

Original Research Article

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Serum Tumor Necrosis Factor-Alpha (TNF- α) Levels in Children with Nephrotic Syndrome and Its Correlation with Biochemical Parameters

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ABSTRACT

Keywords

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The aim of this study is to compare the levels of TNF- α in children with nephrotic syndrome and healthy control children and the correlation of TNF- α with clinical feature of NS. Fifty children with NS were divided into four groups: 14 steroid sensitive infrequent relapse (SSIFR), 12 steroid sensitive frequent relapse (SSFR), 9 steroid resistance (SR) and 15 newly diagnosis (ND) compared to twenty-five unrelated healthy children. The serum IL-13 levels for NS patients and healthy control children were measured by R&D quantitative ELISA kit. Serum levels of TNF- α showed highly significant difference between patients with NS (15.68 ± 2.38 pg/ml) and healthy control (1.066 ± 0.65 pg/ml), and there is no significant different among NS groups. Highly significant correlation between TNF- α mean level and biochemical markers of NS patients also reported in this study.

Introduction

Nephrotic syndrome is an immune mediated renal disease. It is related with dysfunction of T-cell and secondary disorder of B-cell that leads to changes in scales of immunoglobulins (Roy *et al.*, 2009; Bahbah *et al.*, 2015). This systemic disturbance of function of T cell results in the production of humoral factors or lymphokines that responsible for the rise of permeability of glomerular basement membrane (Sahali *et al.*, 2002; Bagga *et al.*, 2005). Nephrotic syndrome is described by huge proteinuria, edema and hypoalbuminemia, the additional features are hyperlipidemia also generally present (Bahbah *et al.*, 2015). Hypercoagulable status, the

dysregulation of fluid, imbalance in electrolyte and capability to infections can caused by large urinary wastage of serum proteins (Kim *et al.*, 2016). Estimates on the annual incidence of nephrotic syndrome range from two to seven per 100,000 children, and prevalence from twelve to sixteen per 100,000 (Eddy and Symons, 2003). Cytokines are functional class of tiny proteins and glycoprotein (molecular weights of approx. 8–80 kDa) and fundamentally they are monomers that function as soluble mediators in an autocrine or paracrine manner. Cytokines are produced by a number of cell types, predominantly leukocytes, and their targets implicate both immune and non-immune cells (Sivangala and Sumanlatha,

2015). Based on their production of cytokine, T-helper lymphocytes can be divided into two types: Th1 and Th2 cells. Th1 cells typically produce TNF (tumour necrosis factor), interleukin-2 (IL-2) and IFN- γ (interferon- γ), which activate cytotoxic and inflammatory reactions. While Th2 cells produce IL-4, IL-5, IL-6, IL-10 and IL-13, which are associated with the strong antibody regulation and allergic responses (Jafar *et al.*, 2011; Mohammed *et al.*, 2015).

Studies on in vitro mitogen-stimulated cytokines production by PBMC from idiopathic NS children have demonstrated an increased production of various cytokines, including IL-1, IL-2, IL-4 and TNF- α , compared with patients in remission or with healthy subjects (Berg and Weening, 2004). There is elevated production of IL-12 and IL-18 by PBMC from MCNS children and reduced production of IL-1 and IL-10 compared with normal controls. To circumvent artefacts induced by stimulation with mitogens, the unstimulated cytokines production by MCNS patients T-lymphocytes and found an elevated production of IL-13, whereas production of IL-4 was normal (Berg and Weening, 2004). Tumor necrosis factor- α (TNF α) is a cytokine secreted from T-cells and macrophages and has multiple immunological functions (Yang, 2015). It is inflammatory mediator that is focal to the inflammatory action of the innate immunity induction: production of cytokine, activation of adhesion molecules, and growth energizing (Turner, 2014). The TNF- α soluble form is cleaved from transmembrane TNF- α . It binds to TNF receptor (TNFR) and transfers intracellular signals through TNFR-associated factors (Yang, 2015). TNFR divided into two types (TNFR1 and TNFR2). TNFR1 is found on all types of cells, while TNFR2 is expressed only on immune system cells (Fischer and Maier, 2015). TNFR1 ligation catalyzes biological function such as induction

of (NF- κ B) system, the triggering of mitogen-activated protein kinase (MAPK)-transduction signaling cascades (Galluzzi *et al.*, 2012; Vandenabeele *et al.*, 2010). TNF- α affect the expression of nephrin and podocyte cytoskeletal rearrangement (Rong *et al.*, 2017).

TNF- α exerts inflammation, apoptosis and proliferation (Shaikh, 2011). TNF- α can stimulate fever, either directly through stimulation of PGE2 synthesis by the vascular endothelium of the hypothalamus, or indirectly by prompting release of IL-1. TNF- α can catalyze the production of PGE2 and collagenase by synovial cells and thus are believed to contribute to harm of joint in inflammatory conditions such as rheumatoid arthritis, TNF- α also shares a substantial inflammatory property with IL-6 and IL-11 (Shaikh, 2011). TNF α mRNA High concentration and elevated TNF- α protein production from monocytes of children with active idiopathic NS, relative to patients in remission, and healthy individual were observed (Bustos *et al.*, 1994).

