Serum Tumor Necrosis Factor-Alpha Levels in Children with Nephrotic Syndrome: A Pilot Study

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ABSTRACT: **Background:** Several studies link the pathogenesis of nephrotic syndrome to tumor necrosis factor-alpha (TNFα). However, data on the serum TNFα level in children with nephrotic syndrome are sparse.

Objective: To investigate serum TNFα levels and the effect of steroid therapy in children with nephrotic syndrome.

Methods: A prospective cohort pilot study of children with nephrotic syndrome and controls was conducted during a 1 year period. Serum TNFα levels were measured at presentation and at remission, or after a minimum of 80 days if remission was not achieved.

Results: Thirteen patients aged 2–16 years with nephrotic syndrome were compared with 12 control subjects. Seven patients had steroid-sensitive and six had steroid-resistant nephrotic syndrome. Mean baseline serum TNFα level was significantly higher in the steroid-resistant nephrotic syndrome patients than the controls (6.13 pg/ml vs. 4.36 pg/ml, $P = 0.0483$). Mean post-treatment TNFα level was significantly higher in the steroid-resistant than in the steroid-sensitive nephrotic syndrome patients (5.67 vs. 2.14 pg/ml, $P = 0.001$). In the steroid-resistant nephrotic syndrome patients, mean serum TNFα levels were similar before and after treatment.

Conclusions: Elevated serum TNFα levels are associated with a lack of response to corticosteroids. Further studies are needed to investigate the role of TNFα in the pathogenesis of nephrotic syndrome.

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KEY WORDS: nephrotic syndrome (NS), steroid resistance, tumor necrosis factor-alpha (TNFα)

Although the pathogenesis of idiopathic nephrotic syndrome (NS) remains unknown, there is evidence that immune system dysregulation plays an important role in the disease. For more than 50 years glucocorticoids have been the mainstay of therapy for children with NS, but their mechanism of action in this disease is not understood. Failure of patients with NS to respond to immunosuppressive therapy is associated with increased risk of end-stage renal disease. More than 50% of children with steroid-resistant NS (SRNS) will acquire end-stage renal disease within 4 years of diagnosis [1].

Tumor necrosis factor-alpha (TNFα) is a cytokine produced by macrophages and T cells and has multiple immune functions. The soluble form of TNFα is cleaved from transmembrane TNFα. It binds to TNF receptor (TNFR) and transmits intracellular signals via TNFR-associated factors [2]. Several studies have linked NS with TNFα activity in humans and animal models of NS [3-7]. High levels of serum TNFα were reported in patients with NS [3]. High levels of TNFα mRNA and elevated TNFα protein production from monocytes of 25 children with active idiopathic NS, relative to children in remission, and healthy children were observed [4]. Additionally, in a biopsy study of membranous nephropathy, TNFα expression was enhanced in glomerular epithelial cells [5].

Researchers recently reported that the administration of anti-TNFα for various indications in patients with coexisting NS led to an unexpected, and complete, remission of the NS. The specific cases involved the use of etancercept in patients with TNFR-associated periodic syndrome [8], and the use of infliximab in a patient with rheumatoid arthritis and amyloidosis [9] and in a patient with inflammatory bowel disease and spondylitis [10,11]. There are also reports of treatment with anti-TNFα agents in patients with NS. Infliximab therapy led to resolution of the disease in a patient with idiopathic NS [12]. Anti-TNFα treatment significantly reduced proteinuria in a child with recurrent post-transplant focal segmental glomerulosclerosis (FSGS) [13].

The possibility that anti-TNFα treatment may be an alternative therapy to corticosteroid treatment is intriguing. A phase 1 trial of adalimumab in FSGS was published in 2010 [14]. However, in a 3 month clinical trial of etancercept for the treatment of membranous nephropathy, only 2 of 12 patients showed complete remission of more than 4 years duration [15].

In view of these data, we undertook a pilot study to investigate the serum TNFα levels in children with NS and the effect of steroid therapy on serum TNFα.

PATIENTS AND METHODS

A prospective cohort study was conducted at Schneider Children's Medical Center of Israel, a tertiary university-affiliated hospital, from March 2011 to March 2012. The study group included children (age 1–18 years) at the first presentation of primary NS. Patients diagnosed with secondary nephrotic syndrome were excluded. Patients with an abnormal estimated glomerular filtration rate (GFR) were excluded as well. NS patients were initially treated with prednisone 60 mg/m^2/day for 6 weeks and then switched to 40 mg/m^2/qid with gradual tapering down. Steroid resistance was defined as lack of remission after 6 weeks of therapy. The control group included children undergoing endocrinologic evaluation for idiopathic short stature or elective cardiac catheterization for congenital heart defects without signs of inflammation or intercurrent illness.

