

## Serum Erythropoietin in Childhood Nephrotic Syndrome

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### ABSTRACT

**Background:** Anemia is a common feature of the nephrotic syndrome that develops before the deterioration of kidney function. Depletion of iron stores (ferritin) and loss of iron binding protein (transferrin) in urine may contribute to the development of anemia, but iron replacement therapy is usually ineffective.

**Objectives:** To investigate erythropoietin (EPO) deficiency as the cause of refractory anemia in children with the nephrotic syndrome.

**Methods:** Forty children with primary nephrotic syndrome (20 with anemia and 20 with normal Hb) and 40 controls (20 with iron deficiency anemia and 20 with normal Hb) were enrolled in the study. The nephrotic children were studied during disease activity. They included 23 steroid responsive, and 17 steroid resistant cases. They were subjected to clinical evaluation, complete blood count, measurement of total serum proteins, serum albumin, serum cholesterol, blood urea, serum creatinine, serum iron, serum ferritin, creatinine clearance, 24 hours urinary proteins and erythropoietin hormone assay by Immulite.

**Results:** Serum EPO levels were significantly lower in both groups of nephrotic patients as compared to each corresponding control group despite similar Hb concentrations. NS anemic children had greater EPO levels than those without anemia (mean  $\pm$  SD =  $24.77 \pm 1.85$  versus  $9.39 \pm 0.47$  mIU/ml;  $p < 0.001$ ) but their response to anemia in terms of EPO production was inappropriately low, almost four folds less than that detected in iron deficiency anemic controls (mean  $\pm$  SD =  $97.97 \pm 10.12$  mIU/ml). There was significant decrease in EPO level in steroid resistant as compared to steroid responsive patients. There was significant positive correlation between EPO level and serum albumin in both groups of nephrotic children. Both serum iron and ferritin levels were significantly decreased in anemic NS patients as compared to those without anemia.

**Conclusions:** EPO was found to be low in NS even before the development of anemia. Other important contributing factors such as the extent of protein loss and iron deficiency help in the development of anemia, which when established, in presence of an inappropriate EPO response to anemia, will furthermore, aggravate the problem.

### INTRODUCTION

The glycoprotein hormone, erythropoietin (EPO) is an essential viability and growth factor for the erythrocytic progenitors. Both hypoxia and anemia stimulate erythropoietin production by the kidney<sup>(1)</sup>.

Besides its well known role in the treatment of anemia of chronic renal failure<sup>(2)</sup>, recombinant human erythropoietin has been used to treat chronic anemia associated with

prematurity, erythroblastosis fetalis, chronic lung disease<sup>(3)</sup>, and anemia of malignancy<sup>(4)</sup>.

Anemia is a common feature of the nephrotic syndrome that develops before the deterioration of kidney function<sup>(5)</sup>. Depletion of iron stores (ferritin) and loss of iron binding protein (transferrin) in urine may contribute to the development of anemia, but iron replacement therapy is usually ineffective<sup>(6)</sup>. Moreover, the loss of blood

through the gastrointestinal tract due to increased incidence of *Helicobacter pylori* infection in nephrotic children<sup>(7)</sup> aggravates the anemic condition. Recently, erythropoietin deficiency is claimed to be the cause of refractory anemia in nephrotic syndrome<sup>(5)</sup>. However, this association has to be studied comprehensively.

### **AIM OF THE WORK**

This study was conducted in a trial to evaluate the role of erythropoietin hormone deficiency in the development of anemia of nephrotic syndrome in children with normal kidney function. Such a study may help to establish new lines for management of refractory anemia in nephrotic children.

### **SUBJECTS AND METHODS**

This study comprised forty children with primary nephrotic syndrome and forty controls. Subject selection followed the stratified non-random method. An informed consent was obtained from their parents before enrollment.

