"URINARY ERYTHROPOIETIN LEVELS IN NEPHROTIC SYNDROME CHILDREN WITH ANEMIA"

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iii

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Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"URINARY ERYTHROPOIETIN LEVELS IN NEPHROTIC SYNDROME CHILDREN WITH ANEMIA",** is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

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5

ABSTRACT

Background and aims

Anemia is one of the many complications seen in persistent Nephrotic Syndrome children. In addition to albumin, there is also excessive urinary losses of iron, transferrin, erythropoietin, etc. leading to a deficiency of substrate necessary for effective erythropoiesis. The aim of this study is to estimate urinary levels of Erythropoietin that can be a potential cause of anemia in Nephrotic Syndrome.

Material and Methods

It is a cross-sectional study carried out in patients who had Nephrotic Syndrome presented to the Pediatric department of Dr. Prabhakar Kore Hospital Belagavi, Karnataka, India. All the Nephrotic Syndrome children aged 1 to 18 years of age attending outpatient services were screened for Hemoglobin levels and Anemic children were enrolled. Routine investigations with iron studies, Urine, and clinical data were collected from Anemic patients. Measurements of Urinary Erythropoietin were performed with LEGEND MAX™ Human EPO ELISA Kit in Basic Science Research Centre JNMC Belgaum.

Results

The mean urinary erythropoietin levels found in our study were 9.61+/-10.94. This finding was consistent with other studies. Urinary erythropoietin levels were compared with different parameters like age groups and gender groups along with different diagnostic types. But no statistical significance was found. But there was a negative relationship between hemoglobin levels and urinary erythropoietin levels suggesting that urinary losses of erythropoietin contribute to the development of anemia along with other factors. The prevalence of anemia in our study is 30% .

Various histological parameters were analyzed in this study. Microcytic hypochromic anemia was the most prevalent morphological variety discovered, followed by normocytic normochromic anemia. One child had macro-ovalocytes in a peripheral smear with a dimorphic picture.

There was significant high transferrin saturation and low total iron binding capacity, which can be due to urinary losses of transferrin. It was also found that there was a significant relationship between serum albumin levels along with transferrin synthesis indicating that synthesis of transferrin is none other than a general response in a nephrotic syndrome where there is a general increase in the production of proteins by the liver.

The incidence of iron deficiency anemia is equally distributed among steroid-sensitive and steroid-dependent cases. Anemia in chronic nephrotic syndrome has been linked to urine losses of erythropoietin, transferrin, soluble transferrin receptor, and iron. While we did not analyze the urine losses of iron and transferrin in this investigation, we did discover a negative association between urinary erythropoietin levels and hemoglobin levels, which may indicate that urinary erythropoietin loss in the nephrotic syndrome is a causative factor of anemia.

Conclusion

In cases of persistent nephrotic syndrome, anemia was found to be one of the many complications. The mean urinary erythropoietin levels found in our study were 9.61+/-10.94. There was a negative correlation between the urinary erythropoietin levels and hemoglobin levels, which is suggestive that urinary erythropoietin loss in turn leads to Anemia in Nephrotic Syndrome. The prevalence of anemia in our study is 30%. Iron deficiency anemia was found to be the most common cause of anemia. The incidence of iron deficiency anemia is equally distributed among steroid-sensitive

and steroid-dependent cases. Urinary erythropoietin levels were found in all the cases with no significant difference between the different groups.

Keywords

Anemia, Nephrotic syndrome, Erythropoietin, Iron Deficiency Anemia.

LIST OF ABBREVIATIONS

KD	-	Kilodalton
EPO	-	Erythropoietin
NS	-	Nephrotic Syndrome
MCNS	-	Minimal Change Nephrotic syndrome
FSGS	-	Focal Segmental Glomeruloslerosis
HIV	-	Human Immunodeficiency
SLE	-	Systemic Lupus erythematosus
HUS	-	Hemolytic Uremic Syndrome
HIF-1	-	Hypoxia-Inducible Factor
DM	-	Diabetes Mellitus
ACEI	-	Angiotensin converting enzyme inhibitor
gm/dl	–	Gram per decilitre
%	-	Percentage
mg	–	Milligram
gm	-	gram
WHO	–	World Health Organisation
ELISA	-	Enzyme linked immune absorbant assay
Hb	-	Hemoglobin
Dr	–	Doctor

RBC	–	Red Blood Cell
SBP	–	Systolic Blood Pressure
DBP	–	Diastolic Blood Pressure
PCV	-	Packed Cell Volume
MCV	–	Mean Corpuscular Volume
MCH	–	Mean Corpuscular Heamoglobin
MCHC	–	Mean Corpuscular Heamoglobin Concentration
RDW	–	Red Cell Distribution width
TIBC	-	Total Iron Binding Capacity
IDA	-	Iron Deficiency Anemia

CONTENTS

LIST OF TABLES

LIST OF FIGURES44

INTRODUCTION

`Nephrotic syndrome is the clinical manifestation of glomerular diseases associated with heavy proteinuria. Nephrotic range proteinuria is defined as proteinuria >3.5g/24hrs or a urine protein creatinine ratio>2. Complications in nephrotic syndrome results from excessive protein loss. In patients with nephrotic syndrome, several plasma proteins necessary for other metabolic functions are passed into the urine of patients, causing complications. Significant loss of plasma proteins such as urinary albumin, coagulation factors, immunoglobulins, and a range of other molecular weights leads to complications associated with morbidity in nephrotic syndrome. The most effective treatment strategy would be controlling this urinary protein loss.

In cases of persistent nephrotic syndrome, anemia was found to be one of the many complications. Anemia may be caused by significant losses of erythropoietin, transferrin, iron, transcobalamin, and metal in the urine. This results in a shortage of substrates required for effective erythropoiesis. Anemia often does not improve with nutritional supplementation alone, suggesting other possible mechanisms.

Anemia was most commonly found in therapy-resistant or persistent cases. Physiology of anemia in cases of chronic kidney disease has been understood and proved through many studies. However, in normal renal function, the physiology of anemia in nephrotic syndrome is both poorly understood and complex.

Excessive urinary leakage can lower plasma concentration and cause deficiencies of such proteins. Also, some mechanisms like water and salt retention along with the expansion of volume significantly change the circulating

concentrations of most proteins in nephrotic syndrome. This leads to a decrease in the amount of proteins circulating in the plasma leading to some serious complications.

The molecular weight of albumin is 68KD, whereas that of transferrin and erythropoietin is 80KDa and 30.4KD, respectively. The molecular weight of erythropoietin is significantly less than that of albumin, and because of this, logically erythropoietin losses in urine are anticipated to be considerably elevated.

Erythropoietin is the renal glycoprotein hormone that drives erythroid progenitor cells to replicate and differentiate into mature reticulocytes. EPO promotes transferrin receptor synthesis, activates hemoglobin gene transcription and translation, and promotes the release of reticulocytes into circulation. EPO deficiency causes hypoproliferative anemia and its overproduction causes erythrocytosis leading to polycythemia.

In earlier studies of nephrotic syndrome patients, an inappropriately low plasma concentration of EPO and significant urinary excretion were found. EPO deficiency was primarily thought to be due to its abnormal filtration in the glomerulus and consequent urinary/renal losses.

The objective of this study is to estimate the urinary erythropoietin levels in nephrotic syndrome children with anemia that can be the potential cause of anemia in nephrotic syndrome.

OBJECTIVES OF THE STUDY

PRIMARY OBJECTIVE:

- To estimate urinary erythropoietin levels in nephrotic syndrome children with anemia

SECONDARY OBJECTIVE:

1)To correlate urinary erythropoietin levels with iron deficiency anemia

2) To estimate vitamin b12 levels in nephrotic syndrome children with megaloblastic anemia

REVIEW OF LITERATURE

NEPHROTIC SYNDROME

Nephrotic syndrome is one of the most common childhood kidney diseases worldwide[1]. It has an annual incidence of nephrotic syndrome ranging from 2-7 per 100,000 children[2] and prevalence from 12-16 per 100,000. There is also epidemiological evidence of a higher incidence of nephrotic syndrome in children from south Asia[3]. Nephrotic syndrome (NS) is characterized by significant urinary protein loss leading to hypoproteinemia and consequent edema. Nephrotic syndrome is characterized by massive proteinuria (3-4+ protein), hypoalbuminemia (less than or equal to 3g/dl), edema, and hyperlipidemia(cholesterol>200mg/dl).

Nephrotic syndrome is usually due to glomerular disease and is currently categorized into primary and secondary forms[4,5]. Most children with nephrotic syndrome children are of idiopathic nephrotic syndrome, and the most common glomerular lesion is minimal change disease. Idiopathic nephrotic syndrome occurs most frequently between the ages of 2-6, occurs more frequently in boys, and is steroid sensitive in the majority of cases[95%]. The cause of idiopathic nephrotic syndrome is unknown. However, there is evidence to suggest that primary T-cell dysfunction leads to glomerular podocyte dysfunction[5]. The clinical presentation of the nephrotic syndrome ranges from mild edema to severe disease with complications, life-threatening infections, and thromboembolism. Nephrotic syndrome with significant glomerular involvement may have high blood pressure, renal failure, and gross hematuria. The overall incidence of MCNS has remained generally stable over the past 30 years. However, the incidence of FSGS appears to be increasing[5].

NS is 15 times more common in children than in adolescents and adults. Up to 90% of children with NS have idiopathic NS, and the remaining 10% have secondary NS associated with systemic diseases, infections, malignancy, and other glomerular disorders. The secondary nephrotic syndrome includes infections like hepatitis B and C, HIV, and parvovirus B19, and systemic causes include SLE, HSP, amyloidosis, and DM.

COMPLICATIONS OF NEPHROTIC SYNDROME[8]

Nephrotic syndrome children are inclined to several complications. Complications in nephrotic syndrome can be due to considerable losses of proteins in plasma or complications associated with immunosuppressive therapy.

Most complications are due to the imposing losses of plasma proteins of varying molecular weights in the urine as described in the below table.

Figure1- **plasma proteins of various molecular weights**

Proteins/micronutrients	Molecular weight (Da)	Comment
Albumin	69,000	The most abundant protein in plasma
Transferrin	80,000	Transports iron in plasma
Soluble transferrin receptor	74,000	Plasma levels elevated in iron deficiency
Ceruloplasmin	132, 000	Transports copper in plasma
Transcobalamin I	120,000	Binds vitamin B12 in plasma
Transcobalamin II	36,000	Transports vitamin B12 in plasma
Vitamin B12	1,355	A non-protein micronutrient
Erythropoietin	30,400	Necessary for erythropoiesis
Immunoglobulin G	150,000	The smallest immunoglobulin
Retinol-binding protein	21,000	Transports vitamin A alcohol from liver to peripheral tissues
Beta-2 microglobulin	11,000	Low molecular weight protein
Alpha-1 microglobulin	26,000	Low molecular weight protein

Anemia as a complication of nephrotic syndrome[7,8]

The anemia of nephrotic syndrome is usually microcytic and hypochromic, typical of iron deficiency, but refractory to iron therapy in nephrotic patients due to substantial urinary loss of transferrin and erythropoietin.

However, although critical data on the prevalence of anemia in patients of nephrotic syndrome is scanty. But data procured suggests that anemia is a complication of recurrent cases.

A prevalence of 59% was found in a study done by Feinstein and his group, with most anemic patients having steroid-resistant nephrotic syndrome[9].

Franca November and his group demonstrated the prevalence of nephrotic syndrome in a research center where about 28% of the population suffered from anemia for the duration of their disease[7].

A common problem for this nephrotic syndrome and anemia patients is refractory or persistent nephrotic syndrome.

Mechanisms of anemia in chronic kidney diseases are well-established, but in nephrotic syndrome cases with anemia and normal kidney function, the pathophysiological mechanisms are perplexing.

