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Research Article

Study of Circulating Vascular Endothelial Growth Factor in Nephrotic Children

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Abstract

Introduction: Although precise aetiology of proteinuria in childhood nephrotic syndrome (NS) remains to be clarified, there is an evidence of a circulating substances, that directly impair glomerular permselectivity and induce proteinuria. Vascular endothelial growth factor (VEGF) is a potent enhancer of vascular wall permeability and it might have some role in the development of glomerular proteinuria by altering glomerular permselectivity. The current study was designed to investigate the relationship between serum level of vascular endothelial growth factor and both proteinuria and steroid responsiveness in children with nephrotic syndrome.

Methods: The study included 55 nephrotic patients recruited from the pediatric nephrology clinic, Ain Shams University Children Hospital. They were 35 males and 20 females with a mean age of (9.02 ± 3.6) years (Age ranged from 3 to 16 years). The patient group comprised 30 steroid sensitive nephrotic patients (SS), 10 steroid dependent (SD), 10 steroid resistant (SR) and 5 newly diagnosed cases of nephrotic syndrome (new). The mean urinary protein/creatinine ratio of the patients was 13.3 ± 17.4. The patients were compared to 24 age and sex matched healthy children as a control group.

Results: The mean serum VEGF of the nephritic patients was significantly higher (279.2 ± 214.3) pg/dl compared to the control group (118.6 ± 62.3) pg/dl ($P < 0.01$). The mean serum VEGF levels of the SS nephrotic syndrome patients (254.9 ± 212.2) pg/dl, SD patients (377.1 ± 209.8) pg/dl, SR patients (310.4 ± 230.6) pg/dl and newly diagnosed cases (265.1 ± 249.4) pg/dl were significantly higher than that of the control group $P < 0.01$. However, no significant difference was detected on comparing the mean serum VEGF of the previous groups with each other or on comparing patients in relapse (329.8 ± 238.7) pg/dl, to patients in remission (222.8 ± 173.6) pg/dl ($P > 0.05$). Moreover, no statistically significant correlation was detected between serum VEGF level and urinary protein/ creatinine ratio in the studied patients.

Conclusion: Childhood nephrotic syndrome is associated with increased level of serum VEGF suggesting its probable role in the disease pathogenesis which apparently has no relation to the disease activity, degree of proteinuria or to the steroid response.

Keywords: *Nephrotic Syndrome; Vascular Endothelial Growth Factor; Vascular Permeability Factor*

Introduction

The term nephrotic syndrome refers to a set of clinical signs and symptoms with glomerular involvement characterized by heavy glomerular proteinuria, hypo-albuminaemia, dysproteinaemia, oedema and varying degree of hypercholesterolaemia [1].

Recently it was realized that proteinuria (albuminuria) is far more deleterious than was originally assumed. Undoubtedly, persistent severe glomerular proteinuria may cause tubular and interstitial renal cell damage, ultimately leading to glomerulosclerosis [2].

The permeability of glomerular capillary wall to serum proteins is limited by properties of the protein themselves (size, negative charge) as well as by intrinsic factors related to the filtration barrier, a proximal electrostatic barrier and a distal size selective barrier. Selective low molecular weight proteinuria is generally responsive to corticosteroids while the non-selective rarely does [3].

Although precise aetiology of proteinuria in childhood nephritic syndrome remains to be clarified, there is an evidence of a circulating substances, that directly impair glomerular permselectivity and induce proteinuria. As VEGF is considered to be a potent enhancer of vascular wall permeability, it might have some role in the development of glomerular proteinuria by altering glomerular permselectivity [4].

VEGF is a potent mitogen that enhances the proliferation of vascular endothelial cells, angiogenesis, micro vascular permeability and monocyte chemotaxis. Its signaling pathway plays pivotal roles in regulating tumor angiogenesis. VEGF as a therapeutic target has been validated in various types of human cancers [6].

In the normal kidneys, it is predominantly expressed by glomerular podocytes and tubular epithelial cells [5]. Several studies have documented altered VEGF expression in various glomerular diseases and suggested that may play some role in the filtering function and integrity of the glomerular basement membrane by acting on glomerular endothelial cells which express its receptors [7].