#### **Patients:**

The nephrotic children were recruited from the Pediatric Nephrology Unit, Children's Hospital, Ain Shams University. The diagnosis of nephrotic syndrome was established according to the criteria of International Study of Kidney disease in Children<sup>(8)</sup>. They were classified into 2 groups:

#### **Group A:**

Included 20 nephrotic children with anemia. Anemia was defined as reduction of the red blood cell volume or hemoglobin concentration below the range of normal values occurring in healthy persons<sup>(9)</sup>. They were 14 males and 6 females. Their ages

ranged from 5-16 years with a mean age of  $9.50 \pm 3.85$  years. The mean duration of nephrotic syndrome was  $4.62 \pm 3.83$  years. These children were followed up and were found to include 9 steroid responsive and 11 steroid resistant cases according to Barratt and Clark (1994)<sup>(10)</sup>.

#### **Group B:**

Included 20 nephrotic children with normal hemoglobin. They were 16 males and 4 females. Their ages ranged from 7 to 12 years with a mean age of  $8.65 \pm 3.03$  years. The mean duration of nephrotic syndrome was  $4.60 \pm 2.68$  years. Fourteen of these children were steroid responsive while 6 were steroid resistant.

#### **Controls:**

Because reference EPO level would vary greatly with anemia, our study included two control groups (group C-a and group C-b), recruited from the Pediatric Outpatient Clinic, Children's Hospital, Ain Shams University.

#### **Group C-a:**

Included 20 children with iron deficiency anemia (14 males and 6 females). Their ages ranged from 5 to 16 years with a mean age of  $9.25 \pm 3.55$  years.

#### **Group C-b:**

Included 20 healthy children with normal hemoglobin (16 males and 4 females). Their ages ranged from 4 to 14 years with a mean age of  $8.45 \pm 3.10$  years.

#### **Exclusion criteria:**

Children with abnormal kidney function (creatinine clearance  $< 80$  ml/min), chronic liver disease, chronic infections, history of or active bleeding, hemolysis, hypoxemia, and recipients of cytotoxic drugs were excluded from the study.

**Methods:**

Both patient and control groups were subjected to:

1. Detailed history especially disease duration, drug therapy and response to treatment.
2. Clinical examination.
3. Routine urine and stool analysis.
4. Complete blood count; using Coulter Counter (Coulter MicroDiff 18, Fullerton CA, USA).
5. Reticulocytic count performed manually by supravital stain.
6. Serum iron by chemical auto-analyzer (absorbent method).
7. Serum ferritin by ELISA.
8. Erythropoietin hormone assay by Immulite.

Further routine investigations done, only for the patients, included: total serum proteins, serum albumin, serum cholesterol, blood urea, serum creatinine, creatinine clearance, and 24 hrs urinary proteins by conventional laboratory techniques.

**Assay of Erythropoietin:**

Serum erythropoietin levels were measured by Immulite automated analyzer with a solid phase, two-site, sequential chemiluminescent enzyme immunoassay<sup>(11)</sup>. The principle depends on binding of murine monoclonal antibody with erythropoietin in the serum sample. The mixture is then incubated for 2 hours. A goat polyclonal antibody is added which upon binding with anti-EPO antibody, a chemiluminescent signal is generated which is detected by specific detectors and is translated to the EPO concentrations by the aid of the EPO adjustors. The erythropoietin DPC Immulite kits was purchased from

Technical Services Company, Los Angeles, USA.

**Statistical Analysis**

Grouping of patients and controls was done after obtaining the results of Hb%, serum iron and serum ferritin. Data were analyzed using statistical software package version 5 (Statsoft, Tulsa, OK, USA). Quantitative variables were presented as mean  $\pm$  standard deviation. Pearson r-correlation test was used for correlating quantitative variables within the studied group. Comparison of mean values of various variables between studied groups was done using student t-test for normally distributed values and Mann-Whitney (u) test for non-parametric data. p-value  $< 0.05$  was considered significant and p-value  $< 0.01$  was considered highly significant.