Vaziri[10] reported data on the regulation and metabolism of erythropoietin (EPO) and transferrin, essential for erythropoiesis in nephrotic children. Urinary loss of EPO causes EPO-deficient anemia and prevents the normal elevation of plasma EPO levels in response to hypoxia and anemia. Transferrinuria and increases in transferrin catabolism cause hypotransferrinemia and iron deficiency anemia. Either

treatment with Subcutaneous administration of recombinant EPO or iron supplementation can be used for treating EPO- and iron-deficiency anemia, respectively. But then, correcting proteinuria will be the ideal approach to reversing these complications.

ANEMIA

Definition of Anemia: A reduction of the hemoglobin concentration or red blood cell (RBC) volume below the range of values occurring in healthy persons. "Normal" hemoglobin and hematocrit (packed red cell volume) vary substantially with age and sex.

Figure 2- **normal mean and lower limits of normal for hemoglobin, hematocrit and MCV**

Table 474.1 Normal Mean and Lower Limits of Normal for Hemoglobin, Hematocrit, and Mean Corpuscular Volume

	HEMOGLOBIN (g/dL)		HEMATOCRIT (%)		MEAN CORPUSCULAR VOLUME (μM^3)	
AGE (yr)	Mean	Lower Limit	Mean	Lower Limit	Mean	Lower Limit
0.5-1.9	12.5	11.0	37	33	77	70
2-4	12.5	11.0	38	34	79	73
5-7	13.0	11.5	39	35	81	75
8-11	13.5	12.0	40	36	83	76
12-14 female	13.5	12.0	41	36	85	78
12-14 male	14.0	12.5	43	37	84	77
15-17 female	14.0	12.0	41	36	87	79
15-17 male	15.0	13.0	46	38	86	78
18-49 female	14.0	12.0	42	37	90	80
18-49 male	16.0	14.0	47	40	90	80

NORMAL IRON HOMEOSTASIS AND ERYTHROPOIESIS[7]

In the typical scenario, iron from the aged erythrocytes will be recycled, which is a significant contribution of iron for erythropoiesis, and it is a process driven by macrophages, with a minute benefaction from intestinal absorption (Fig. 1). Interestingly, systemic iron is regulated primarily at the level of absorption by enterocytes.

Figure 3-**Regulation of Intestinal Uptake Of Iron**

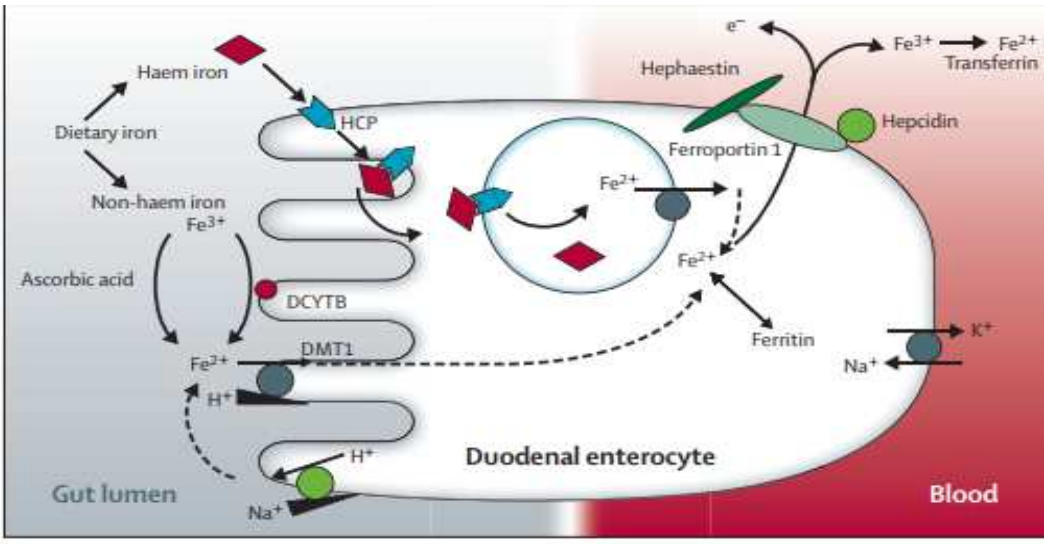
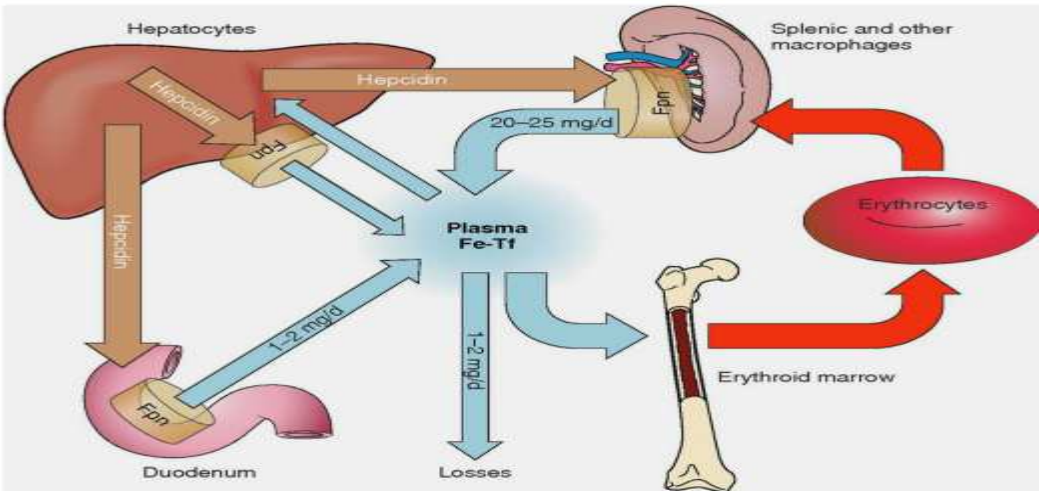


***Figure:* Regulation of intestinal iron uptake**
Haem iron is taken up by the haem iron transporter (HCP), undergoes endocytosis, and Fe^{2+} (ferrous iron) is liberated within the endosome or lysosome. Non-haem iron includes Fe^{2+} and Fe^{3+} (ferric iron) salts. Fe^{3+} is reduced to Fe^{2+} by ascorbic acid in the lumen or by membrane ferrireductases that include duodenal cytochrome B (DCYTB). At the apical membrane, the acid microclimate provides an H^+ electrochemical gradient that drives Fe^{2+} transport into the enterocyte via the divalent metal-ion transporter (DMT1). At the basolateral membrane, iron transport to transferrin in the circulation is mediated by ferroportin 1, in association with hephaestin. Hepcidin, produced by th liver, binds to ferroportin 1, causing its internalisation and degradation and decreasing iron transfer into the blood

The liver and spleen are the main storage organs of iron, where iron is stored as ferritin in macrophages and hepatocytes.

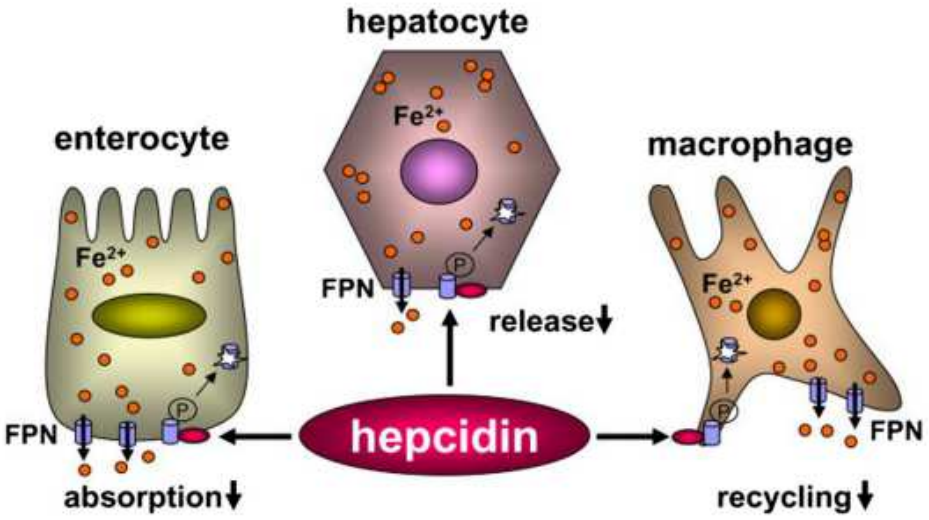
A transmembrane protein known as ferroportin, located on the surface of these cells, functions as an exporter of iron to be released from the storage cells.

Figure 4- **Regulation of Hepcidin**



Hepcidin is a key regulator of iron transport in the body. This is a 2.7-kDa peptide containing 25 amino acids. Hepcidin is synthesized by hepatocytes, secreted into the plasma, and finally excreted by the kidneys.

Figure 5- **Hepcidin as a main regulator**



Iron transport protein transferrin is the primary medium for transporting iron. Iron bound to transferrin is carried to the bone marrow, which erythrocyte precursors take up for erythropoiesis.

Erythropoiesis is a process driven primarily by erythropoietin, a glycoprotein produced mainly by the kidney. Erythropoietin circulates in the plasma and has a plasma half-life of seven–eight hours. Erythropoietin binds to the marrow's erythroid progenitor cells. This binding allows the maturation of these precursor cells into erythrocytes[11,12].

ERYTHROPOIETIN- Production, Regulation, and Actions

EPO is a glycoprotein consisting of a large carbohydrate moiety and a single strand of 165 amino acids. EPO gene is located on chromosome 7[13]. EPO production is increased under conditions that result in reduced oxygen supply to EPO-producing cells(hypoxia), such as anemia, altitude elevation, and certain hemoglobinopathies[14].

Hypoxia-induced upregulation of EPO production was recently shown to be facilitated by hypoxia-inducible factor (HIF-1). It acts as a transcription factor for several hypoxia-induced genes, including many other glycolytic enzymes. HIF-1 also functions as a transcription factor for EPO by binding to oxygen-sensitive enhancer sequences located immediately downstream from the EPO gene[15,16]. EPO messenger RNA (mRNA) is found in fibroblasts, near the base of proximal tubular epithelial cells in the renal cortex[17]. EPO gene transcription in these cells occurs in an all-or-none manner depending on the number of cells expressing EPO mRNA. EPO is also produced by non-renal tissues like macrophages and hepatocytes which account for approximately 10% of the body's total EPO production.

The binding of EPO to the EPO receptors triggers a cascade of protein phosphorylation events, leading to the release of second messengers and cell proliferation. When adequately supplied with vitamin B12, folate, and iron, erythroblasts proliferate at a constant rate, then become reticulocytes, and finally mature red blood cells.

EPO-Metabolism in Nephrotic Syndrome

Significant urinary excretion of EPO was found in a group of patients with nephrotic syndrome in a study by VAZIRI et al[10]. In the adult population, EPO losses in the urine were linked to significantly low plasma EPO concentrations and a decline in hematocrit, but there was no sign of blood loss, hemolysis, or deficiencies in hematopoietic factors. This investigation came to the conclusion that EPO shortage in those cases was what caused the anemia that was seen. This study came to the conclusion that nephrotic syndrome was a probable factor in the development of EPO-deficiency anemia.

The entire metabolism, regulation, and pharmacokinetics of EPO in nephrotic and control rats were then studied in another work by VAZIRI et al[18] to investigate the many mechanisms behind the nephrotic syndrome. These studies similarly demonstrated considerable urine EPO excretion in nephrotic animals in comparison to the healthy control group, along with a notable fall in plasma levels and a modest decline in hematocrit. In these test animals, urine EPO excretion was inversely correlated with plasma EPO concentrations. Glomerular filtration rates were normal in both the control group and the affected individuals, ruling out renal insufficiency as a potential cause of the resulting EPO shortage. The nephrotic condition was the only

factor contributing to the observed decrease in plasma EPO concentrations in nephrotic rats in this investigation.

