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.

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 etanercept 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 etanercept 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/m²/day for 6 weeks and then switched to 40 mg/m²/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 (800xg 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 TNFα 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).
endothelial cells respond to TNFα by a number of pro-inflammatory, anti-survival, differentiation, proliferation and cell death. Vascular endothelial cells lead to a range of cellular responses, including migration, proliferation, and vascular permeability, and may promote thrombosis. The central role of TNFα in inflammation has been demonstrated in 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 mean serum TNFα levels in the patients with SRNS. This assumption is further supported by the lack of a reduction in mean serum TNFα levels in the patients with SRNS.
Capsule

Gamma frequency entrainment attenuates amyloid load and modifies microglia

Changes in gamma oscillations (20–50Hz) have been observed in several neurological disorders. However, the relationship between gamma oscillations and cellular pathologies is unclear. Iaccarino et al. showed reduced, behaviorally driven gamma oscillations before the onset of plaque formation or cognitive decline in a mouse model of Alzheimer’s disease. Optogenetically driving fast-spiking parvalbumin-positive (FS-PV)-interneurons at gamma (40Hz), but not other frequencies, reduces levels of amyloid-β (Aβ1-40 and Aβ1-42 isoforms). Gene expression profiling revealed induction of genes associated with morphological transformation of microglia, and histological analysis confirmed increased microglia co-localization with Aβ. Subsequently, the authors designed a non-invasive 40Hz light-flickering regime that reduced Aβ1-40 and Aβ1-42 levels in the visual cortex of pre-depositing mice and mitigated plaque load in aged, depositing mice. These findings uncover a previously unappreciated function of gamma rhythms in recruiting both neuronal and glial responses to attenuate Alzheimer’s disease-associated pathology.

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Capsule

Natural genetic variation profoundly regulates gene expression in immune cells and dictates susceptibility to CNS autoimmunity

Regulation of gene expression in immune cells is known to be under genetic control, and likely contributes to susceptibility to autoimmune diseases such as multiple sclerosis (MS). How this occurs in concert across multiple immune cell types is poorly understood. Using a mouse model that harnesses the genetic diversity of wild-derived mice, more accurately reflecting genetically diverse human populations, Beareff et al. provide an extensive characterization of the genetic regulation of gene expression in five different naive immune cell types relevant to MS. The immune cell transcriptome is shown to be under profound genetic control, exhibiting diverse patterns: global, cell-specific and sex-specific. Bioinformatic analysis of the genetically controlled transcript networks reveals reduced cell type specificity and inflammatory activity in wild-derived PWD/PhJ mice, compared with the conventional laboratory strain C57BL/6J. Additionally, candidate MS-GWAS (genome-wide association study candidate genes for MS susceptibility) genes were significantly enriched among transcripts over-represented in C57BL/6J cells compared with PWD. These expression level differences correlate with robust differences in susceptibility to experimental autoimmune encephalomyelitis, the principal model of MS, and skewing of the encephalitogenic T cell responses. Taken together, these results provide functional insights into the genetic regulation of the immune transcriptome, and shed light on how this in turn contributes to susceptibility to autoimmune disease.

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“Do unto those downstream as you would have those upstream do unto you”
Wendell Berry (born 1934), American novelist, poet, environmental activist, cultural critic, and farmer