**RESULTS*****Results of serum EPO assays in the studied groups:***

Serum EPO levels were significantly decreased in each group of nephrotic patients as compared to the corresponding control group with similar Hb concentrations (p  $< 0.001$  in each comparison) (Table 1). Also, the EPO levels of the anemic nephrotic group (group A) was significantly higher than that of the nephrotic non anemic group (group B) (Table 2). However, still the observed reduction in Hb values in the anemic nephrotic group was not associated with the expected rise in plasma EPO concentrations as evident when compared with the observed response in the iron deficiency anemic controls (group C-a) (Fig. 1).

The expected physiologic negative

correlation between Hb% and serum EPO was significantly found in both control groups. On the contrary, there was a disordered significant positive correlation between Hb% and serum EPO in both groups of nephrotic patients (Fig. 2).

**Results of other factors related to the development of anemia in nephrotic syndrome:**

Group A patients had significant lower levels of serum iron, serum ferritin, serum albumen, and significant increase in 24 hours urinary proteins as compared to group B patients (Table 2).

**Serum EPO concentrations in relation to serum albumen and 24 hours urinary proteins:**

We could report significant positive correlation between serum EPO and serum albumen levels in both group A and group B ( $r = 0.98, p < 0.001$ ;  $r = 0.89, p < 0.001$  respectively).

Also, serum EPO levels showed significant negative correlations with the 24 hours urinary proteins in both group A and group B patients ( $r = -0.96, p < 0.001$ ;  $r = -0.99, p < 0.001$  respectively).

**Steroid responsiveness and EPO levels:**

Steroid responsive patients had significantly higher EPO levels when compared to steroid resistant patients within each group of nephrotic children (Table 3).

**Table 1: Comparison of Hb% and serum EPO in the patients and controls.**

	Anemic subjects (n = 40)		Non-anemic subjects (n = 40)	
	Group A (n = 20)	Group C-a (n = 20)	Group B (n = 20)	Group C-b (n = 20)
<b>Hb% (gm/dl)</b>	Mean ± SD		Mean ± SD	
	9.28 ± 0.88	9.08 ± 0.92	12.29 ± 0.75	12.54 ± 1.02
	z = -0.70	p > 0.05	t = -0.87	p > 0.05
<b>Serum EPO (mIU/ml)</b>	Mean ± SD		Mean ± SD	
	24.77 ± 1.85	97.97 ± 10.12	9.39 ± 0.47	18.89 ± 0.95
	z = -5.41	p < 0.001**	t = -39.95	p < 0.001**

p > 0.05 = not significant, p < 0.05\* = significant, p < 0.01, 0.001\*\* = highly significant, Hb = hemoglobin, EPO = erythropoietin.

**Table 2: Comparison of some studied parameters in group A and group B patients.**

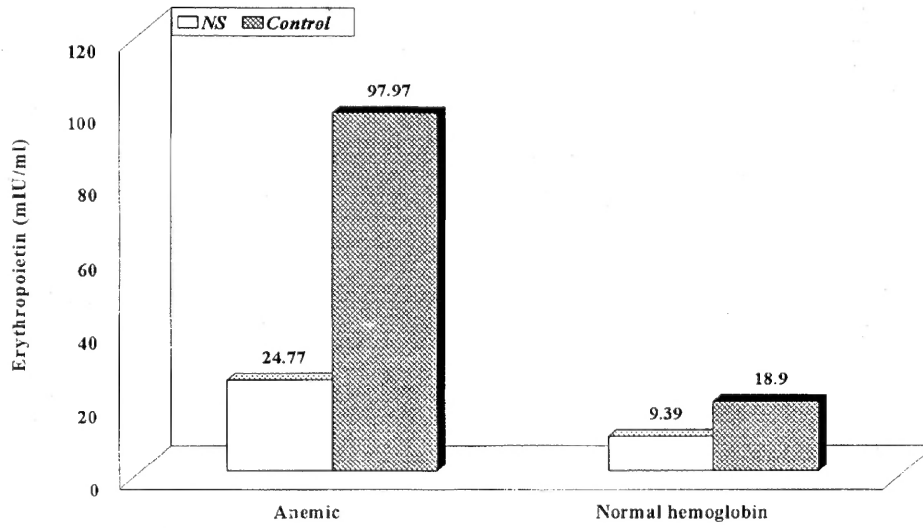
	Group A (n = 20)	Group B (n = 20)	z/t <sup>#</sup>	p
	Mean ± SD	Mean ± SD		
Age (in years)	9.50 ± 3.85	8.65 ± 3.03	0.58	> 0.05
Duration of NS (in years)	4.62 ± 3.83	4.60 ± 2.68	0.36	> 0.05
S. albumen (gm/dl)	2.79 ± 0.05	3.41 ± 0.10	-4.41	< 0.001**
24 hrs urinary proteins (gm)	9.04 ± 1.55	6.07 ± 1.90	4.78	< 0.05*
Serum iron (ug/dl)	33.22 ± 3.19	64.95 ± 8.44	-5.08	< 0.001**
Serum ferritin (ng/ml)	40.43 ± 4.62	94.00 ± 9.63	-22.41 <sup>#</sup>	< 0.001**
Hb% (gm/dl)	9.28 ± 0.88	12.29 ± 0.75	-5.41 <sup>#</sup>	< 0.001**
EPO (mIU/ml)	24.77 ± 1.85	9.39 ± 0.47	35.88	< 0.001**