The aforementioned study also evaluated the variations in plasma EPO concentration brought on by exposure to hypoxia experimental anemia of various degrees of severity brought on by phlebotomy and volume replacement. The research revealed that following a 6-hour exposure to hypobaric hypoxia, nephrotic animals' plasma EPO concentration increased only marginally and insignificantly, and their urine EPO excretion increased significantly above baseline. In contrast, control animals had no detectable urine excretion of EPO[1] but displayed a sharp rise in plasma EPO concentration in response to the drop in erythrocyte mass.

A study by Feinstein et al[9], was done in 19 nephrotic syndrome children. They have concluded that anemia is a feature of persistent nephrotic syndrome and develops before kidney function deterioration. They concluded that anemia was partly due to the depletion of stores of iron, but iron replacement therapy was ineffective. Anemia in nephrotic patients also had a deficiency of EPO with a blunted response to supplementation to iron.

In an educational review by Franca et al[7], anemia due to erythropoietin losses in urine was discussed. The blunted response of serum erythropoietin levels to anemia could be partly attributable to the marked loss of erythropoietin in urine, as was shown in recent studies[19-21]. Since large urine losses are anticipated in individuals with persistent nephrotic syndrome, it was advised that patients with anemia who exhibit low or normal serum erythropoietin levels consider trying erythropoietin therapy.

TRANSFERRIN- Synthesis and Function

Transferrin has two homologous domains, each possessing a binding site for a ferric iron atom. It is an 80-kd monomeric glycoprotein of which 6% is carbohydrate. The liver synthesizes transferrin. It should be noted that some organs like the spleen, brain, testicles, and kidneys also produce modest amounts of transferrin[22-24].

Schade and Caroline characterized transferrin as an iron-binding component of human plasma and mentioned its bacteriostatic function when they initially isolated it from plasma. The half-life of transferrin is eight-twelve days. A conformational transition from the open to the closed configuration is caused by ferric iron binding to the two apo transferrin domains. In people in good health, plasma transferrin concentrations range between 2 and 4 g/L.

Transferrin production elevated in- hypoxia, iron deficiency, pregnancy and estrogen.

Transferrin production is decreased in- malnutrition, inflammation and iron overload.

Iron reserves in the body are inversely correlated with hepatic transferrin mRNA expression.

Transferrin quickly binds iron released from the iron reserves in macrophages or absorbed from the GI tract to extracellular fluid. The transferrin receptor, a 180-kD transmembrane glycoprotein homodimer comprising two subunits connected by a disulfide bond, is then responsible for delivering iron carried by transferrin to the target cells. Except for mature erythrocytes, all cells have the transferrin receptor, which has the best affinity for diferric transferrin. One transferrin molecule is bound to each transferrin receptor subunit on the cell surface. The ligand-receptor combination is then absorbed inside the freshly created endosome.

TRANSFERRIN- Metabolism in Nephrotic Syndrome

In both humans and animals with nephrotic syndrome, serum transferrin levels are commonly decreased, primarily due to this protein being lost through urine[25-30]. Conversely, Reduced serum iron levels brought on by urinary transferrin losses might infrequently result in microcytic anemia and iron insufficiency.

It should be highlighted that the reported cases of iron shortage were determined by plasma iron indices and peripheral blood tests rather than by detecting stainable iron in bone marrow or ferritin levels. Since transferrin is the main protein used to carry iron to erythroid cells for hemoglobin formation, severe hypotransferrinemia can result in microcytic anemia even in the absence of a real iron deficiency.

Renal injury secondary to transferrinuria occurs due to its potential contribution in promoting the generation of free radicals which in turn cause injury to renal tubules. In the presence of luminal fluid pH less than 6, Iron is capable of separating from filtered transferrin. Additionally, iron might be released in the proximal tubular epithelial cells by the enzymatic cleavage of the reabsorbed transferrin. Free iron liberated within the cytoplasm of tubular epithelial cells or the lumen of the proximal tubule can accelerate the production of hydroxyl radicals, causing tubulointerstitial damage and developing nephropathy.

Due to the strong relationship between albumin and transferrin elimination in the urine, the proportionate nephrotoxicity of proteinuria may be partially attributed to the corresponding transferrinuria. To investigate this matter, specialized studies are necessary. In addition to urine losses, the nephrotic syndrome is characterized by a

marked elevation in transferrin catabolism, which can potentially cause hypotransferrinemia.[31]

As said, hypotransferrinemia rather than genuine iron shortage causes hypochromic microcytic anemia and lower serum iron levels in nephrotic syndrome.

PATHOPHYSIOLOGY OF ANEMIA IN NEPHROTIC SYNDROME

Altered iron-transferrin homeostasis

Children and adults with nephrotic syndrome have both shown signs of iron deficiency anemia. As demonstrated by several studies[32-35], this form of anemia has been accompanied by significant iron and transferrin depletion from the urine in nephrotic syndrome. Iron is eliminated with transferrin and remains attached to it in alkaline urine, where it is eventually excreted. Six out of nine patients with nephrotic syndrome showed a considerable loss of iron through the urine in a case study of adults by Brown et al[33].

Urinary transferrin loss can be a possible reason for anemia in nephrotic syndrome patients, and these losses are often associated with low serum transferrin levels. Transferrin losses in urine has been associated with anemia in both pediatric and adult populations[32, 35-38]. Decreased plasma concentrations of transferrin results in enhanced hepatic production of transferrin as well as increased soluble transferrin receptors. However, in nephrotic syndrome individuals with active disease[37], this upregulation is insufficient to restore urine losses; instead, only stabilization of serum transferrin concentration is attained with the management of proteinuria.

Steroid-sensitive nephrotic syndrome exhibits significant concentrations of soluble transferrin receptors and increased urine transferrin loss, which are similar to

those seen in iron deficiency anemia[39]. The method by which iron deficit prevents the development of iron deficiency anemia is the overexpression of this soluble transferrin receptor[40,41].

Urinary losses of erythropoietin

A study by Feinstein et al researched how EPO affects anemia in children with nephrotic syndrome who have normal renal function[9]. In this study, various erythropoiesis precursors, including serum erythropoietin concentrations, iron levels, and vitamin B12 concentrations, were examined in children with nephrotic syndrome. The results were then compared between nephrotic children who had anemia and nephrotic children who did not. Additionally, these patients were paired with two groups of controls, which included control group of children who are healthy and children with iron deficiency anemia and normal renal function. It was concluded that nephrotic syndrome without anemia was steroid sensitive. In contrast, nephrotic syndrome anemia had resistant to immunosuppressive therapy.

However, compared to controls who had iron deficiency anemia without kidney disease, the erythropoietin levels in nephrotic children with anemia were incredibly low[9]. Patients with nephrotic syndrome who had anemia were given erythropoietin along with concurrent iron supplementation, and this had no negative effects on the patient's hemoglobin levels. As shown in both animal and human research, the muted response in nephrotic syndrome may be partially due to the increased substantial loss of erythropoietin[38,18,20,21]. As a result, EPO insufficiency should always be taken into account as a potential factor that could contribute to the emergence of anemia in nephrotic patients.

Metals and Drugs and their role in development of anemia

Copper- The body uses copper as a cofactor in a variety of enzymatic processes, one of which is the mitochondrial respiratory chain enzyme cytochrome c oxidase[42]. Because copper is essential for erythropoiesis, deficiencies are attributed to hematological disorders including anemia[43,44]. Copper deficiency will lead to ineffective erythroid differentiation and hypochromic microcytic anemia[45-47].

Like many of the proteins lost in the urine in patients with nephrotic syndrome, urinary losses of ceruloplasmin which is a plasma protein that transports copper can result in copper deficiency. Anemia results from this considerable loss of ceruloplasmin in children with nephrotic syndrome. Majority of the patient's anemia was corrected by copper supplementation.[38,48]. Copper deficiency should be taken into consideration in treatment-resistant anemia, and serum copper concentrations should be measured to rule out this possibility.

By reducing circulating erythropoietin levels, ACEIs impede erythropoiesis, which can either cause or aggravate anemia[49-51]. Immunosuppressant drugs such as mycophenolate mofetil have broad antiproliferative effects, which result in bone marrow suppression and can cause severe anemia[52]. Discontinuation of such drugs in such patients should be considered.

Vitamin B12 deficiency anemia

Cobalamin, often known as vitamin B12, is a cofactor for numerous enzymatic processes required for maturation. In order to produce methionine from homocysteine and convert methylmalonyl-CoA to succinyl-CoA, vitamin B12 functions as a cofactor. By creating a compound with intrinsic components in the stomach, vitamin

B12 is absorbed and delivered in the bloodstream coupled to the protein transcobalamin[53]. Cubulin, is necessary for the reabsorption of the vitamin B12 complex protein from the kidney's proximal tubule. It is a peripheral membrane protein. CUBN- cubulin gene mutation was associated with increased urinary protein excretion and also associated with megaloblastic anemia[54,55].

Megaloblastic anaemia is brought on by a vitamin B12 shortage, which also affects DNA synthesis and causes hematopoietic cells to die before they have had a chance to mature[44,56]. Children with nephrotic syndrome experience considerable losses of transcobalamin and vitamin B12 in their urine, which results in much lower serum levels of vitamin B12, similar to the significant protein loss seen in nephrotic syndrome.[9,39,58].

MATERIAL AND METHODS

METHODOLOGY

"This study was conducted from January 2021 to December 2021 in the Department of Paediatrics, KLES Dr. Prabhakar Kore Hospital, and Medical Research Centre, Belagavi, on all the nephrotic syndrome children aged 1-18 years attending outpatient service".

Study Design

"Hospital-based cross-sectional study"

Study duration and period

"January 2021 to December 2021"

Place

"This study was conducted in the Department of Paediatrics, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi a tertiary care teaching hospital attached to KAHER Jawaharlal Nehru Medical College, Belagavi".

Source of Data

"All cases of Nephrotic syndrome attending outpatient services at KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi at the Diviosion of Paediatric Nephrology and fulfilling the ISKDC (international study of kidney disease in children) criteria for nephrotic syndrome[59]".

"The sample size was calculated using the formula mentioned below".

N (sample size) =

$$n = \frac{P \times (100 - P) \times Z^2}{d^2}$$

Where ,

N= sample size

p=prevalence of anemia in nephrotic syndrome in a recent study, which was 28%

Q= 100-p when p is in percentage

Relative error- d=10%, and confidence level of 90%,

Z=1.(constant)

$$n = \frac{P \times (100 - P) \times Z^2}{d^2}$$

$$n - \frac{28 \times (100 \quad 28) \times 1.645^2}{10^2}$$

$$n = 51$$

the sample size would be n=51

SAMMPLE SELECTION CRITERIA:

INCLUSION CRITERIA:1) All Nephrotic Syndrome children aged from 1 to 18 years of age attending outpatient services.

2)Including children on treatment for Nephrotic Syndrome.

3)Including children with relapse.

EXCLUSION CRITERIA:

1)eGFR OF <90ml/min/1.73m2.

2)Nephrotic Syndrome patients in remission, which means no proteinuria for 3 consecutive days.

Method of collection of data

Following clearance from the institution's clinical ethical committee, this investigation was carried out.

All nephrotic syndrome children aged 1 to 18 years of age attending outpatient services will be subjected to hemoglobin levels and serum creatinine.

The study's purpose was explained to the parents of the children who met the eligibility requirements, and before the children were enrolled in the study, their signed informed permission was obtained.

Interviews with the parents or caregivers of the kids who met the selection criteria were conducted, and a thorough history was gathered, including

demographic information, the course of the illness, and a history of the treatments used.