Aim of the Study

This study is aimed at investigating the relationship between VEGF and both proteinuria and steroid responsiveness in children with NS. Understanding the pathogenesis of proteinuria in NS may pave the way for early detection and prevention of the disease.

Methods

The study comprised 55 patients with different types of idiopathic nephrotic syndrome from the pediatric nephrology clinic of Ain Shams University children's Hospital. The group of patients comprised 35 males and 20 females, their ages ranged from 3 to 16 years with a mean value of 9.02 ± 3.6 years. The patients were subclassified according to their response to corticosteroids into the following groups:

1. **Group I:** Included 30 patients of steroid sensitive nephrotic syndrome (SS). They were 18 males and 12 females, their ages ranged from 4 to 15 years with a mean value of 9.26 ± 3.47 years this group is furtherly classified into:
    a. **Ia:** 15 patients steroid sensitive nephrotic syndrome in relapse (SL).
    b. **Ib:** 15 patients steroid sensitive nephrotic syndrome in remission (SM).
2. **Group II:** Included 10 patients with steroid dependent nephrotic syndrome (SD) they were 9 males and 1 female. Their ages ranged from 4 to 15 years with a mean value 8.6 ± 3.6 years.
3. **Group III:** Included 10 patients with steroid resistant nephrotic syndrome (SR) they were 6 males and 4 females. Their ages ranged from 3 to 16 years with a mean value 9.4 ± 4.1 years.
4. **Group IV:** Included 5 newly diagnosed nephrotic syndrome patients (new). They were 2 males and 3 females. Their ages ranged from 3 to 14 years with mean value 7.6 ± 4.4 years.

The control group (C) comprised 24 age and sex matched healthy children. They were 14 females and 10 males and their age ranged from 4 - 14 years with a mean value of (9.0 ± 3.2) years.

Children in the study were subjected to:

1. Thorough history taking laying stress on symptoms of nephrotic syndrome and its complications. Detailed information about drug therapy particularly response to steroid therapy.
2. Clinical examination to confirm the diagnosis and to detect signs of remission or relapse. Measurement of the arterial blood pressure and the body weight were done.

3. Laboratory investigation:

    a. Laboratory investigations to verify the diagnosis and the disease state:

        i. Complete blood picture (Coulter Counter T660 - Coultronic France).

        ii. Complete urine analysis.

        iii. Serum cholesterol, serum creatinine levels (Colorimetric method, Synchrome Cx7).

        iv. Quantitative estimation of urinary protein by measuring urinary protein and urinary creatinine in a random urine sample [Urinary Protein/Creatinine ratio] (Colorimetric method, Synchrome Cx7 system).

    b. Circulating serum vascular endothelial growth factor by ELISA using Quantikine, human VEGF (R and D System, Minneapolis, MN, USA).

From each patient and control 3 ml of venous whole blood was withdrawn. Sera were separated and preserved at -20^{0}C for estimation of VEGF till time of assay. A monoclonal antibody specific for VEGF has been pre coated onto a microplate. Standard samples are pipetted into wells. A wash follows to remove any unbound antibody enzyme reagent then substrate solution is added to the wells and color develops in proportion to the amount of VEGF bound in the initial step. The color development is stopped and the intensity of the color is measured. The concentration of the samples were calculated from the standard curve.

Statistical Methods

The following methods were used for the statistical analysis of the collected data:

- Data were analyzed using statistical software package for window (version 5) (Stat soft, Tulsa, OK, USA).
- Quantitative data were presented as mean ± SD (median an quartile range for non-parametric values).
- Comparison of variables between various groups was done using student t-test for normally distributed variables and Mann Whitney U-test for non-parametric variables.
    - P > 0.05: Non-Significant.
    - P < 0.05: Significant.
    - P < 0.01: Highly Significant.
- Correlation of various variables was done using Pearson R correlation test and Spearman rank coefficient for normal and non-parametric variables respectively.
- Chi square test was used for comparison of qualitative variables.

Results

A highly significant increase in the mean VEGF level was detected on comparing the nephrotic patients (279.2 ± 214.3) to the normal controls (118.6 ± 62.36) P < 0.01 (Figure 1).