p > 0.05 = not significant, p < 0.05\* = significant, p < 0.01, 0.001\*\* = highly significant, Hb = hemoglobin, EPO = erythropoietin.

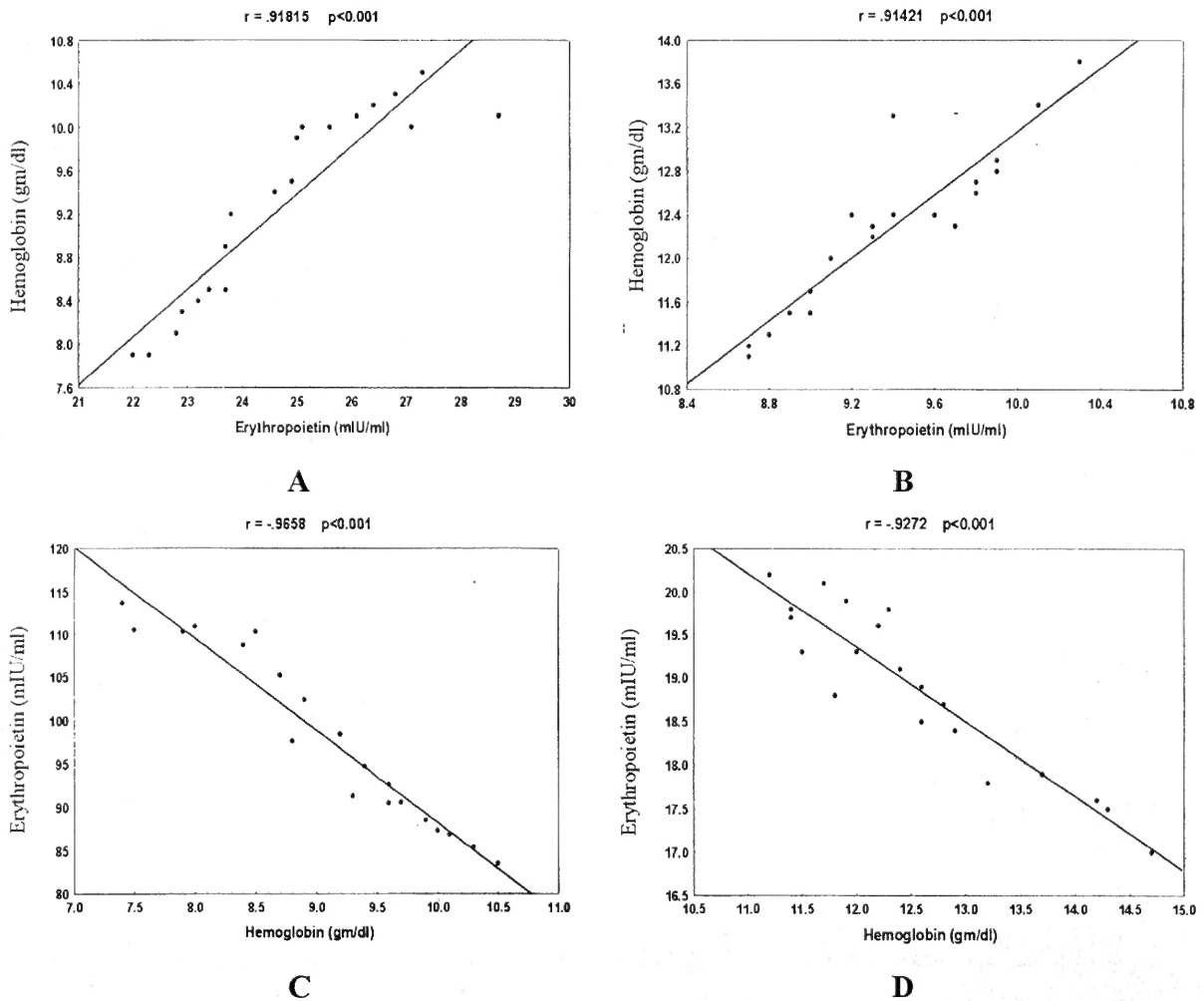
**Table 3: Comparisons of some studied parameters in steroid sensitive and steroid resistant patient subgroups.**