In addition to vital signs, anthropometry, and a thorough evaluation of the afflicted system in relation to the symptoms, parents and caregivers of the children who satisfied the selection criteria were questioned about the current complaints recorded. A proforma that had been previously prepared and tested contained all of the findings.

Each patient enrolled was subjected to the following investigations

ROUTINE: Urine Albumin, urine routine examination, serum creatinine, serum albumin, serum cholesterol.

SPECIFIC:

1)Urinary erythropoietin by sandwich-ELISA method

2)complete hemogram and iron studies

All the investigations except urinary erythropoietin levels were processed.

On the same day of enrolment, blood samples were collected from the patients along with urine samples. Urine samples were stored at the Basic Science Research Center at a temperature of < -70°C at KAHER Jawaharlal Nehru Medical College, Belagavi. Blood samples were processed on the same day n our center

LEGEND MAX™ ELISA Kit with Pre-coated Plate was procured from BioLegend, Inc company.

Figure 6- ELISA kit

After the completion of sample collection, all the samples were processed on the same day in Basic Science Research Center.

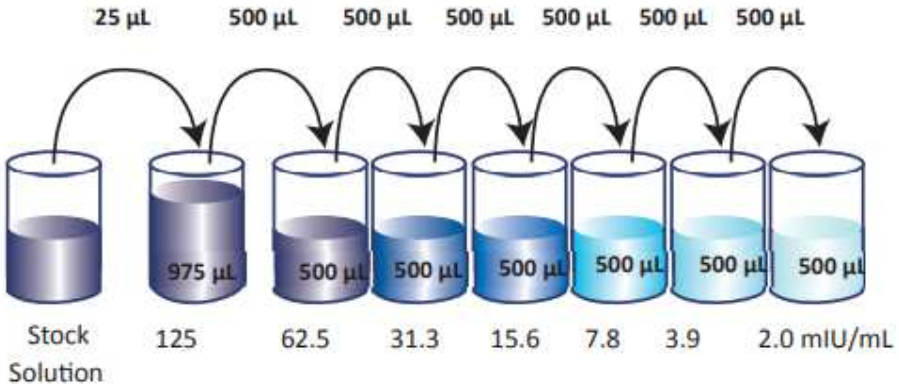
Figure 7- samples

Assay Procedure:

1. All reagents were brought to room temperature before use. It was strongly recommended that all standards and samples be run in duplicate. A standard curve is required for each assay.

2. “Prepare 1,000 µL of the 125 mIU/mL top standard by diluting 25 µL of the standard stock solution in 975 µL of Assay Buffer A. Perform six two-fold serial dilutions of the 125 mIU/mL top standard in separate tubes using Assay Buffer A as the diluent. Thus, the human EPO standard concentrations in the tubes are 125 mIU/mL, 62.5 mIU/mL, 31.3 mIU/mL, 15.6 mIU/ mL, 7.8 mIU/mL, 3.9 mIU/mL and 2.0 mIU/mL, respectively. Assay Buffer A serves as the zero standard (0 mIU/mL)”.

Figure 8- Sample Dilution Method



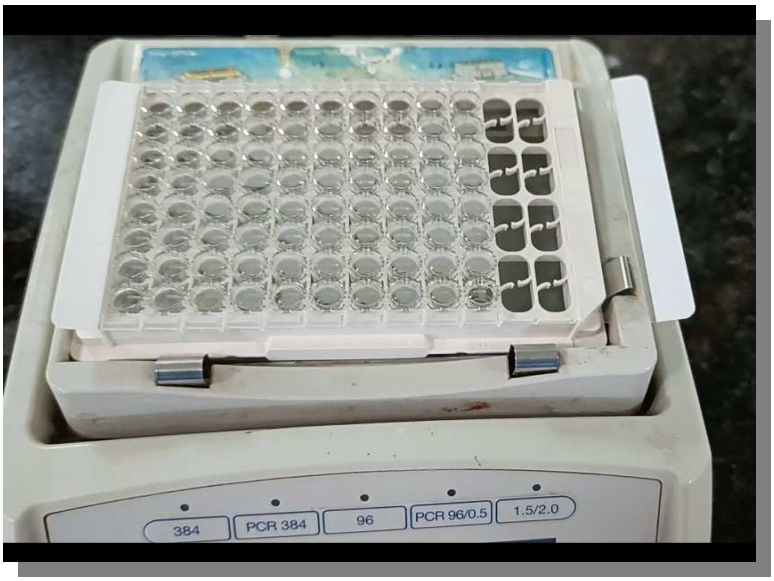
3. For measuring samples:

a) 50 µL of Assay Buffer A was added to each well containing standards or samples.

b) 50 µL of standard dilutions were added to the wells for standards. 50 µL of samples was added to the wells for samples.

4. The assay plate was sealed with a Plate Sealer included in the kit and incubated at room temperature for 2 hours while shaking at 200 rpm.

Figure-9- Incubated at room temperature for 2 hours while shaking at 200 rpm.



5. **The contents of the plate were discarded into a sink, then the plate was washed four- times with 1X-Wash Buffer. The plate was washed with at least 300 µL of 1X Wash Buffer per well** (Figure 10) and blotted any residual buffer by firmly tapping plate upside down on absorbent paper. All subsequent washes were performed similarly.

6. 100 µL of Human EPO Detection Antibody solution was added to each well, sealed the plate, and incubated at room temperature for 2 hours while shaking

7. The contents of the plate were discarded into a sink, then washed the plate four times with 1X Wash Buffer as in step 5.

8. 100 µL of Avidin-HRP solution was added to each well, sealed the plate, and incubated at room temperature for 30 minutes while shaking .

10. The contents of the plate were discarded into a sink, then washed the plate five times with 1X Wash Buffer as in step 5 .

11. 100 µL of Substrate Solution F was added to each well and incubated for 15 minutes in the dark. [**Wells containing human EPO should turn blue in color with intensity proportional to concentration**- Figure 11]

12. 100 µL of Stop Solution was added to each well to stop the reaction. [The well colour should change from blue to yellow.]

13. ELISA reader present at the basic science research center was used. ELISA reader present at the basic science research center was used for reading of plate at 450nm and 570nm within 20mins- Figure- 12

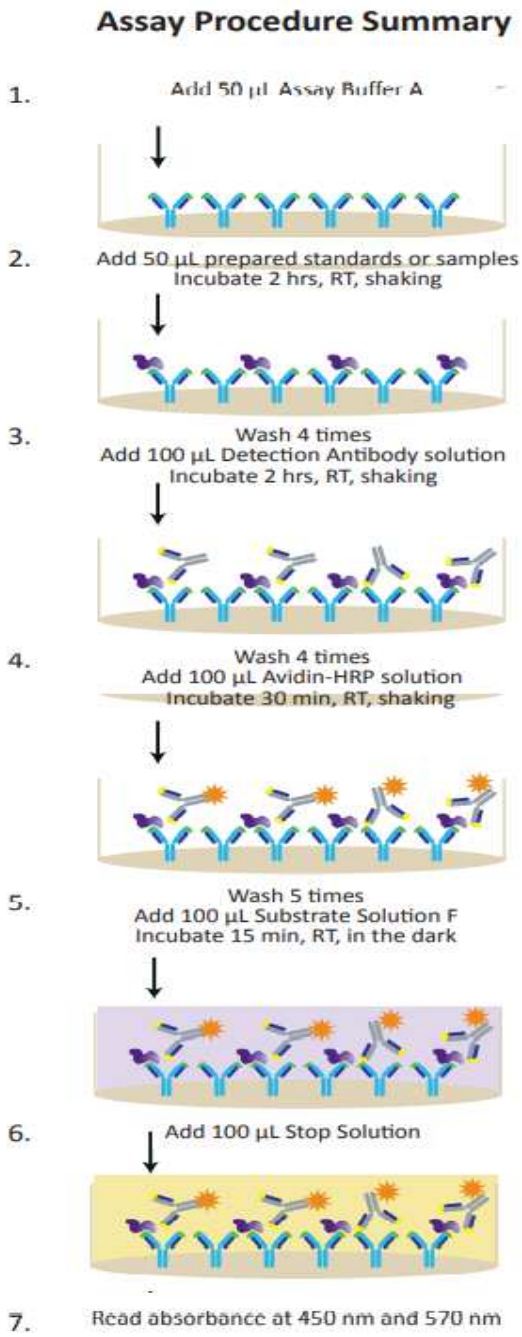


Figure 13- **Assay Procedure Summary**

CASE DEFINITIONS:

1) STEROID SENSITIVE NEPHROTIC SYNDROME: Therapy with prednisolone results in complete remission of proteinuria in 85-90% of patients, termed steroid-sensitive nephrotic syndrome (SSNS).

2) STEROID RESISTANT NEPHROTIC SYNDROME: Lack of complete remission despite therapy with daily prednisolone at 2 mg/kg (or 60 mg/m2) daily for six weeks.

3) STEROID DEPENDENCE: Two consecutive relapses when on alternate-day steroid, or within 14 days of its discontinuation.

4)RELAPSE: Urine protein ≥3+ (Up/Uc >2 mg/mg) for three consecutive early morning specimens, having been in remission previously.

5)FREQUENT RELAPSES: Two or more relapses in the first six months after stopping initial therapy; ≥3 relapses in any 6 months; or ≥4 relapses in one year.

6)ANAEMIA: Anemia is defined as a reduction of the hemoglobin concentration or red blood cell (RBC) volume below the range of values occurring in healthy persons . "Normal" hemoglobin and hematocrit (packed red cell volume) vary substantially with age and sex .

Figure 2- similar as above

Table 474.1 Normal Mean and Lower Limits of Normal for Hemoglobin, Hematocrit, and Mean Corpuscular Volume

	HEMOGLOBIN (g/dL)		HEMATOCRIT (%)		MEAN CORPUSCULAR VOLUME (μM^3)	
AGE (yr)	Mean	Lower Limit	Mean	Lower Limit	Mean	Lower Limit
0.5-1.9	12.5	11.0	37	33	77	70
2-4	12.5	11.0	38	34	79	73
5-7	13.0	11.5	39	35	81	75
8-11	13.5	12.0	40	36	83	76
12-14 female	13.5	12.0	41	36	85	78
12-14 male	14.0	12.5	43	37	84	77
15-17 female	14.0	12.0	41	36	87	79
15-17 male	15.0	13.0	46	38	86	78
18-49 female	14.0	12.0	42	37	90	80
18-49 male	16.0	14.0	47	40	90	80

7) SEVERITY OF ANEMIA[60]:

8)HEMATOCRIT: According to age and gender (above table)

9)MEAN CORPUSCULAR VOLUME: According to age and gender (above table)

10)MEAN CORPUSCULAR HEMOGLOBIN: "Mean corpuscular hemoglobin (MCH) represents the mean mass of hemoglobin in one red blood cell and is expressed in the mass unit picograms (pg= 10-12 gr). It is calculated by dividing the total mass of hemoglobin by the number of red blood cells. The normal range is 27-31 pg".

11)MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION: "Mean corpuscular hemoglobin concentration (MCHC) is the mean concentration of hemoglobin in the red cell or average concentration of hemoglobin in one liter of red blood cells. It is calculated by dividing the hemoglobin by the hematocrit. MCHC full-fill the meaning of MCH considering the size of the cell. The normal range is 31.5-35 g/dl".

12)RED CELL DISTRIBUTION WIDTH: "RDW or red cell distribution width is a parameter that measures variation in red blood size or red blood cell volume. The reference ranges for RDW for an adult is 11.6%-14.6%".