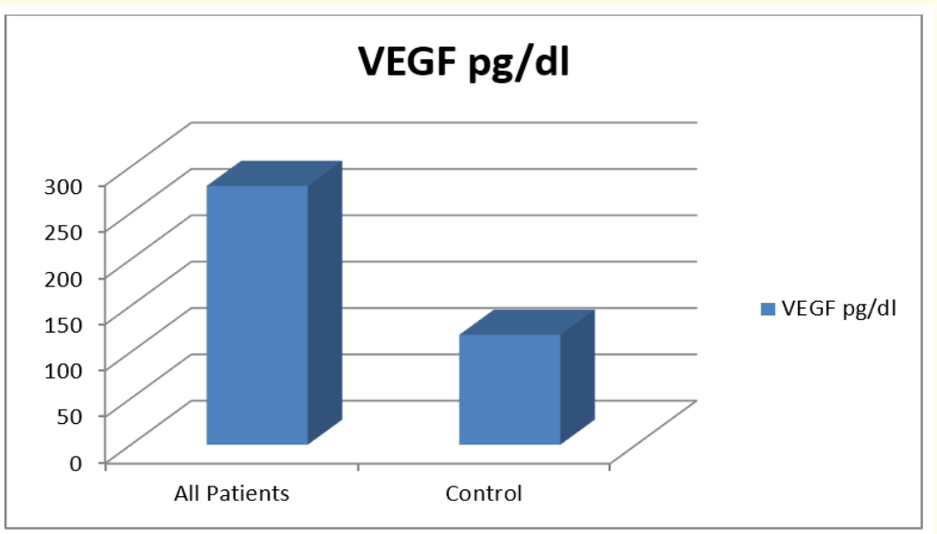

Figure 1: *Comparison of VEGF level between all patients and control.*

The mean urinary protein/creatinine ratio was significantly higher in the studied group of patients (13.33 ± 17.4) in comparison to normal control group (0.04 ± 0.02) P < 0.01.

The mean serum VEGF levels of the studied subgroups: SS nephrotic syndrome patients (254.9 ± 212.2) pg/dl, SD patients (377.1 ± 209.8) pg/dl, SR patients (310.4 ± 230.6) pg/dl and newly diagnosed cases (265.1 ± 249.4) pg/dl, were significantly higher than that of the control group P < 0.01. However, no significant difference was detected on comparing the mean serum VEGF of the previous groups with each other (Figure 2).

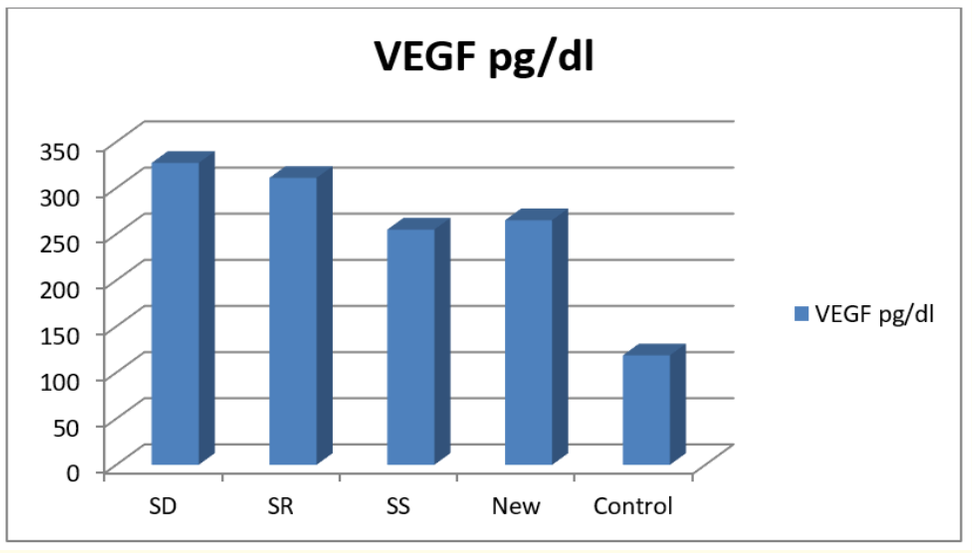

Figure 2: *Comparison of VEGF level between SD, SR, SS, new and control groups.*

On comparing the mean serum VEGF in patients during relapse (329.8 ± 238.7) pg/dl, to patients in remission (222.8 ± 173.6) pg/dl, there was no significant difference (P > 0.05) (Figure 3).