	Group A (n = 20)		Group B (n = 20)	
	Steroid sensitive (n = 9)	Steroid resistant (n = 11)	Steroid sensitive (n = 14)	Steroid resistant (n = 6)
Serum albumin (gm/dl)	Mean ± SD		Mean ± SD	
	2.83 ± 0.03	2.76 ± 0.04	3.45 ± 0.07	3.32 ± 0.10
	t = 3.84	p < 0.001**	t = 3.10	p > 0.001**
24 hrs urinary proteins (gm)	Mean ± SD		Mean ± SD	
	7.86 ± 1.12	10.00 ± 1.15	5.39 ± 0.57	7.66 ± 2.81
	t = -4.17	p < 0.001**	t = -2.88	p < 0.001**
Hb% (gm/dl)	Mean ± SD		Mean ± SD	
	9.93 ± 0.47	8.75 ± 0.79	12.54 ± 0.55	11.70 ± 0.86
	t = 3.89	p > 0.001**	t = 2.36	p > 0.05*
Serum EPO (mIU/ml)	Mean ± SD		Mean ± SD	
	26.11 ± 0.54	23.67 ± 1.29	9.55 ± 0.36	9.03 ± 0.53
	t = 5.41	p < 0.001**	t = 2.51	p < 0.05*

p < 0.05\* = significant, p < 0.01, 0.001\*\* = highly significant, Hb = hemoglobin, EPO = erythropoietin.



**Fig. 1: Erythropoietin levels in the studied groups.**



**Fig. 2: Correlations between Hb% and serum erythropoietin in the studied groups.**

(A = Correlations in group A), (B = Correlations in group B), (C = Correlations in group C-a), (D = correlations in group C-b);  $p < 0.001$  = highly significant.

## DISCUSSION

The nephrotic syndrome is associated with a significant alteration of protein metabolism. While lowering the plasma concentration of certain proteins, the disease often raises the level of other certain proteins. Nephrotic syndrome anemia may result from profound alteration of the metabolism and regulation of iron, ferritin and erythropoietin which are essential for erythropoiesis<sup>(12)</sup>.

The results presented demonstrate that serum EPO concentrations in both groups of nephrotic children were significantly decreased as compared to each corresponding control group. Our results agree with those of Feinstein and colleagues<sup>(5)</sup>, who reported EPO deficiency in nephrotic patients.

In the current study, the exact cause of EPO deficiency has to be defined. Possible contribution of a reduction in EPO biosynthesis, an abnormal rate of EPO degradation/excretion, and/or an expanded volume of distribution are all to be considered.