The study was approved by the institutional ethics board, and informed consent was obtained from the parents prior to enrollment.

MEASUREMENTS

At enrollment of patients and controls, 2.5 ml blood was collected from a forearm vein and placed into a non-heparinized glass tube and centrifuged (800x*g* for 10 min). Serum was immediately separated and stored at -70°C until analysis. Serum concentration of TNFα was measured with a quantitative high sensitivity sandwich enzyme-linked immunosorbent assay (ELISA) (Human TNFa HS, Quantikine kit, R&D Systems Inc., Minneapolis, MN, USA) according to the manufacturer's instructions. Each sample was tested in duplicate and the accepted result was the mean value; if results were discrepant the test was repeated. The detection limit for TNFα was 0.5 pg/ml. Cytokine concentration (pg/ml) was calculated from a standard curve of the corresponding recombinant human cytokine.

A repeated blood test was performed as part of the routine follow-up of NS at remission of the disease (steroid-sensitive NS, SSNS) or after a minimum of 80 days (range 80–258) if no remission was achieved (SRNS).

DATA ANALYSIS

Statistical analysis was performed with IBM SPSS statistics software, version 20.0. Paired *t*-test was used to evaluate differences in serum TNFα level within the patient group before and after therapy, and unpaired *t*-test was used to evaluate differences in continuous variables between patients and controls. A two-tailed P value < 0.05 was considered statistically significant. Fisher's exact test was used to compare categorical data.

RESULTS

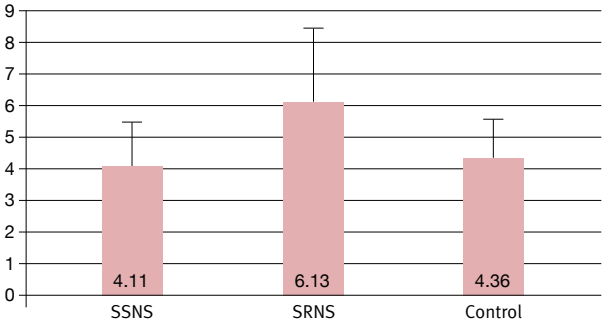
Fourteen patients with primary NS were enrolled in the study; one of them was lost to follow-up and was excluded from the analysis. The final study group comprised four girls and nine boys with a mean age of 6.1 years (range 2–16 years). The control group consisted of 12 children, 2 girls and 10 boys, with a mean age of 10.1 years; 9 were undergoing endocrinologic evaluation, and 3 cardiac catheterization. The mean age was significantly lower in the study group than the control group (P = 0.02). There was no significant between-group difference in female-to-male ratio (P = 0.38).

Seven children in the study group had SSNS and six had SRNS (five FSGS and one diffuse mesangial proliferation). There was no significant difference in mean age between the SSNS and SRNS patients (6.2 and 6.0 years, respectively, P = 0.92). Mean baseline (pretreatment) serum level of TNFα was significantly higher in the SRNS patients than the control group (P = 0.0483). There was no significant difference between the SSNS patients and controls (P = 0.68) [Figure 1]. Mean baseline serum TNFα level was higher in the SRNS than the SSNS patients, but the difference did not reach statistical significance (6.13 ± 2.59 pg/ml vs. 4.11 ± 1.48 pg/ml, P = 0.078).

After treatment, mean TNFα level was significantly higher in the SRNS patients than the SSNS patients (P = 0.001) [Figure 2]. Post-treatment (at remission) mean TNFα level was significantly lower in the SSNS patients than in the control group (2.14 ± 1.13 vs. 4.36 ± 1.23 pg/ml, P = 0.0011). Post-treatment TNFα in the SRNS patients was higher than the control group, but the difference was not statistically significant (5.67 ± 1.79 vs. 4.36 ± 1.23 pg/ml, P = 0.085).