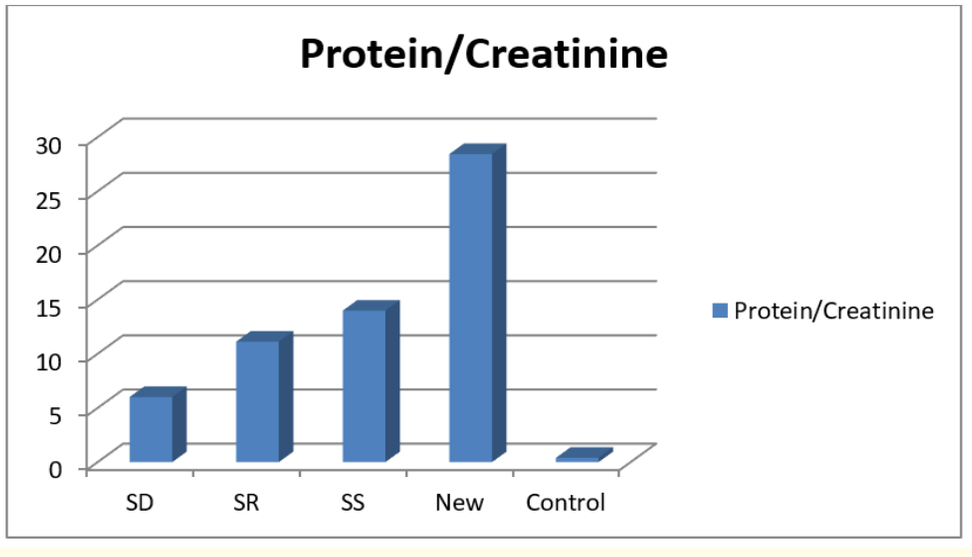

Figure 3: *Comparison of Protein/Creatinine between SD, SR, SS, new and control groups.*

The relapse status was studied further in the steroid sensitive nephrotic patients only. Despite higher mean VEGF level recorded in patients with relapse (308.45 ± 238.7) pg/dl compared to those in remission (201.54 ± 173.6) pg/dl, the difference was not statistically significant ($p > 0.05$).

When different reported pathologies were concerned, no statistical significant difference in the mean serum VEGF level could be detected between patients with FSGS (250.3 ± 245.8) pg/dl and patients with MCNS (394.29 ± 244.4) pg/dl ($p > 0.05$) (Figure 4).

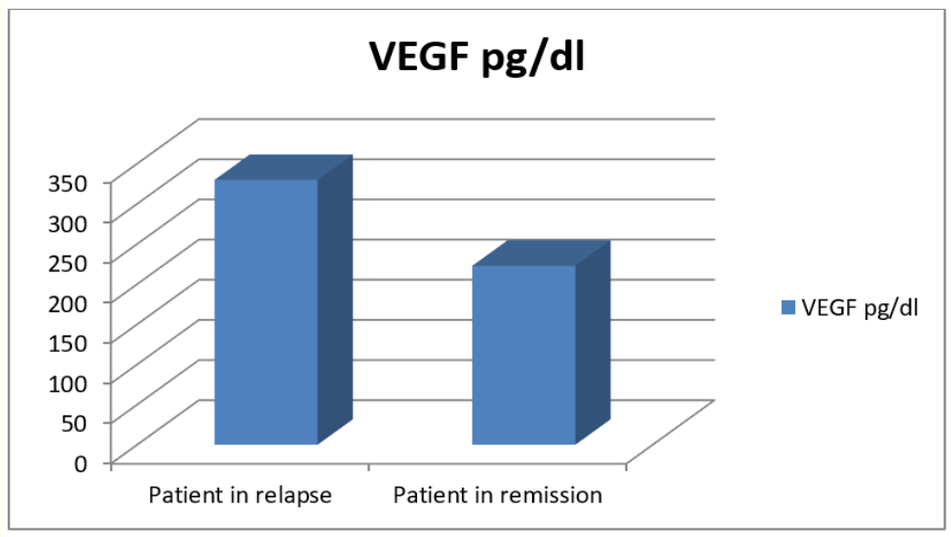

Figure 4: *Comparison of VEGF level between patients in relapse and patients in remission.*

