# A Study of Some Immunological and Hematological Aspects in Children with Renal Disease

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## Abstract:

This study was carried out between September 17, and December 25, 2023. It included 62 pediatric patients at the age range (1-12) years of both genders: 26 with chronic renal failure (CRF) and 36 with nephrotic syndrome (NS), who were outpatients and in-patients in the dialysis unit in both Al-Mansour pediatric teaching hospital and Child's central teaching hospital. The control group consisted of 26 children. Blood samples were collected from patients and controls. Immunological tests of interleukin-6 (IL-6) and erythropoietin (Epo) measurement were done by using ELISA technique. Biochemical tests, including blood urea and serum creatinine, were applied to evaluate renal function. Hematological aspects referred to anemia presence or not; by measuring PCV and Hb. The results explained significant increase in serum IL-6 levels ($P<0.001$), significant decrease in serum Epo levels ($P<0.001$), significant increase in both urea and creatinine concentrations ($P<0.001$) in serum and significant decrease in both PCV and Hb ($P<0.001$) in NS and CRF patients in comparison with the control group.

**Keywords:** Cytokines, Interleukin-6, Erythropoietin, Renal disease, Renal function, Anemia

## 1. Introduction:

Chronic kidney disease (CKD) is a worldwide public health problem progresses towards end stage renal disease (ESRD) as non-curable and progressive condition in childhood can lead to death by early adulthood (www.kidney-international.org). Nephrotic syndrome (NS) is an important CKD in children which is characterized by the presence of proteinuria, hypoalbuminemia, hyperlipidemia and edema. The other important CKD in childhood is chronic renal failure (CRF) which is a progressive irreversible destruction of the kidney tissues leading to the loss of renal function, and if not treated, it will result in death (IAP, 2022). Anemia is a major complication of CKD and ESRD in children that can frequently develop (www.ncbi.nlm.nih.gov). It results from erythropoietin (Epo) deficiency due to its decreased production from kidneys as Epo is probably not only hematopoietic factor but also a cytokine produced mainly in the kidneys (Abid, 2023). A wide variety of immunological abnormalities of cellular or humoral immunity has been found in renal disease (Atul, 2018). It is important to ask whether the physiological abnormalities are cause or consequence of the state of CKD, for example the proinflammatory cytokines, interleukin-6 (IL-

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6), which is secreted by many body cells, is reported to have a central role in the pathophysiological process in patients with renal disease (Gordillo and Spitzer, 2009). Its activities range from the control of the immune response to the involvement in the pathological states (Macioszek et al., 2021). So, this study was carried out to:

- Examine the kidney functions in children with NS and CRF to assess whether and to what degree the kidney may be affected.
- Estimate the levels of IL-6 in the sera of patients and controls.
- Determine serum Epo level and evaluate its correlation with anemia parameters (PCV and Hb).
- Find the correlation between some cytokines production such as IL-6 and Epo as that was not found in previous local studies.

## 2. Experimental Procedure:

### 2.1 Equipments and materials

From different manufacturing companies.

### 2.2 Methods

#### 2.2.1 Study groups

This study was carried out between September 17, and December 25, 2023. The pediatric patients were 26 of CRF and 36 of NS at the age of 1 to 12 years of both genders, who were outpatients and inpatients in the dialysis unit in both Al-Mansour pediatric teaching hospital and Child's central teaching hospital. The control group consisted of 26 children of both genders and at the same age range of the study groups.

#### 2.2.2 Blood samples

Five ml of venous blood were obtained from each child in the study and control group and distributed in suitable containers (Plain tubes and capillary tubes) according to the use in different tests. Eppendorff tubes were used for serum storage at -20°C after centrifugation at 3000 rpm for 10 minutes (Geoerger et al., 2021).