13)SERUM IRON[61]:

Figure 15: **Reference intervals of serum iron in pediatric population**

Pediatric reference intervals (2.5th–97.5th percentiles) of serum/Plasma iron concentrations for females and for males age 0–18 years

Iron	Male, *n*	Intervals		Female, *n*	Intervals	
Age		µg/dl	µmol/l		µg/dl	µmol/l
0–90 days	44	72–203	12.9–36.3	32	75–235	13.4–42.1
91 days–12 months	54	23–142	4.1–25.4	38	60–192	10.7–34.4
13 months–3 years	66	25–126	4.5–22.6	56	55–162	9.8–29.0
4–10 years	77	15–128	2.7–22.9	66	28–122	5.0–21.8
11–14 years	59	32–107	5.7–19.2	57	25–102	4.5–8.3
15–18 years	47	30–130	5.4–23.3	51	25–107	4.5–19.2

14)SERUM FERRITIN: Serum ferritin-5 years or younger <12

Children older than five years < fifteen

In all age groups in the presence of infection < thirty are cutoff values for iron deficiency anemia

15)TOTAL IRON BINDING CAPACITY[61]:

Figure 16: **Reference intervals of TIBC**

Pediatric reference intervals (2.5th–97.5th percentiles) of total iron binding capacity for females and for males age 0–18 years

TIBC	Male, *n*	Intervals		Female, *n*	Intervals	
Age		Ag/dl	Amol/l		Ag/dl	Amol/l
0–90 days	58	155–330	27.7–59.1	38	165–275	29.5–49.2
91 days–12 months	80	150–380	26.9–68.0	37	250–455	44.8–81.4
13 months–3 years	80	215–420	38.5–75.2	60	160–415	28.6–74.3
4–10 years	79	185–415	33.1–74.3	72	260–385	46.5–68.9
11–14 years	70	265–410	47.4–73.4	75	250–420	44.8–75.2
15–18 years	40	270–415	48.3–74.3	41	285–410	51.0–73.4

16)TRANSFERRIN SATURATION: Transferrin saturation <16% is the selected cutoff value to define iron deficiency anemia

17)IRON DEFICIENCY ANEMIA:

CRITERIA INCLUDE 1) LOW SERUM IRON,2) LOW SERUM FERRITIN, 3) LOW TRANSFERRIN SATURATION, 4)HIGH TOTAL IRON BINDING CAPACITY

STATISTICAL ANALYSIS:

Data were entered into Microsoft excel and analyzed using R software version 4.1.1. Categorical variables are given in the form of a frequency table. Continuous variables are given in Mean ± SD/ Median (Min, Max) form. The Chi-square test is used to check the dependency between categorical variables. Two sample t-tests were used to compare the mean of variables over the group. A P-value less than or equal to 0.05 indicates significance.

RESULTS

The cross-sectional study was conducted from January 2021 to December 2021 in the Department of Paediatrics, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi.

In this study, 170 nephrotic syndrome children were screened based on inclusion and exclusion criteria, and 51 children were included in the study fitting the criteria. All 51 children were cases of nephrotic syndrome children with anemia. The prevalence of anemia was found to be 30% of the study population.

Table 1: Anthropometry of Subjects

Parameter	Mean±SD	Median	Minimum	Maximum	95% C. I	
					Lower	Upper
Age (Years)	5.55 ± 3.86	5.0	1.0	16.0	4.5	6.6
Weight(Kg)	19.37 ± 9.23	16.0	8.5	52.0	16.8	22.0
Height(Cm)	103.67 ± 21.32	101.0	66.0	157.0	97.7	109.7

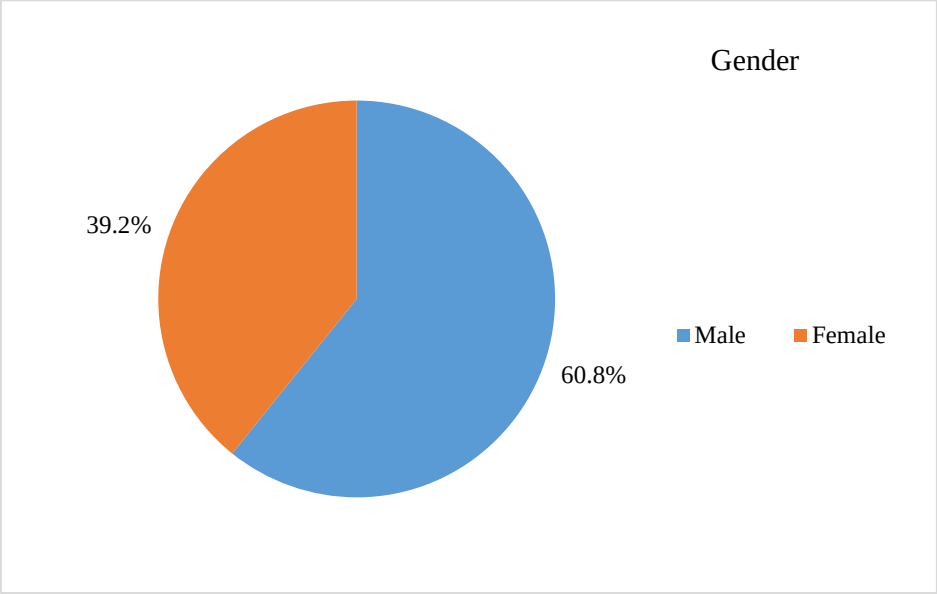
51 patients with nephrotic syndrome anemia were analyzed, and the age distribution of cases ranged from 1 to 18 years. The mean age of presentation was 5.55 ± 3.86 years.

Table 2: Demographic characters of the study population

Demography characters		**n**	**%**
Gender	Male	31	60.78%
	Female	20	39.22%
Age Group	< 5 Years	32	62.75%
	5-10 Years	14	27.45%
	11-16 Years	5	9.80%
Diagnosis	Infantile Nephrotic syndrome	3	5.88%
	Newly Diagnosed	4	7.84%
	Steroid Sensitive	37	72.55%
	Steroid Resistant	7	13.73%
Frequent and Infrequent Relapses	1st Episode	4	7.84%
	Frequent	20	39.22%
	Infrequent	27	52.94%

Out of the 51 subjects enrolled it was found that the majority of subjects constituted of male gender 31 (60.78%) while females were 20 (39.22%). The most common age group was 1-5years , which counted for 62.75%, followed by 6-10years counting for 27.45%, and 11-18years, which counted for 9.8%. In this study, steroid-sensitive nephrotic syndrome accounted for a maximum number of cases -72.5%, followed by steroid-resistant cases, newly diagnosed nephrotic syndrome, and infantile nephrotic syndrome with 13.75%, 7.8%, and 5.8% respectively. It is found that out of 51 patients enrolled 20 patients were frequent relapsers, 27 patients were infrequent relapsers and 4 patients were presenting for the first time.

Figure 17: Pie chart of gender in the study population



M: F ratio of 1.55:1

Figure 18: Pie chart of Age group in the study population

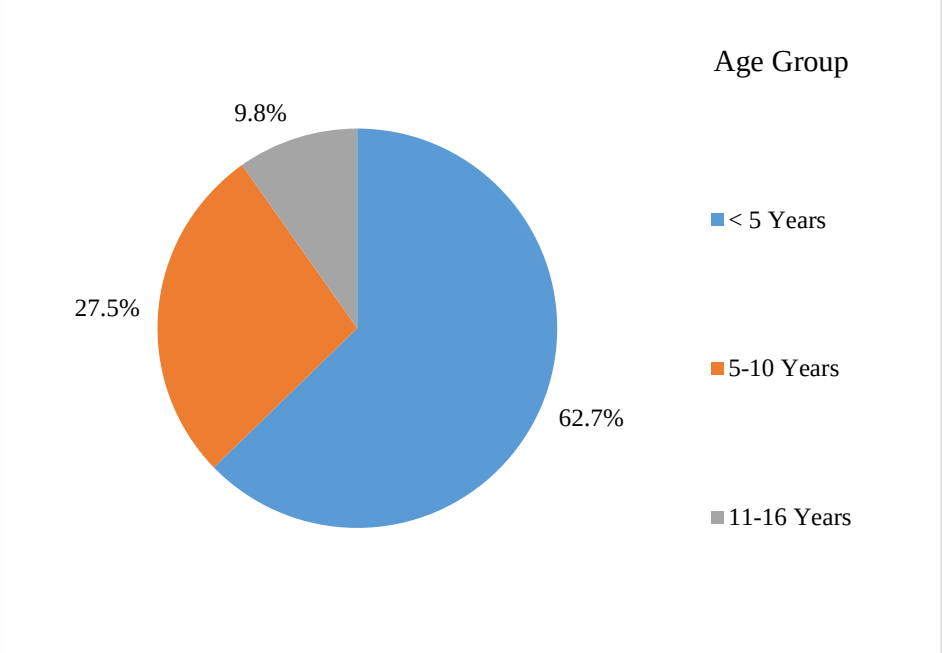


Figure 19: Pie chart of Diagnosis in the study population

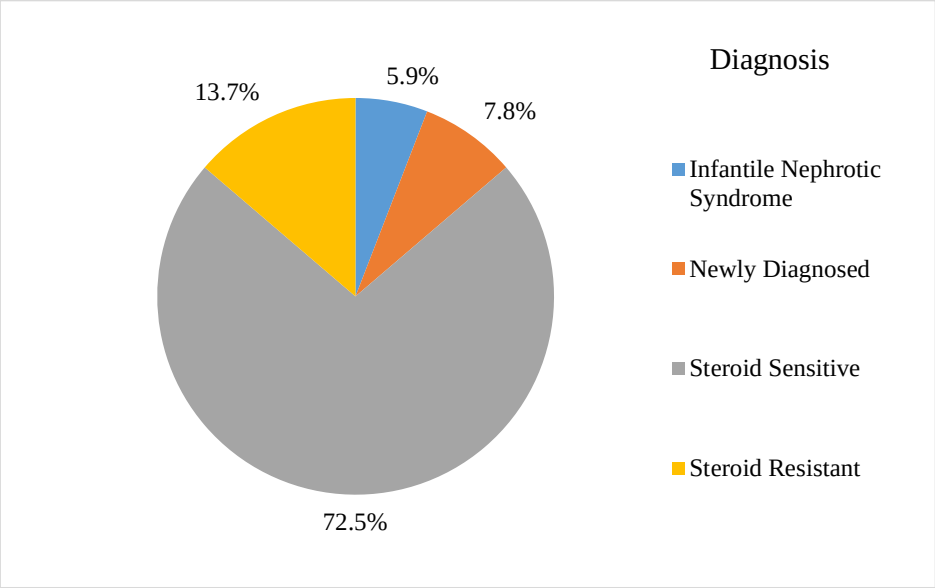


Figure 20: Bar chart of Frequent/Infrequent Relapses in the study population

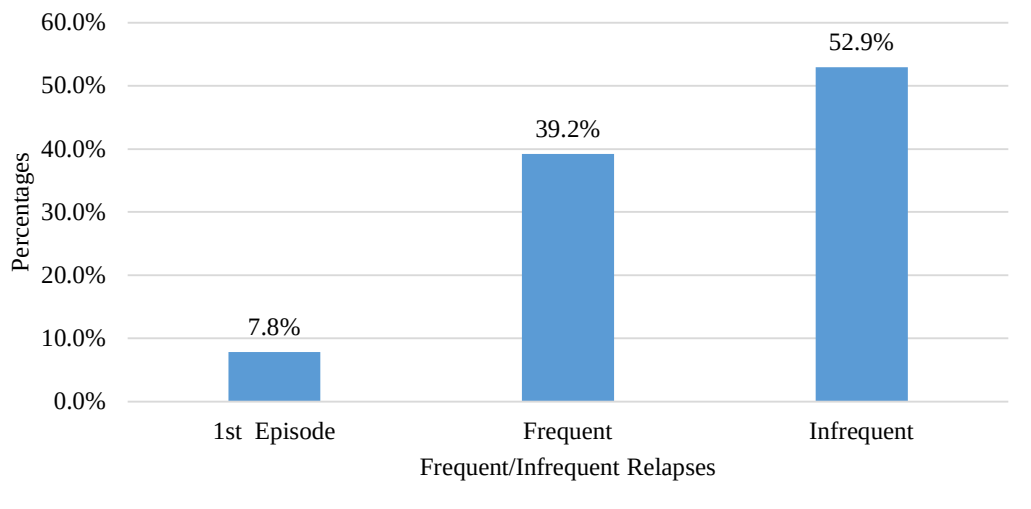


Table 3: Descriptive analysis of all the Parameters in the study population.