EPO producing cells in the kidney are fibroblast-like type 1 interstitial cells located in the peritubular space in the cortex and outer medulla. These cells showed increased apoptosis<sup>(13)</sup> and significant decrease of EPO m RNA expression<sup>(14)</sup> in a mouse model of idiopathic NS with normal kidney function. It is possible that EPO-producing cells become damaged by interstitial fibrosis before the development of overt renal failure.

The effect of renal pathology in the NS on EPO biosynthesis might explain our results in the form of significant increase in EPO level in steroid responsive nephrotic children when compared with steroid

resistant patients. To our knowledge, no study was done as regards the link between EPO level in NS and the renal biopsy results, which need to be assessed in future studies.

The M.W. of EPO (30,400 d) is almost half that of albumin. In nephrotic syndrome, there likely is a massive loss of EPO in urine, as well as other low-molecular-weight biologically active proteins. Substantial urinary losses of EPO were shown in rats with puromycin-induced NS<sup>(15)</sup> and in a case report of an anemic patient with minimal change nephrotic syndrome<sup>(16)</sup>. On the contrary, other investigators<sup>(5)</sup> could not detect EPO in the urine of anemic nephrotic patients with low serum EPO levels.

The rate of EPO production is regulated by oxygen tension within the EPO producing cells. A reduction in oxygen supply associated with either hypoxia or reduced erythrocyte mass stimulates EPO production, which in turn, serves to promote erythropoiesis<sup>(5)</sup>. This explains our results in the form of significant increase of the EPO level in the anemic nephrotic group (group A) as compared to the nephrotic non anemic group (group B). However, the reduction in Hb values in the anemic nephrotic group was not associated with the expected rise in serum EPO concentrations as evident when compared with the significant higher response in the iron deficiency anemic controls (group C-a).

The expected physiologic negative correlation between the Hb and EPO concentrations was found in both control groups in our study. In contrast, this intact EPO response to anemia was lacking in our nephrotic groups who showed an abnormal

relationship of EPO levels to Hb concentrations in the form of positive correlation between Hb and EPO in both nephrotic groups. This blunted EPO response to anemia in NS and an abnormal relationship between EPO and Hb was also evident in other published reports in the literature<sup>(5,16)</sup>. This abnormal relationship suggests that the EPO deficiency in NS is not only due to decreased total mass of EPO producing cells by the presumptive interstitial fibrosis, but we are also confronted with disordered function of these cells in the form of lack of the normal response to anemia.

In our study, a significant positive correlation links serum albumin and EPO level in both studied groups of nephrotic patients. Moreover, a significant negative correlation links 24 hours urinary protein to EPO levels in group A. Our results support the postulate of a possible negative effect of proteinuria and the resultant decline in serum albumin and oncotic pressure on EPO biosynthesis<sup>(12)</sup>.

Our findings do not conform with the findings of Shibasaki and associates<sup>(17)</sup> who could not establish a link between serum EPO and serum proteins or proteinuria in NS.

The relationship between the extent of

protein loss and the development of anemia is evident in our results in the form of significant increase in the 24 hours urinary proteins and significant decrease in serum albumin in group A, as compared to group B.

In NS urinary losses of ferritin and transferrin can reduce serum iron concentrations and cause iron deficiency anemia<sup>(6)</sup>. This coincides with our results in the form of significant decrease of serum iron and ferritin in group A as compared to group B.

In conclusion, our study showed that anemia is a common manifestation of nephrotic syndrome. Depletion of iron stores caused by prolonged transferrin and iron losses may contribute to the development of anemia, however, EPO deficiency with an inappropriate response to anemia seems to be an important factor in either the development of anemia or its persistence if initiated by a different factor. Therefore, the definitive treatment of nephrotic syndrome anemia should be directed towards correction of the underlying proteinuria. Nephrotic children with persistent anemia after replenishment of iron stores should receive recombinant human EPO therapy. Dosage schedules need to be assessed in future studies.

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