Patients and Methods

Fifty blood samples of patients suffering from nephrotic syndrome during the period from September 2017 to January 2018 from the Al-Emamain Al-Kadhemain Teaching hospital and Welfare Teaching hospital in medical city of Baghdad and twenty-five blood samples have been collected from unrelated healthy children. The age of NS patients was from 2 to 14 years with mean 7.78 ± 3.52 years and from 5 to 14 years with mean 8.64 ± 3.08 years for healthy control. Out of 50 cases, 35 cases (70%) were males and 15 cases (30%) were females and the male to female ratio was 2.3:1. Pathological types based on response to steroid nephrotic syndrome among patients revealed that 14 cases (28%) steroid sensitive infrequent relapse (SSIFR), 12 cases (24%)

steroid sensitive frequent relapse (SSFR), 9 cases (18%) steroid resistance (SR) and 15 cases (30%) newly diagnosis (ND). The biochemical test was done to determine the levels of blood urea, serum creatinine, serum albumin and serum cholesterol in patients and healthy control. Serum TNF- α levels were detected for NS patients and healthy control by using R&D ELISA kit.

The Statistical Analysis System- SAS (2012) program was used to effect of difference factors in study parameters. Chi-square test was used to significant compare between percentage and least significant difference – LSD test (ANOVA) or T-Test was used to significant compare between means. Estimate of correlation coefficient between variables in this study.

Results and Discussion

Fifty children with NS compared to twenty-five healthy children as a control group were recorded in this study. Pathological types based on response to steroid nephrotic syndrome among patients revealed that 14 cases (28%) steroid sensitive infrequent relapse (SSIFR), 12 cases (24%) steroid sensitive frequent relapse (SSFR), 9 cases (18%) steroid resistance (SR) and 15 cases (30%) newly diagnosis.

The mean level of serum albumin, serum creatinine and blood urea was significantly decreased in patients in compared with healthy control, while the mean level of urine albumin and serum cholesterol was significantly increased in patients in compared with healthy control. The results of mean TNF- α level in healthy serum ($1.066 \pm 0.65\text{pg/ml}$) was significantly lower than children with nephrotic syndrome ($15.68 \pm 2.38\text{pg/ml}$) (Table 1).

Comparison between the TNF- α concentration among types of NS and healthy control

demonstrated that insignificant difference between ND ($15.03 \pm 2.39\text{pg/ml}$), SR ($14.39 \pm 1.14\text{pg/ml}$), SSFR ($14.75 \pm 3.64\text{pg/ml}$) and SSIR ($18.01 \pm 7.71\text{pg/ml}$) (Figure 1).

This study showed a highly significant ($P < 0.01$) correlation between serum TNF- α levels and Serum albumin, proteinuria and RBCs in urine of the nephrotic groups.

There was a significant ($P < 0.05$) correlation between TNF- α and Urea level in NS patient. No significant correlation was reported for TNF- α with Creatinine and pus cell in urine (Table 2).

Increased levels of TNF- α was agreed with Suranyi *et al.*, (1993) which reported that TNF- α was found to be significantly increased in the plasma and urine of patients with nephrotic syndrome above that found in healthy control.

Elevated levels of TNF- α were found in supernatant of culture of mitogen-stimulated mononuclear cells of peripheral blood from infected children with FSGS. Four out of ten children with primary FSGS showed remission of proteinuria in a (phase I) trial of a TNF α -neutralizing antibody (Bierzynska and Saleem, 2017).

Bitzan *et al.*, (2012) showed that TNF- α pathway dependent alteration in podocyte cytoskeletal alterations and also alterations in focal adhesion complexes of podocyte.

TNF- α interacts with 2 different receptors, specified TNFR1 and TNFR2, which are differentially expressed on tissues and cells and begin both distinct and overlapping pathways of signal transduction (Bradley, 2008). These varied signaling cascades progress to a range of cellular responses, inclusive survival, migration, proliferation, differentiation and death of cell.