#### 2.2.3 Biochemical tests

- Blood urea

Principle: (BioMérieux Company)

Serum concentration of urea in the current study was determined by enzymatic method (Urease –Modified Berthelot Enzymatic-Colorimetric) according to the following reaction:

$$Urea + H_2O \xrightarrow{Ureas} 2NH_3 + CO_2$$

In an alkaline medium, the ammonium ions react with salicylate and hydrochloride to form a green colored complex:

$$NH_3 + Salycilate \xrightarrow{Nitroptrusside} Indophenol$$

Assay procedure:

1) Working solution was prepared by mixing one vial of R2 enzyme into one bottle of R3; so gently mixed as to dissolve contents.
2) The following solutions were pipetted into three test tubes as mentioned in the kit directions: Working solution, sample and standard solution.
3) All the tubes were mixed separately and then incubated for 5 minutes at 20–25°C.
4) A volume of 200 µL of R4 was added to all tubes, then they were shaked and Incubated for 10 minutes at 20-25°C.
5) The absorbance (A) was read against reagent blank at 580 nm.

Calculation:

$$Urea\ (mg/dl) = \frac{A\ Sample}{A\ Standard} \times Standard\ Concentration$$

* *Normal value of blood urea in children = (20-45) mg / dl*

- Serum creatinine

Principle: (Randox Company)

This assay was done by using calorimetric method. Creatinine

in alkaline solution reacts with picrate to form a colored complex.

Assay procedure:

1) Working solution was prepared by mixing 4 parts of sodium hydroxide with 1 part of picric acid.
2) One ml of TCA was added to 1 ml of serum and they were mixed well by using a glass rod until the dispersion of the precipitate. Then mixture was centrifuged at 2500 rpm for 10 minutes, and then supernatant was poured off.
3) The following solutions were pipetted into three test tubes as mentioned in the kit directions: Working solution, sample and standard solution.
4) All solutions were incubated at 25°C for 25 minutes after separated mixing.
5) The absorbance (A) was read against reagent blank at 550 nm.

Calculation:

$$Creatinine\ (mg/dl) = \frac{A\ Sample}{A\ Standard} \times Standard\ Concentration$$

*Normal values of creatinine in children = (0.7 – 1.4) mg/dl.*

### 2.2.4 Immunological tests

- Interleukin-6 determination:

Principle: (Immunotech Company)

This assay is intended for quantification of human IL-6. This test is a one immunological step, sandwich type assay.

Assay procedure:

As descried in the leaflet of the kit manufactured by Immunotech Company, samples and calibrators were incubated in the microtiter plate coated with the first monoclonal antibody anti IL-6, in the presence of the second anti-IL-6 monoclonal antibody linked to acetylcholine esterase (ACE). After incubation, the wells were washed and the bound enzymatic activity was detected by addition of a chromography substrate. The intensity of the coloration or the absorbance (OD) at 450 nm was proportional to the IL-6 concentration in the sample or calibrator.

Calculation of results:

The samples results were calculated by interpolation from a calibrator curve that was performed in the same assay as that of the sample. The curve was drawn by plotting the IL-6 concentration of the calibrators on the horizontal axis and the absorbance on the vertical axis. By using the computer, the use of a curve fit quadratic mode was recommended. There was no normal range of human serum IL-6 in the leaflet of the kit, so the levels of patient's serum IL-6 were compared with those of the control group.

- Erythropoietin (Epo) determination:

Principle: (D.R.G Company)

This assay utilizes two different monoclonal antibodies to human Epo specific for well-defined regions on the Epo molecules. One mouse monoclonal antibody to human Epo is biotinylated and the other mouse monoclonal antibody to human Epo is labeled with horse radish peroxidase (HRP) for detection.

Assay procedure:

All reagents were prepared to become ready to use, then the procedure was done according to the directions of D.R.G Company using ELISA technique.

Calculation of results (Automatic method):

Computer programs can generally give a good fit to quantify the concentration of Epo. The reference ranges were (4.3 - 32.9) mU/ml for Epo in the serum.