Parameter	Mean ± SD	Median	Minimum	Maximum	95% C.I	
					Lower	Upper
Sr Creatinine	0.35 ± 0.11	0.3	0.2	0.6	0.3	0.4
Sr Albumin	1.72 ± 0.47	1.7	0.7	3.4	1.6	1.9
Sr Cholesterol	413.12±123.11	406.0	203.0	705.0	378.5	447.7
Hb	9.19 ± 1.32	9.2	5.3	11.4	8.8	9.6
PCV	30.1 ± 5.02	29.6	20.6	41.3	28.7	31.5
RBC	4.21 ± 0.8	3.9	3.1	6.3	4.0	4.4
MCV	73.12 ± 12.33	72.6	34.8	98.3	69.7	76.6
MCH	22 ± 4.45	21.9	13.0	31.4	20.8	23.3
MCHC	29.57 ± 3.04	30.1	20.3	34.2	28.7	30.4
RDW	19.71 ± 5.24	19.0	12.7	31.1	18.2	21.2
Platelet(In Thousands)	510.45±210.59	488.0	110.0	1055.0	451.2	569.7
TLC	14103.53±7003.48	12400.0	6500.0	46000.0	12133.7	16073.2
Sr Iron	43.08 ± 27.18	38.0	12.0	142.0	35.4	50.7
TIBC	182.84 ± 126.95	142.0	36.0	502.0	147.1	218.6
Transferrin Saturation	31.75 ± 21.13	26.0	7.0	82.0	25.8	37.7
Sr Ferritin	221.78 ± 232.23	156.5	14.5	1206.0	156.5	287.1
Urinary Erythropoietin	9.61 ± 10.94	6.5	0.0	55.9	6.5	12.7

MEAN URINARY ERYTHROPOIETIN LEVELS FOUND IN OUR STUDY POPULATION IS **9.61 ± 10.94** mU/ml

\

Table 4: Comparison of Parameters between gender.

Parameter	Gender (Mean± SD)		(IST)
	Male (N=31)	Female (N=20)	P value
HB	9.27 ± 1.03	9.08 ± 1.7	0.616
PCV	29.85 ± 4.14	30.5 ± 6.25	0.660
RBC	4.21 ± 0.88	4.21 ± 0.66	0.992
MCV	74.3 ± 11.61	71.29 ± 13.45	0.399
MCH	22.53 ± 4.83	21.18 ± 3.74	0.295
MCHC	30.09 ± 3.08	28.78 ± 2.87	0.133
RDW	19.29 ± 5.59	20.35 ± 4.72	0.488
Sr Iron	37.39 ± 19.09	51.9 ± 35.11	0.062
TIBC	184.1 ± 124.71	180.9 ± 133.6	0.931
Transferrin Saturation	26.71 ± 16.92	39.55 ± 24.84	0.033
Sr Ferritin	229.05 ± 256.5	210.51 ± 194.47	0.784
Urinary Erythropoietin	9.22 ± 11.59	10.22 ± 10.12	0.752

Table 5: Comparison of Parameters between Age Groups.

Parameter	Age Group			ANOVA F value	P Value
	<5 Years(N=32)	5-10 Years(N=14)	11-16 Years(N=5)		
HB	9.28 ± 1.27	9.36 ± 1.25	8.16 ± 1.65	1.76	0.183
PCV	30.33 ± 5.25	30.64 ± 4.84	27.18 ± 3.7	0.96	0.392
RBC	4.31 ± 0.77	4.06 ± 0.92	4.03 ± 0.59	0.641	0.531
MCV	71.17 ± 12.51	77.23 ± 11.69	74.14 ± 12.29	1.21	0.308
MCH	21.22 ± 4.05	23.81 ± 4.66	21.96 ± 5.76	1.70	0.193
MCHC	29.11 ± 3	30.78 ± 2.72	29.2 ± 3.88	1.55	0.223
RDW	20.38 ± 5.04	18.21 ± 5.05	19.62 ± 7.2	0.83	0.442
Sr Iron	45.16 ± 29.74	40.79 ± 22.6	36.2 ± 24.63	0.295	0.746
TIBC	172.44 ± 132.79	214 ± 130.34	162.2 ± 69.24	0.585	0.561
Transferrin Saturation	36.28 ± 22.27	25.21 ± 19.45	21 ± 6.82	2.15	0.128
Sr Ferritin	177.92 ± 189.53	345.19 ± 311.23	156.92 ± 83.54	2.96	0.061
Urinary Erythropoietin	11.22 ± 12.25	7.91 ± 8.71	4.05 ± 4.33	1.17	0.319

ANOVA and multivariate tests showed no statistically significant difference for all hematological parameters and urinary erythropoietin levels between gender and different age groups.

Table 6: Mean of Urinary Erythropoietin between Different Diagnosis of NS.

Parameter	Diagnosis				ANOVA F value	P Value
	Infantile Nephrotic Syndrome (N=3)	Newly Diagnosed (N=4)	Steroid Sensitive (N=37)	Steroid Resistant (N=7)		
Urinary Erythropoietin	3.09 ± 1.08	9.4 ± 5.72	10.84 ± 12.2	6.00 ± 5.46	0.755	0.525

Levels of urinary erythropoietin were compared between the types of nephrotic syndrome and no statistically significant difference was found.

Table 7: Post Hoc (Pairwise) Comparison of mean of Urinary Erythropoietin between Diagnosis.

Pairs	Mean Difference	(LSD)P-value
Infantile Nephrotic Syndrome Vs Newly Diagnosed	-6.31	0.457
Infantile Nephrotic Syndrome Vs Steroid Sensitive	-7.75	0.247
Infantile Nephrotic Syndrome Vs Steroid Resistant	-2.90	0.704
Newly Diagnosed Vs Steroid Sensitive	-1.44	0.805
Newly Diagnosed Vs Steroid Resistant	3.41	0.624
Infantile Nephrotic Syndrome	7.75	0.247
Steroid Sensitive Vs Steroid Resistant	4.85	0.292

Table 8: Mean of Urinary Erythropoietin between Frequent/Infrequent relapses

Parameter	Frequent/Infrequent Relapses			ANOVA F value	P Value
	1st Episode **(N=4)**	Requent**(N=20)**	Infrequent**(N=27)**		
Urinary Erythropoietin	9.4 ± 5.72	11.47 ± 15.78	8.26 ± 6.33	0.480	0.620

There was also no statistically significant difference in urinary erythropoietin levels when compared between frequent and infrequent relapsers.

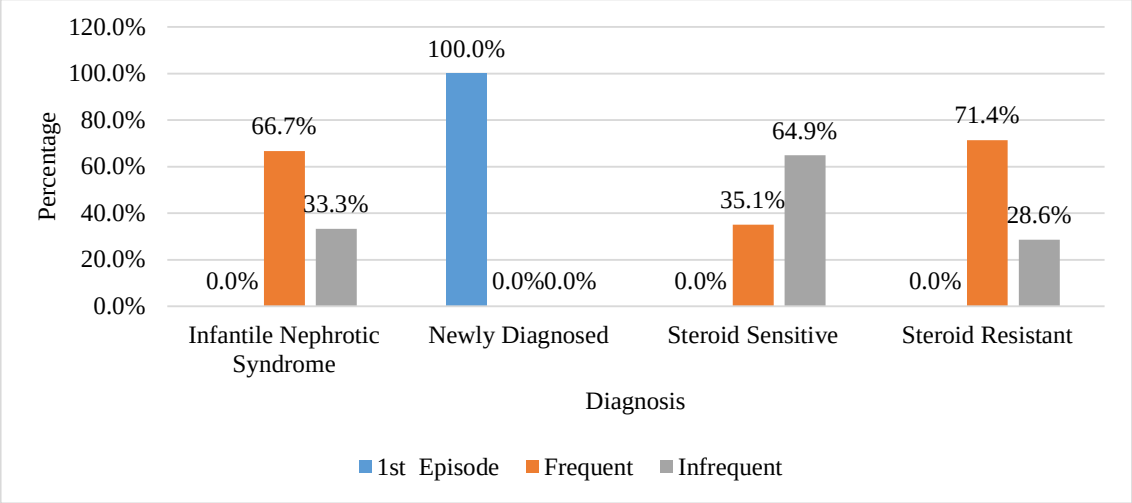
Table 9: Post Hoc (Pairwise) Comparison of mean of Urinary Erythropoietin between Frequent/Infrequent Relapses.

Pairs	Mean Difference	(Post Hoc LSD) P-value
1st Episode Vs Frequent	-2.06	0.735
1st Episode Vs Infrequent	1.14	0.848
Frequent Vs Infrequent	3.21	0.331

Table 10: Comparison of diagnosis

Frequent/Infrequent Relapses	Diagnosis			
	Infantile Nephrotic Syndrome (N=3)	**Newly Diagnosed (N=4)**	**Steroid Sensitive (N=37)**	**Steroid Resistant (N=7)**
1st Episode	0 (0%)	4 (100%)	0 (0%)	0 (0%)
Frequent	2 (66.67%)	0 (0%)	13 (35.14%)	5 (71.43%)
Infrequent	1 (33.33%)	0 (0%)	24 (64.86%)	2 (28.57%)

Figure 21: Cluster bar chart of comparison of diagnosis across frequent/infrequent relapses



In this study, 64.9% of children with steroid-sensitive nephrotic syndrome had frequent relapses and 35% had infrequent relapses. All cases enrolled in our study were relapsers

Table 11: Comparison of mean of Urinary Erythropoietin between Different diagnosis and their Frequent/Infrequent Relapses.

Parameter	Frequent/Infrequent Relapses (Mean± SD)		P value
Newly Diagnosed (N=4)	1st Episode (N=4)		*
Urinary Erythropoietin	9.4 ± 5.72		
Infantile Nephrotic Syndrome (N=3)	Frequent (N=2)	Infrequent (N=1)	
Urinary Erythropoietin	3.71 ± 0.25	1.87 ± 0	*
Steroid Sensitive (N=37)	Frequent (N=13)	Infrequent (N=24)	
Urinary Erythropoietin	14.1 ± 18.83	9.08 ± 6.23	0.238
Steroid Resistant (N=7)	Frequent (N=5)	Infrequent (N=2)	
Urinary Erythropoietin	7.74 ± 6.95	1.64 ± 2.16	0.300

**No statistical test applied due to the nature of the data (sample size is very small for groups)*

Urinary erythropoietin levels were also compared between steroid-sensitive nephrotic syndrome and steroid-resistant nephrotic syndrome along with frequent and infrequent relapsers as a subtype. There was no statistically significant difference found between the above-said groups.