Studying the relationship between the degree of proteinuria in nephrotic patients and serum VEGF revealed no statistically significant correlation between protein/creatinine ratio and VEGF in nephrotic patients (P) group ($r = 0.201$, $P > 0.05$) (Figure 5). The situation was similar in different subgroups of the nephritic patients, N group ($r = 0.24$, $P > 0.05$), SD group ($r = 0.26$, $P > 0.05$), SR group ($r = 0.501$, $P > 0.05$), SS group ($r = 0.41$, $p > 0.05$), SL group ($r = 0.43$, $P > 0.05$) and SM group ($r = 0.435$, $P > 0.05$).

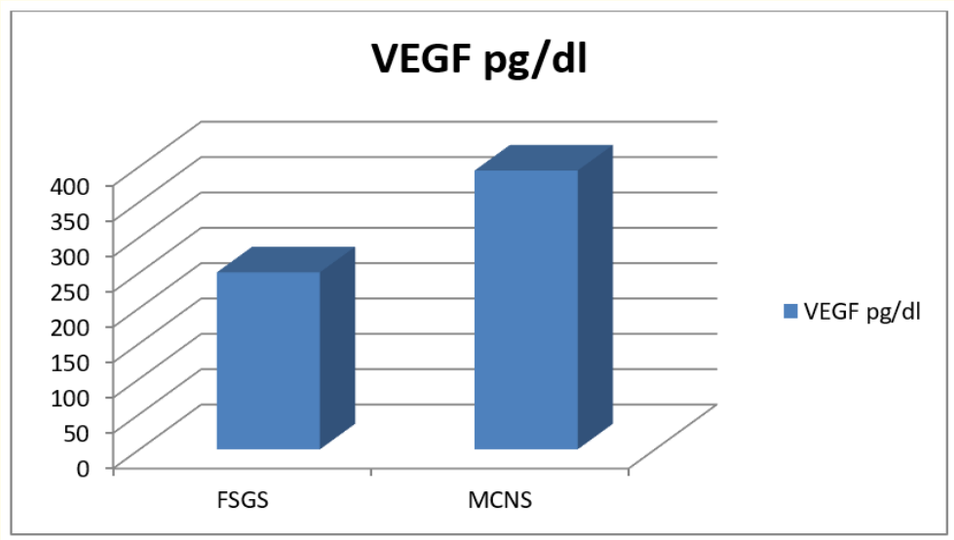

Figure 5: *Comparison of VEGF level between patients with FSGS and patients with MCNS.*

To define the patients who had abnormally high level of VEGF a cut off value was calculated according to the following equation: (cut off value = Mean + 2 SD of the control = 243.32 pg/dl). The positivity rate of VEGF in the studied patients was 60% in SD group, 50% in SR group, 30% in SS group and 40% in N group. When compared to each other, there was no statistically significant qualitative difference between them ($p > 0.05$).

