients with acute kidney injury (ARF). Each group was studied before starting treatment and after three months of follow-up. NF-κB was measured in transcutaneous renal biopsies for all patients before starting treatment. Clinical and laboratory parameters of the studied groups before treatment are shown in Table (1) and clinical and laboratory characteristics after treatment are shown in Table (2).

By comparing NF-κB in different groups it was highest in group 3 including patients with acute renal failure (1085.7±463.4 density/mm²) (fig. 1). Results showed no significant difference in relation to the two pathological entities that were found, acute interstitial nephritis (mean: 1102.7 density/mm², ±SD 548.4) and acute tubular necrosis (mean 1222.5, ±SD 432.0).

On evaluation of NF-κB levels in patients with nephrotic syndrome resulted in, that patients with MPGN had the highest levels, mean 795 density/mm² ±643.5, followed by the MesPGN patients, mean 598.7, SD±166.5. The lowest levels were found in patients with membranous nephopathy, mean 387.5 and SD±101.1. In patients with FSGN the level was 628.4±183.1 density/mm².

Taking the patients as one group before treatment, NF-κB showed significant positive correlation with, mean BP, the serum creatinine level (r=0.531, p=<0.01) (fig. 2) and inverse correlation with proteinuria (r=0.415, p=<0.05).

While when correlating NF-κB assessed in renal biopsies before treatment to post treatment laboratory follow up investigations a significant positive correlation with serum creatinine (r=0.568, p=<0.05) and an inverse correlation with proteinuria (r=-0.503, p=<0.01) was found.

Within group 1, only pretreatment MBP was significantly correlated with NF-κB (r=0.729, p=<0.05).

Within group 2, proteinuria showed a significant positive correlation with NF-κB before treatment (r=0.670, p=<0.05).

Within group 3, after three months of treatment, there was only a significant positive correlation with SCr (r=0.785, p=<0.01).

Logistic Regression analysis was done to evaluate the NF-κB as a predictor of outcome showing that NF-κB isn’t predictor for outcome in any group of the three (Table 3).

<table>
<thead>
<tr>
<th>Pathology results (n)</th>
<th>Group 1 (N=10)</th>
<th>Group 2 (N=10)</th>
<th>Group 3 (N=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Focal Segmental (FSGS)</td>
<td>10 (100%)</td>
<td></td>
<td>Acute tubulointerstitial (7)</td>
</tr>
<tr>
<td>Mesangio proliferative (MesPGN)</td>
<td>4 (40%)</td>
<td></td>
<td>Acute tubular necrosis (3)</td>
</tr>
<tr>
<td>Membranoproliferative (MPGN)</td>
<td>3 (30%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Membranous GN (MGN)</td>
<td>3 (30%)</td>
<td></td>
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</tbody>
</table>
This study shows that NF-κB in different groups was highest in group 3 including patients with acute renal failure. The results showed no significant difference in relation to the two pathological entities that were found, acute interstitial nephritis and acute tubular necrosis.

Evaluation of NF-κB levels in patients with nephrotic syndrome resulted in, that patients with MPGN had the highest levels, followed by the MesPGN patients. The lowest levels were found in patients with membranous nephropathy. Taking the patients as one group before treatment, NF-κB showed significant positive correlation with, mean BP, the serum creatinine level and inverse correlation with proteinuria.

While when correlating NF-κB assessed in renal biopsies before treatment to post treatment laboratory follow-up investigations a significant positive correlation with serum creatinine and an inverse correlation with proteinuria was found. However logistic Regression analysis showed that NF-κB isn’t predictor for outcome in any group of the three.

Previous studies showed elevated levels of NF-κB in minimal change disease and membranous nephropathy [6]. The results are also in accordance with Zhao et al., who studied the NF-κB activity in kidneys of children with primary nephrotic syndrome [7].

It has been suggested that elevated activity of NF-κB was related to types and severity of primary nephrotic syndrome, which was also cited by other workers [8].

Studies showed that higher activities of NF-κB in non minimal change nephrotic syndrome and nephritic syndrome may be the cause of their poor response to steroid therapy, because activated NF-κB exerts an inhibitory effect on glucocorticoid receptors [9].

However, during the last few years there have been suggestions from the literature, regarding NF-κB in various tissues to be regarded as a sign of injury [6].

Also, a close correlation has been shown between tubulointerstitial injury and the outcome of renal dysfunction. NF-κB has been shown to play a key role in proteinuria-induced tubulointerstitial injury [10]. Studies show that resistance to glucocorticoid treatment is mediated by the activation
of transcription factors as NF-κB and the production of lymphokines that initiate and sustain an inflammatory response mediated in great part by T cells [11].

Data from the literature show that the enhancement of renal TGF-beta production and the persistent increase of NF-κB in renal cortex tubulointerstitium post-ATN may explain the impaired recovery of renal function observed in patients post-ATN [12].

In human renal disease, there is histologic evidence of NF-κB activation in diabetic nephropathy, glomerular disease, and AKI. In general, NF-κB activates in macrophages and glomerular (including podocytes in proteinuric diseases) and tubular parenchymal cells and correlates with parameters of severity of disease such as proteinuria or inflammation [4].

These data have been interpreted as supportive for the role of NF-κB in promoting inflammation; however, inflammation itself will promote NF-κB activation, and because these data are descriptive, the precise role of the various NF-κB complexes in human kidney injury remains uncertain. Functional evidence of NF-κB activation has been obtained by transcriptomics-based pathway mapping [4].

**Conclusion:**

The study shows that NF-κB levels are higher in patients with severe degrees of tubulointerstitial injury. Hence, it can be utilized as a marker of disease severity within this regard. Our results have also showed that, higher levels of NF-κB were detected MPGN) more than other disease forms with the membranous nephropathy having the lowest levels. Our results didn’t prove NF-κB as a prognostic tool in glomerulonephritis and acute injury probably due to small numbers of patients.

Further studies are recommended to evaluate the NF-κB in larger groups and correlating it to the therapeutic response in different types of idiopathic nephrotic syndrome.

**References**