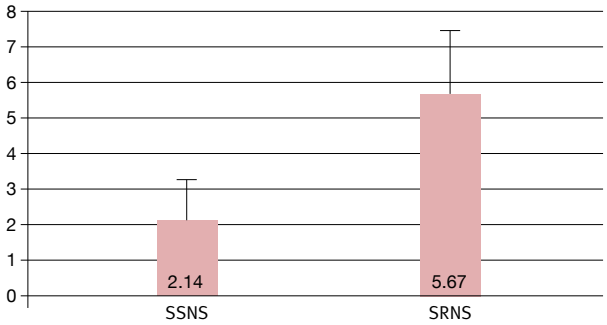
Mean post-treatment serum TNFα level in the patients with SSNS was significantly lower than mean pretreatment TNFα level (P = 0.019). There was no significant difference in mean serum TNFα level in the SRNS patients before and after treatment (6.13 ± 2.59 vs. 5.67± 1.79 ng/ml, respectively, P = 0.9).

Figure 1. Mean (±SD) pre-treatment serum TNFα levels (pg/ml) in SRNS and SSNS controls. Mean TNFα level in the SRNS patients was significantly higher than the controls (P = 0.048). No significant difference was seen in TNFα levels between the SSNS patients and the controls (P = 0.68)



TNFα = tumor necrosis factor-alpha, SRNS = steroid-resistant nephrotic syndrome, SSNS = steroid-sensitive nephrotic syndrome

Figure 2. Mean (±SD) post-treatment serum TNFα levels (pg/ml) in patients with SSNS and SRNS. Mean level was significantly higher in the SRNS patients than in the SSNS patients (P = 0.001)



TNFα = tumor necrosis factor-alpha, SRNS = steroid-resistant nephrotic syndrome, SSNS = steroid-sensitive nephrotic syndrome

DISCUSSION

In the children with steroid-sensitive nephritic syndrome, mean TNFα level decreased at remission after corticosteroid therapy. This finding suggests that disease activity, at least in some NS children, may be correlated with TNFα serum levels. This assumption is further supported by the lack of a reduction in mean serum TNFα levels in the patients with SRNS.

TNFα interacts with two different receptors, designated TNFR1 and TNFR2, which are differentially expressed on cells and tissues and initiate both distinct and overlapping signal transduction pathways [16]. These diverse signaling cascades lead to a range of cellular responses, including migration, survival, differentiation, proliferation and cell death. Vascular endothelial cells respond to TNFα by a number of pro-inflammatory changes which increase leukocyte adhesion, transendothelial migration, and vascular permeability, and may promote thrombosis. The central role of TNFα in inflammation has been demonstrated by the successful use of TNFα blocking agents in the treatment of various inflammatory conditions, such as rheumatoid arthritis, ankylosing spondylitis, inflammatory bowel disease, and psoriasis [15]. It has long been recognized that glucocorticoids suppress TNFα production by human monocytes [17]. One study showed that dexamethasone reduced both bioactive TNFα levels in peripheral blood mononuclear cells and its soluble inhibitors [18].

The mechanism whereby TNFα may interfere with glomerular basement membrane permeability to albumin is unknown. In one study, levels of various cytokines, including IL-1β, IL-2, interferon (IFN)-α, IFNγ, and TNFα were measured in patients with primary NS and findings of minimal change nephropathy (MCN), FSGS, or membranous nephropathy on biopsy [3]. Of all the cytokines, only TNFα showed a significant increase in plasma and urine in patients with FSGS compared to healthy controls and patients with MCN [3]. Using an animal model, researchers reported a significant increase in albuminuria in rats following infusion of TNFα [7]. In addition, incubation of isolated rat glomeruli with TNFα increased glomerular basement permeability to albumin compared to controls [6]. This finding was not observed when TNFα was combined with anti-TNFα antibodies or oxidizing radical neutralizers. Thus, it was speculated that TNFα is linked to NS by its effect on glomerular basement membrane permeability, via a complex mechanism that involves superoxide production [6]. In the present study, steroid treatment in the SSNS group induced a decrease in the serum TNFα, whereas in the SRNS group the steroids failed to reduce serum TNFα. This may indicate that the high TNFα serum level is associated with increased glomerular capillary wall permeability.

Our study was limited by the small number of participants, although the results were statistically significant. A second limitation is the higher mean age of the control group compared with the study group. However, it is unlikely that this age difference significantly influenced TNFα blood values. The comparison of patients with SRNS and SSNS and of pretreatment and post-treatment levels of TNFα in the same patients circumvented a possible effect of mean age in the controls.

In summary, serum TNFα levels decrease after corticosteroid treatment in children with SSNS but not in SRNS. Further studies of the effects of serum TNFα and TNFR in the kidney in patients with NS, and of the impact of TNFα on glomerular permeability are needed.

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