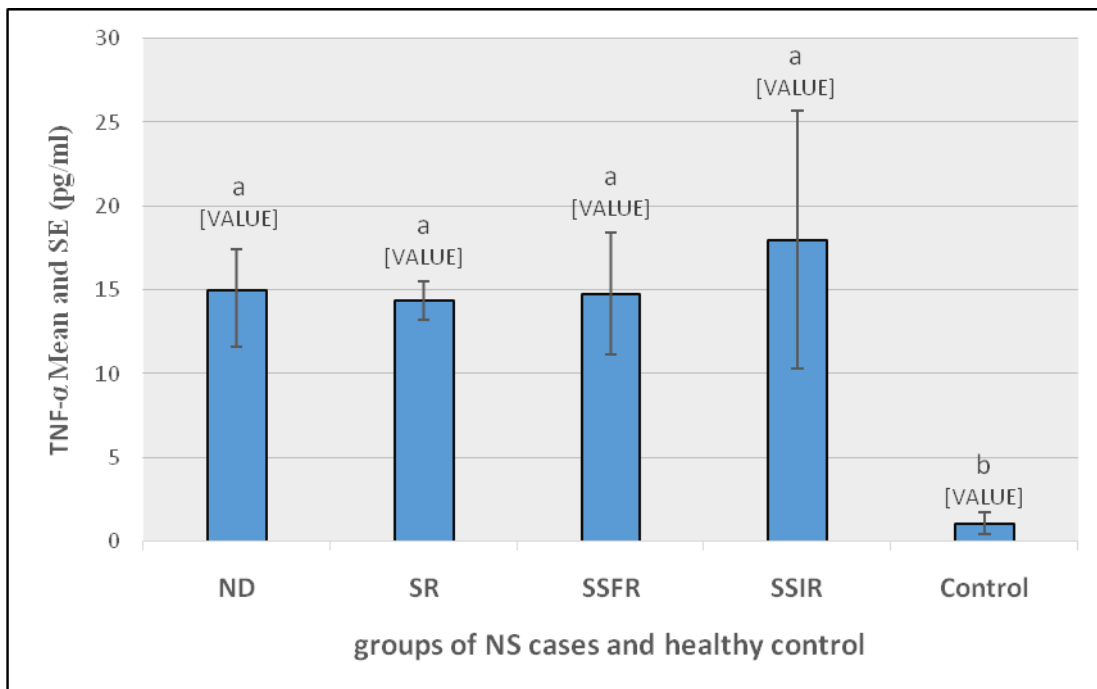
Table.1 Compare between patients and control in TNF- α levels

Group	Mean \pm SE
	TNF- α (Pg/ml)
Patients	15.68 \pm 2.38
Control	1.066 \pm 0.65
T-Test	6.820 **
P-value	0.0001
** (P<0.01).	

Table.2 Correlation between TNF- α and diagnostic features in patients

Laboratory parameters for diagnosis NS	TNF- α
Urea	-0.21 *
Creatinine	-0.15 NS
Serum albumin	-0.43 **
Cholesterol	0.16 NS
Urine albumin	0.29 **
Pus cell in urine HPF	-0.11 NS
RBC in urine HPF	0.46 **
* (P<0.05), ** (P<0.01), NS: Non-Significant.	

Fig.1 Serum TNF- α levels among groups of NS and control



ND: Newly diagnosis, SR: steroid resistance, SSFR: Steroid sensitive frequent relapse, SSIR: Steroid sensitive infrequent relapse. Different letters mean significant differences between means at P<0.01

Vascular endothelial cells comply to TNF- α by a number of pro-inflammatory changes which rise white blood cells adhesion, vascular permeability and trans endothelial migration, and may thrombosis promoting. The central role of TNF- α in inflammation has been elucidated by the successful use of TNF- α blocking agents in the therapy of many inflammatory conditions, like rheumatoid arthritis, inflammatory bowel disease, ankylosing, psoriasis and spondylitis (Lonaki *et al.*, 2009).

Nephrotic syndrome recurrence is linked with glucocorticoid receptor (GR) expression levels, overexpression of GR β and decrease in GR α expression. Renal syndrome is caused by numerous factors, including infections, which may trigger the return of renal syndrome by inducing the glucocorticoid receptor to alter the efficiency of glucocorticoid (GC). Immunological inflammatory interface and transcription factors are pivotal to the organizing of GR-mediated GC efficacy. Various inflammatory response exciter are able to minimize or even prevent the binding of glucocorticoid to target cells. This episode is the clinical cause for return nephrotic syndrome. Infection is the most prevalent reason of relapse. Components of Bacterial cell, including lipopolysaccharide and lipoteichoic acid, are capable to stimulate the monocyte/macrophage intra cellular indicative transduction pathways and prompt the transcription and overexpression of TNF- α encoding genes. During the precocious phases of inflammation, TNF- α may play as a key medium to activate the downstream factors expression, including λ -IFN, IL-1, IL-2, IL-4 and IL-8 and thus start a cascade reaction, which then promotes the nephrotic syndrome return by affecting glucocorticoid response (Liang *et al.*, 2015).

Liang *et al.*, (2015) also reported that TNF- α may also induce the decrease or even

inhibition of glucocorticoid activity and therefore directly affect the glucocorticoid receptor. The serum concentration of TNF- α in patients with nephrotic syndrome return were significantly higher compared with those in the normal groups. This proposes that elevated serum levels of TNF- α are associated with the nephrotic syndrome recurrence.

Berghea *et al.*, (2017) reported many studies that tried to recognize the circulating factors released from T-lymphocyte that elevate the glomerular permeability to serum albumin. elevated levels of IL-2, soluble IL-2 receptor, IL-4, IL-12, IL-13, IL-18, tumor necrosis factor (TNF)- α , and vascular endothelial growth factor (VEGF) were related with MCD relapse. The specific action of a cytokine on the proteinuria development was not studied extensively. Using an animal model, Laflam and Garin (2006) recorded a significant elevated in albuminuria in rats following infusion of TNF- α .

Conclusion: increased serum TNF- α concentration in children with NS and there is high correlation between its level and decrease serum albumin and elevate serum cholesterol. Further studies of the effect of TNF- α in the kidney in children with NS and the effect of TNF- α on permeability of glomeruli are needed.

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