### 2.2.5 Hematological tests

- Packed cell volume (PCV) test or microhematocrit method:

Principle:

The PCV is the measurement of the ratio of the volume which is occupied by the RBCs to the volume of whole blood in a sample of capillarian or venous blood (Portolés et al., 2021). Following centrifugation, this ratio is measured and expressed as a percent or decimal fraction.

Procedure and Calculation of results:

They were done and calculated in children as described in scientific references (Portolés et al., 2021), (Hazin, 2020).

- Hemoglubin (Hb) concentration:

It was calculated by the use of the following equation (Connor et al., 1994) because it is a simple and wide dependent method:

$$Hb\ (g/dl) = (PCV\ (L/L)\ - 1)\ /\ 3$$

Normal values of Hb in children were calculated in children (Hazin, 2020).

#### 2.2.6 Statistical analysis

Data were translated into a computerized database structure. Statistical analysis was computer assisted using SPSS (Statistical Package for Social Sciences) 2019, version 20. The charts were done by using curve estimation system (the quadratic mode). The statistical significance of association between two variables within the same group was assessed by Chi-square. LSD was used in comparison between two different groups. p-value less than 0.05 was considered statistically significant.

## 3. Results and Discussion:

### 3.1 The biochemical tests results:

#### 3.1.1 Blood urea values

As recorded in table 1, the blood urea levels were significantly higher (P<0.001) in both NS and CRF groups than that in the controls' blood. Our results agree with the findings of many studies (Kolvek, 2014), (Pandya et al., 2016) about CRF patients, and agree with (Sundaram et al., 2023), (Jönsson, 2021) regarding NS patients. Impaired renal function and damaged tissues of the kidney cause high blood urea level (Yosa and Wibowo, 2019).

#### 3.1.2 Serum creatinine values

The levels of creatinine were much higher in the both sick groups' sera (P<0.001) than those of the healthy ones. In table 1 the measured parameters were compared among patients with control children. This result agreed with many results (Kolvek, 2014), (Pandya et al., 2016) about CRF. Also it agreed with others about NS patients (Sundaram et al., 2023), (Jönsson, 2021). Any rise in blood creatinine is a sensitive indicator of kidney malfunction, because it is normally and rapidly removed from the blood and excreted (Sen, 2020).

**Table 1. Biochemical tests results of patients and control group**

| Test | Group | Mean | S.D ± | Min | Max |
|---|---|---|---|---|---|
| Urea (mg/ dl) | NS | 59.5 | 10.3 | 44.97 | 74.2 |
| | CRF | 96.2 | 14.4 | 75.9 | 115.1 |
| | Cont | 29.5 | 6.9 | 20 | 44 |
| Creatinine (mg/ dl) | NS | 1.85 | 0.43 | 1.33 | 2.73 |
| | CRF | 4.93 | 1.52 | 2.3 | 6.9 |
| | Cont | 0.87 | 0.14 | 0.7 | 1.2 |

*NS= nephritic syndrome; CRF= chronic renal failure; Cont= controls*

The increase in urea and creatinine levels in serum (called renal impairment) could be due to the decrease in the number of functioning nephrons in addition to the subsequent hypertrophy of them (Yosa and Wibowo, 2019).

### 3.2 The immunological tests results:

#### 3.2.1 Interleukin-6 (IL-6) values:

High levels of IL-6 were markedly detected in the sera of NS and CRF in comparison with controls' (P<0.001 in both patients groups). Table 2 shows the levels of IL-6 in the sera of each group in this study. This matches other results (McCranora et al., 2014) about CRF patients. It also matches the results (Al-Radeef et al., 2018) about NS pediatric patients. Several lines of evidences have reported that a kind of immune complex underlines the effects of IL-6 in the renal injury, besides viral infection and genetic factors (Atul, 2018).