Table 12: Comparison of mean of Parameter between Frequent/Infrequent Relapses – Iron Deficiency Anemia- N- 26

Parameter	Frequent/Infrequent Relapses (Mean± SD)			F value	P Value
	1St Episode(N=4)	Frequent (N=11)	Infrequent (N=11)		
Hb	9.38 ± 1.19	8.35 ± 1.2	9.26 ± 1.34	1.77	0.192
Sr Iron	29.25 ± 6.08	29.45 ± 10.56	33 ± 15.03	0.73	0.764
TIBC	110.75±80.21	132.36 ± 76.85	239.36±180.89	2.33	0.120
Transferrin Saturation	32.5 ± 11.12	30.55 ± 21.49	20.09 ± 14.73	1.25	0.306
Sr Ferritin	29.35 ± 7.1	210.38±188.57	215.96 ± 335.84	0.89	0.425
Urinary Erythropoietin	9.4 ± 5.72	9.17 ± 13.67	7.46 ± 6.07	0.10	0.906

Table 13: Comparison of mean of Parameter between Frequent/Infrequent Relapses- Non Nutritional Anemia- N- 24

Parameter	Frequent/Infrequent Relapses (Mean± Sd)		P value
	Frequent (N=8)	Infrequent (N=16)	
Hb	9.55 ± 0.7	9.56 ± 1.53	0.991
Sr Iron	54.75 ± 21.34	58.69 ± 37.07	0.785
TIBC	179.75 ± 90.81	199.44 ± 130.05	0.706
Transferrin Saturation	36 ± 19.6	39.63 ± 24.96	0.724
Sr Ferritin	229.4 ± 192.85	290.75 ± 213.54	0.501
Urinary Erythropoietin	15.62 ± 19.28	8.82 ± 6.64	0.210

Table 14: Comparison of mean of Parameter between Different Diagnostic types along with Frequent/Infrequent Relapses with Iron Deficiency Anemia- N- 26

Diagnosis	**Frequent/Infrequent Relapses (Mean± SD)**		**P value**
Newly Diagnosed (N=4)	**1st Episode (N=4)**		
Hb	9.38 ± 1.19		
Sr Iron	29.25 ± 6.08		
TIBC	110.75 ± 80.21		
Transferrin Saturation	32.5 ± 11.12		
Sr Ferritin	29.35 ± 7.1		
Urinary Erythropoietin	9.4 ± 5.72		
Infantile Nephrotic Syndrome (N=2)	**Frequent (N=1)**	**Infrequent (N=1)**	
Hb	6.9 ± 0	9.2 ± 0	*
Sr Iron	35 ± 0	23 ± 0	*
TIBC	87 ± 0	95 ± 0	*
Transferrin Saturation	40 ± 0	24 ± 0	*
Sr Ferritin	234.5 ± 0	40 ± 0	*
Urinary Erythropoietin	3.88 ± 0	1.87 ± 0	*
Steroid Sensitive (N=16)	**Frequent (N=7)**	**Infrequent (N=9)**	
Hb	8.46 ± 1.35	9.3 ± 1.49	0.263
Sr Iron	34 ± 8.29	36.44 ± 14.2	0.693
TIBC	134.57 ± 89.79	269.33 ± 187.94	0.104
Transferrin Saturation	36.14 ± 23.72	20.67 ± 16.09	0.142
Sr Ferritin	174.77± 206.48	238.41 ± 369.41	0.690
Urinary Erythropoietin	9.54 ± 16.88	8.9 ± 5.76	0.916
Steroid Resistant (N=4)	**Frequent (N=3)**	**Infrequent (N=1)**	
Hb	8.6 ± 0.72	9 ± 0	*
Sr Iron	17 ± 5.57	12 ± 0	*

TIBC	142.33 ± 64.36	114 ± 0	*
Transferrin Saturation	14.33 ± 8.5	11 ± 0	*
Sr Ferritin	285.43 ± 191.58	189.8 ± 0	*
Urinary Erythropoietin	10.08 ± 7.99	0.12 ± 0	*

No statistical test applied due to nature of the data (sample size is very small for groups)

Table 15: Comparison of mean of Parameter between Different Diagnostic Anemia along with Frequent/Infrequent Relapses with Non-Nutritional Anemia N-24

Diagnosis	**Frequent/Infrequent Relapses (Mean± SD)**		**P value**
Steroid Sensitive (N=21)	**Frequent (N=6)**	**Infrequent (N=15)**	
Hb	9.38 ± 0.66	9.48 ± 1.55	0.886
Sr Iron	62.83 ± 17.36	60.2 ± 37.85	0.873
TIBC	215.17 ± 72.82	199.33 ± 134.62	0.790
Transferrin Saturation	31.33 ± 12.77	40.67 ± 25.47	0.408
Sr Ferritin	253.2 ± 222.14	267.73 ± 199.44	0.885
Urinary Erythropoietin	19.42 ± 21.12	9.19 ± 6.69	0.101
Steroid Resistant (N=3)	**Frequent (N=2)**	**Infrequent (N=1)**	
Hb	10.05 ± 0.78	10.7 ± 0	*
Sr Iron	30.5 ± 10.61	36 ± 0	*
TIBC	73.5 ± 33.23	201 ± 0	*
Transferrin Saturation	50 ± 36.77	24 ± 0	*
Sr Ferritin	158 ± 2.12	636 ± 0	*
Urinary Erythropoietin	4.23 ± 4.97	3.17 ± 0	*

No statistical test applied due to nature of the data (sample size is very small for groups)

Table 16: Descriptive analysis of bp normal/hypertensive

Bp Normal/Hypertensive	n	%
Hypertensive	24	47.06%
Normal	27	52.94%

Hypertension was found in 47% of subjects in the study.

Figure 22: Bar chart of Bp Normal/Hypertensive in the study population

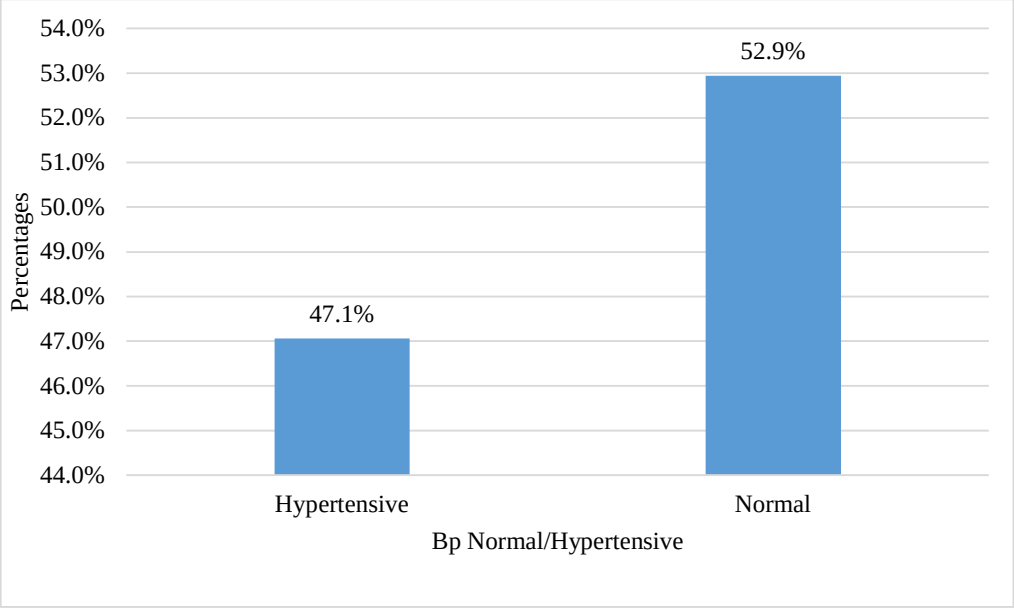


Table 17: Comparison of mean of urinary erythropoietin between bp normal/hypertensive

Parameter	Bp Normal/Hypertensive (Mean± SD)		P value
	Normal (N=27)	Hypertensive (N=24)	
Urinary Erythropoietin	7.17 ± 5.72	12.35 ± 14.44	0.092

The urinary erythropoietin levels between normal and hypertensive patients were compared but there was no statistical significance was found.

Table 18: Descriptive analysis of biopsy report if done in the study population

Biopsy Report If Done	n	%
Crescentic Glomerulonephritis	1	1.96%
FSGS	9	17.65%
IGA Nephropathy	1	1.96%
MCD	9	17.65%
Not Done	31	60.78%

Out of the enrolled cases only 20 patients underwent in the recent past. Out of these biopsied children, 9 were of MCD and FSGS each. Other etiologies were found in the remaining cases.

Table 19: Comparison of mean of urinary erythropoietin between biopsy report if done

Parameter	Biopsy Report If Done (Mean± SD)		P value
	FSGS (N=9)	MCD (N=9)	
Urinary Erythropoietin	14.18 ± 18.81	12.01 ± 14.24	0.787

Urinary erythropoietin levels were compared between the two common histological patterns found in our study, and no statistical significance was found.

Table 20: Comparison of bp normal/hypertensive between biopsy report if done

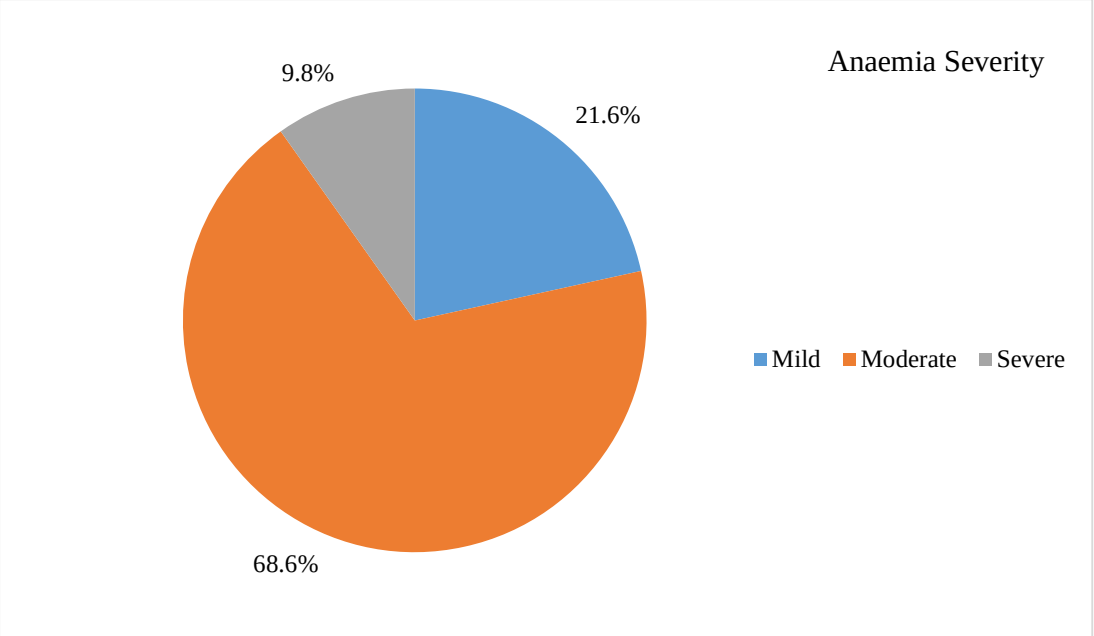
Bp Normal/Hypertensive	Biopsy Report If Done		Chi-square	Fisher's exact P value
	FSGS (N=9)	MCD (N=9)		
Normal	2 (22.22%)	5 (55.56%)	2.104	0.335
Hypertensive	7 (77.78%)	4 (44.44%)		

Out of 9 cases of MCD 5 patients had normal bp and 4 patients were hypertensive. Whereas out of 9 cases of FSGS 2 patients has normal bp and 7 patients were hypertensive.

Table 21: Descriptive analysis of anemia severity

Anemia Severity	n	%
Mild	11	21.57%
Moderate	35	68.63%
Severe	5	9.80%

Figure 23: Pie chart of Anemia Severity in the study population



About 69% of children in this study have moderate anemia, whereas 21.6% of children have mild anemia and only 9.8% of children have severe anemia.