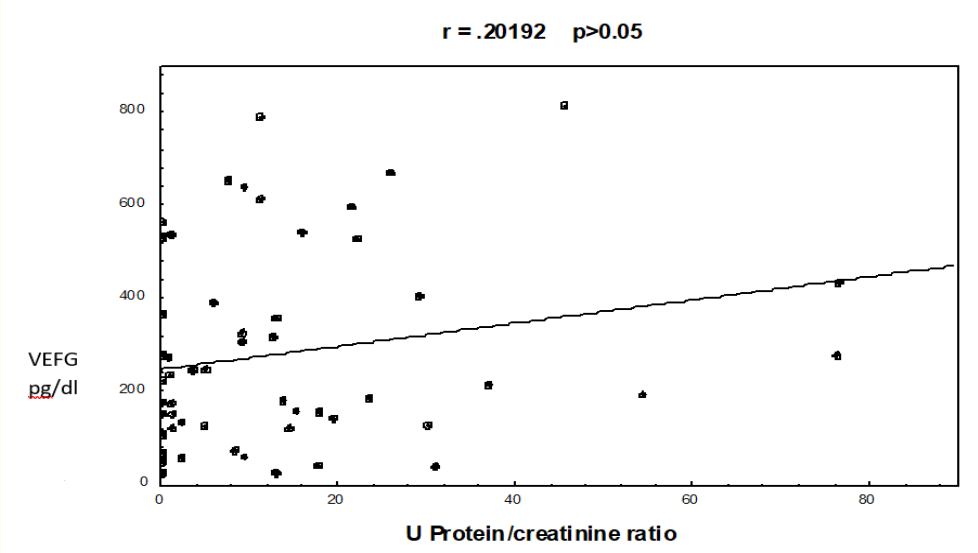

Figure 6: *Correlation between U.Pr/U.Cr ratio and VEGF among all patient.*

Discussion

Nephrotic syndrome is the clinical presentation of various pathologic process [8]. There is evidence of a circulating substance, that directly impairs glomerular permselectivity and induces proteinuria. For example, FSGS may recur immediately after renal transplantation in some patients, and plasmapheresis can induce the remission of proteinuria in some of these cases [9]. This may provide indirect but strong evidence for the presence of a circulating factor that alter glomerular perm selectivity and can be removed by plasmapheresis [10]. VEGF, sometimes also called vascular permeability factor (VPF), is a potent substance that can increase the permeability of vascular walls and can be expressed by peripheral blood mononuclear cells, activated neutrophils, and platelets [11]. It could possibly have some role as a circulating factor in the development of glomerular proteinuria by altering glomerular permselectivity. Moreover, several studies have documented altered VEGF expression in various glomerular diseases, and suggested that it may play some role in the pathogenesis [12].

In the present study serum VEGF was detected to be significantly higher in the nephrotic children, compared to normal control. Horita., *et al.* [13] report an increase in the expression of VEGF in mesangial cells, visceral and partial glomerular epithelial cells.

Safouh., *et al.* [14] demonstrated high serum VEGF levels in proteinuric patients and these levels were higher in SS nephrotic syndrome patients than in those with FSGS and SLE and suggested that VEGF may be involved in the development of proteinuria in different renal diseases.

Studying level of VEGF in different types of nephrotic syndrome classified according to steroid response revealed no significant difference and despite the reports about the inhibitory effect of corticosteroids on VEGF expression [15], the difference in steroid response

was not associated with comparable difference in VEGF level. This is matched with Cheong., *et al.* [16] who found that no significant difference between steroid responders and non-responders and concluded that VEGF is associated with proteinuria in childhood primary NS regardless of steroid responsiveness.

On studying VEGF status in relation to disease activity, no significant difference could be detected between patients in relapse and those in remission in nephrotic patients as well as in the subgroup of SS nephrotic patients. In a study conducted by Iijima., *et al.* [17], intravenous infusion of VEGF into rats increased urine albumin excretion however, Webb., *et al.* [7], could not detect either proteinuria or glomerular histologic changes after VEGF administration to rats. Cheong., *et al.* [16] reported increase in that peripheral VEGF expression in patients with active nephrotic syndrome than in those in remission and they attributed that to the association between VEGF and proteinuria.

These controversies in the results possibly denoting that VEGF level has no relation with the activity of the disease or alternatively, the inhibitory effect of the steroid therapy on VEGF could mask the difference in its level in the two states as corticosteroids inhibit VEGF expression in human vascular smooth muscle cells [17]. Considerable number of patients in this study was on steroids at the time of enrolment.

There was no statistically significant correlation between VEGF and the degree of proteinuria. Nicholus., *et al.* [7] concluded that circulating VEGF levels are not responsible for the degree of proteinuria observed during relapses of SS nephrotic syndrome and such finding supports our results.

Regarding the pathological parameter investigated in this study, no significant difference was found between MCNS and FSGS in agreement Cheong., *et al* [16]. This is may be due to decrease in the number of VEGF expressing cells in sclerotic areas as detected by Shulman., *et al* [18].

Conclusion

In conclusion, childhood nephrotic syndrome is associated with increased level of serum VEGF which is likely to have an important role in glomerular permselectivity dysfunction. However, it has no relation to the disease activity, degree of proteinuria or to the steroid response.

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