#### 3.2.2 Erythropoietin (Epo) values:

Epo levels in the patients' blood were significantly lower than those in the healthy group (P<0.001 in both CRF and NS

patients in comparison with controls). The values of serum Epo in sick children were in the normal limits of the kit. In table 2. Normal values were compared with those of the control group.

Low concentrations of serum Epo were also detected in NS patients in the study of (Valderrabano, 1996) and (Al-Radeef et al., 2016) in CRF patients. This might be attributed to the decreased production of Epo from the kidney (its major source) or the urinary loss of Epo in proteinuria (Gordillo and Spitzer, 2009).

**Table (2): The interleukin-6 and Epo levels of patients' sera and controls' sera.**

| Test | Group | Mean | S.D ± | Min | Max |
|---|---|---|---|---|---|
| IL-6 (pg/ ml) | NS | 75.5 | 7.2 | 65.2 | 87.98 |
| | CRF | 58.2 | 7.5 | 43.51 | 69.4 |
| | Cont | 37.86 | 6.5 | 29.1 | 47.3 |
| Epo (mU/ ml) | NS | 11.86 | 1.65 | 9.5 | 14.32 |
| | CRF | 10.2 | 1.3 | 8.53 | 12.2 |
| | Cont | 16.5 | 3.6 | 11.35 | 23.75 |

### 3.3 The hematological tests results:

In comparison with normal values, low hematological parameters suggest a kind of anemia, called "renal anemia" or "anemia associated with chronic kidney disease" (www.ncbi.nlm.nih.gov). The hematological parameters are present in table 3.

#### 3.3.1 Packed cell volume (PCV) or hematocrit values:

In table 3, PCV values in both CRF and NS groups were significantly lower than those of healthy children (P<0.001 in CRF and NS children). This is similar to what was obtained by (Al-Radeef et al., 2016a) regarding CKD. Hematocrit less than 33% refers to anemia (www.ncbi.nlm.nih.gov). Low hematocrits observed may be related to low levels of Epo needed in the erythropoiesis activity because the low endogenous production of Epo in chronic disorders of kidneys affects the erythropoiesis (Macioszek et al., 2021).

#### 3.3.2 Hemoglobin (Hb) values:

There was a statistic significance in Hb decreased levels in the renal patients groups reverse controls (P<0.001 in both patients groups). This is shown in table 3 and the normal values of PCV and Hb were as in (Hazin, 2020). Hb concentrations less than 11 g/dl were considered as anemia indicator according to definition of anemia (www.ncbi.nlm.nih.gov). This sounds what was recorded in (Sinha and Bagga, 2022) about CKD. This was more likely to show parallel pattern to hematocrits because Hb values were derived from PCV values of each patient by an equation according to (Connor et al., 1994).

**Table (3): Hb and PCV levels in patients and control group.**

| Test | Group | Mean | S.D ± | Min | Max |
|---|---|---|---|---|---|
| PCV (L/L) | NS | 33.9 | 4.61 | 21 | 40 |
| | CRF | 26.03 | 3.9 | 18.87 | 31.73 |
| | Cont | 39.5 | 2.85 | 36.5 | 44.35 |
| Hb (g/dl) | NS | 10.96 | 1.54 | 6.7 | 13 |
| | CRF | 8.38 | 1.36 | 5.96 | 11 |
| | Cont | 12.82 | 0.95 | 11.83 | 14.45 |

### 3.4 Correlation between Epo and PCV:

A highly significant, positive correlation was found between these two parameters in the group of pediatric NS patients as seen in table 4. No references were obtained about this correlation; except (Shahab and Khan, 2020) in which no correlation was found. Epo concentrations are related to the hematocrit in an exponential manner; so the lowest Epo levels may be attributed to the urinary loss of it as a suggestion, besides the with malnutrition and lack of vitamin B12 or folic acid (Sen, 2020).

**Table (4): Correlation between Epo and PCV**

| Gr. | NS | CRF | Cont. |
|---|---|---|---|
| Chi-square | 0.687** | 0.254 | -0.368 |
| p-value | (P<0.001) | 0.210 | 0.064 |
| N | 36 | 26 | 26 |

** *Correlation is significant at the 0.01 level (2-tailed).*

### 3.5 Correlation between Epo and Hb:

A positive significant correlation was found between Epo and Hb in NS group only as shown in table 5. This is similar to the result in (Hazin, 2020) about NS children. In NS group, the lowest Hb concentration is possibly caused by low levels of Epo in plasma which can result from increased urinary loss in NS or Erythropoietin resistance or insufficient produced levels of hemoglobin (Nelms et al., 2021).

Table (5): Correlation between Epo and Hb

| Gr. | NS | CRF | Cont. |
|---|---|---|---|
| Chi-square | 0.687** | 0.230 | -0.369 |
| p-value | (P<0.001) | 0.258 | 0.064 |
| N | 36 | 26 | 26 |

** *Correlation is significant at the 0.01 level (2-tailed).*

### 3.6 Correlation between urea and creatinine:

The correlation between serum urea and serum creatinine is represented in table 6. No significant correlation was found in both sick groups, while the correlation in control group was highly significant that explains intact renal function. A significant correlation was found in the normal controls in comparison with renal disease patients in the (Pandya et al., 2016). The urea: creatinine ratio can be useful in determining the cause of renal dysfunction (Yosa and Wibowo, 2019). A linear relationship existed between these two variables in normal subjects. So, this linear relationship may refer to either the two portions of the nephrons (the glomerulus and the tubules) are functioning or their function is impaired, besides contributions of child general state, nutrition and the impact of prolonged medications usage (ISN, 2012).

Table (6): Correlation between Urea and creatinine

| Gr. | NS | CRF | Cont. |
|---|---|---|---|
| Chi-square | -0.187 | 0.071 | 0.555** |
| p-value | 0.276 | 0.730 | 0.003 |
| N | 36 | 26 | 26 |

** *Correlation is significant at the 0.01 level (2-tailed).*

### 3.7 Correlation between IL-6 and Epo:

This correlation was not significant in CRF patients (p-value was 0.923); but it was significant in NS patients (p-value was 0.049). The results are shown in table 7. Those results were similar to study of (Deepa et al., 2021) concerning CKD which suggested that either the high levels of blood urea or the dialysis procedure has effect on some cytokines . The significant correlation between IL-6 and Epo in NS children was recorded in (Rodriguez-Ballestas and Reid-Adam, 2022) in which IL-6, in combination with other cytokines, acts on the bone marrow and may suppress erythropoiesis, which might be also suppressed by certain nephropathy therapeutic agents (ISN, 2012), (Rodriguez-Ballestas and Reid-Adam, 2022).

Table (7): Correlation between IL-6 and Epo

| Gr. | NS | CRF | Cont. |
|---|---|---|---|
| Chi-square | -0.331* | 0.020 | -0.282 |
| p-value | 0.049 | 0.923 | 0.162 |
| N | 36 | 26 | 26 |

* *Correlation is significant at the 0.05 level (2-tailed).*

## 4. Conclusions:

- Significant increase was proved in serum interleukin-6 levels in NS (P<0.001) and CRF patients (P<0.001) in comparison with controls.
- Erythropoietin levels in both renal diseases groups were significantly less than that in control children (P<0.001), which may indicate renal anemia.
- Significant increase in the concentrations of blood urea (P<0.001 in CRF and NS patients) and serum creatinine (P<0.001 in CRF and NS patients) in both sick groups.
- Significant decrease in anemia parameters (PCV and Hb) was present in both sick groups.
- High significant correlations were observed between Erythropoietin and both of PCV and Hb in NS patients (P<0.001), in contrast of CRF patients (P=0.21 and 0.258 respectively).
- Correlation between IL-6 and Epo was significant in NS group only (P= 0.049).

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