Table 22: Descriptive analysis of the etiology of anemia

Etiology Of Anemia	**n**	**%**
Iron Deficiency Anemia	26	50.98%
Iron Deficiency Anemia and Vitamin B12 Deficiency	1	1.96%
Non-Nutritional Anemia	24	47.06%

Figure 24: Pie chart of Etiology of Anemia in the study population

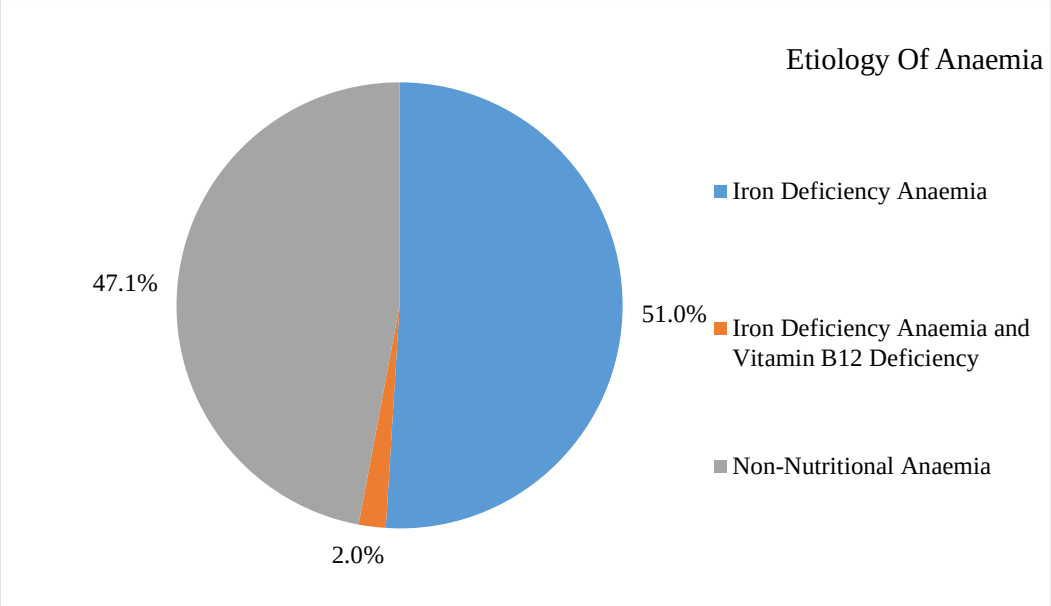


Table 23: Comparison of mean of urinary erythropoietin between etiology of anemia

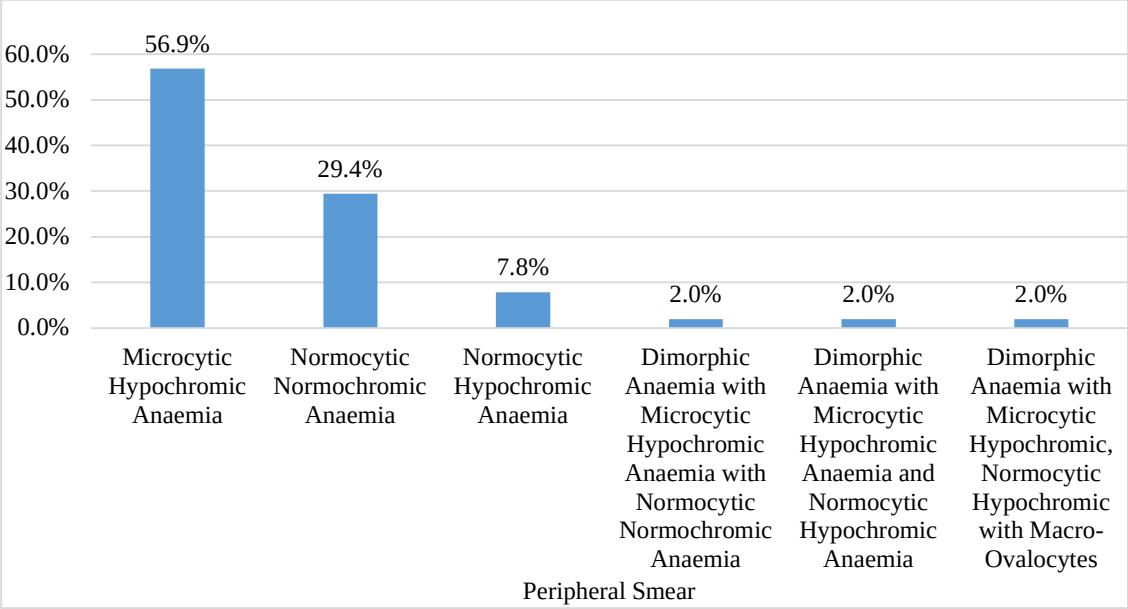
Parameter	Etiology Of Anemia (Mean± SD)		P value
	Iron Deficiency Anemia (N=26)	Non-Nutritional Anemia (N=24)	
Urinary Erythropoietin	8.48 ± 9.71	11.08 ± 12.35	0.410

In this study, 51% of children had iron deficiency anemia followed by 47% of children who had non-nutritional anemia with one child having iron deficiency anemia with vitamin b12 deficiency. No statistical significance was found between the two etiological types of anemia.

Table 24: Descriptive analysis of peripheral smear in the study population.

Peripheral Smear	n	%
Microcytic Hypochromic Anemia	29	56.86%
Normocytic Normochromic Anemia	15	29.41%
Normocytic Hypochromic Anemia	4	7.84%
Dimorphic Anemia with Microcytic Hypochromic Anemia with Normocytic Normochromic Anemia	1	1.96%
Dimorphic Anemia with Microcytic Hypochromic Anemia and Normocytic Hypochromic Anemia	1	1.96%
Dimorphic Anemia with Microcytic Hypochromic, Normocytic Hypochromic with Macro-Ovalocytes	1	1.96%

Figure 25: Bar chart of Bp Normal/Hypertensive in the study population



Most of the children had microcytic hypochromic anemia-57% followed by normocytic hypochromic anemia-29% and normocytic hypochromic anemia-8%.

Microcytic hypochromic anemia in our study was depicted as the most common morphological type in all the age groups

Table 25: Comparison of etiology of anemia between peripheral smear.

Etiology Of Anemia	Peripheral Smear		Chi-square	P value
	Microcytic Hypochromic Anaemia (N=29)	Normocytic Normochromic Anaemia (N=15)		
Iron Deficiency Anemia	21 (72.41%)	1 (6.67%)	17.094	<0.001
Non-Nutritional Anemia	8 (27.59%)	14 (93.33%)		

IDA was documented in close to 72.4% of children with microcytic hypochromic anemia.

Table 26: Comparison of mean of Parameter between peripheral smear.

Parameter	PERIPHERAL SMEAR (Mean± SD)		P value
	Microcytic Hypochromic Anemia (N=29)	Normocytic Normochromic Anemia (N=15)	
HB	8.79 ± 1.43	9.73 ± 0.77	0.022
MCV	66.43 ± 9.14	85.14 ± 6.67	<0.001
MCH	19.6 ± 3.52	26.51 ± 1.77	<0.001
MCHC	28.78 ± 3.34	31 ± 1.47	0.019
RDW	21.93 ± 4.88	15.15 ± 1.49	<0.001
Urinary Erythropoietin	8.78 ± 9.57	11.36 ± 14.58	0.483

The mean MCV, MCH, and RDW showed a significant difference between normocytic normochromic and microcytic hypochromic types. The mean MCV and MCH were significantly lower in the microcytic group when compared with the normocytic group. The mean RDW is higher in the microcytic group in comparison with the normocytic group. No statistical significance in urinary erythropoietin levels was found between different morphological types of anemia.

Table 27: Comparison of mean of Parameter between peripheral smear

Parameter	PERIPHERAL SMEAR (Mean± SD)		P value
	Microcytic Hypochromic Anemia (N=29)	Normocytic Normochromic Anemia (N=15)	
Sr Iron	39.72 ± 16.87	44.4 ± 20.79	0.426
Sr Ferritin	139.18 ± 126.32	277.6 ± 226.41	0.012
TIBC	182.34 ± 135.04	191.53 ± 139.02	0.833
Transferrin Saturation	29.93 ± 17.51	35.33 ± 25.36	0.411

Iron studies were compared with different morphological types of anemia but there was no statistical significance found between the two groups.

Table 28: Comparison of mean of Parameter between etiology of anemia.

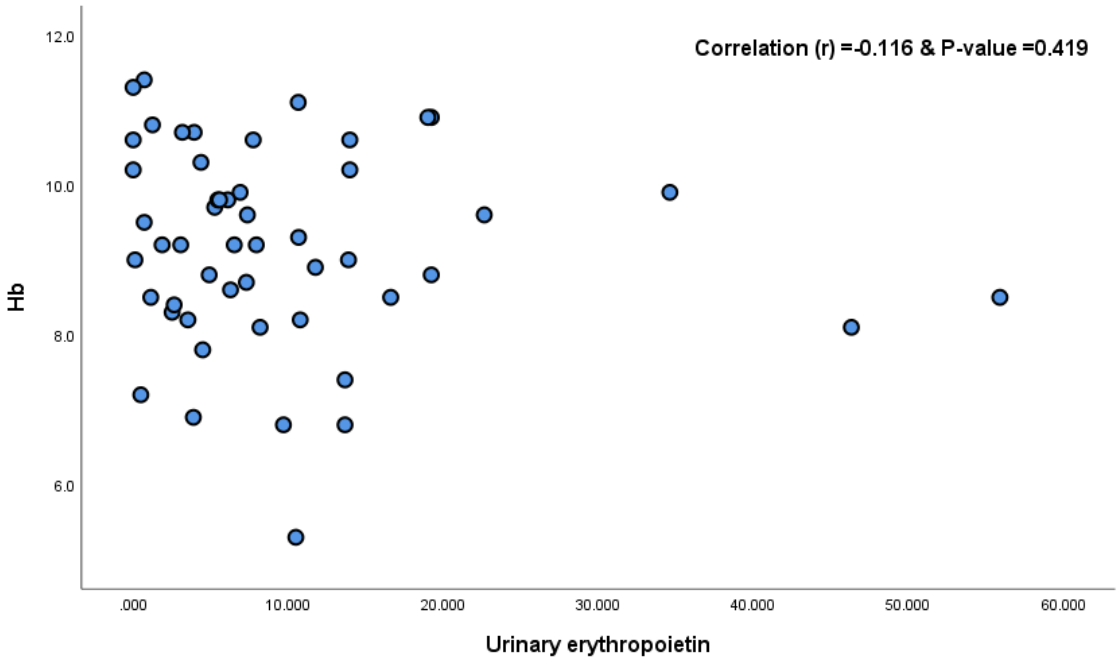
Parameter	Etiology Of Anemia (Mean± SD)		P value
	Iron Deficiency Anemia (N=26)	Non-Nutritional Anemia (N=24)	
HB	8.9 ± 1.3	9.55 ± 1.3	0.079
MCV	68.32 ± 9.49	79.1 ± 12.4	0.001
MCH	21.01 ± 3.41	23.38 ± 4.99	0.054
MCHC	29.94 ± 2.81	29.3 ± 3.31	0.462
RDW	20.67 ± 4.99	18.39 ± 5.28	0.124
Urinary Erythropoietin	8.48 ± 9.71	11.08 ± 12.35	0.410
Sr Iron	30.92 ± 11.95	57.38 ± 32.22	<0.001
Sr Ferritin	184.89 ± 252.84	270.3 ± 204.77	0.198
TIBC	174.31 ± 139.66	192.88 ± 116.75	0.614
Transferrin Saturation	26.42 ± 17.82	38.42 ± 22.94	0.043

Different hematological parameters were compared between two etiological types of anemia along with iron studies. Mean MCV and serum iron levels were showing statistically significant data.

Table 29: Correlation between Hb and list of parameters

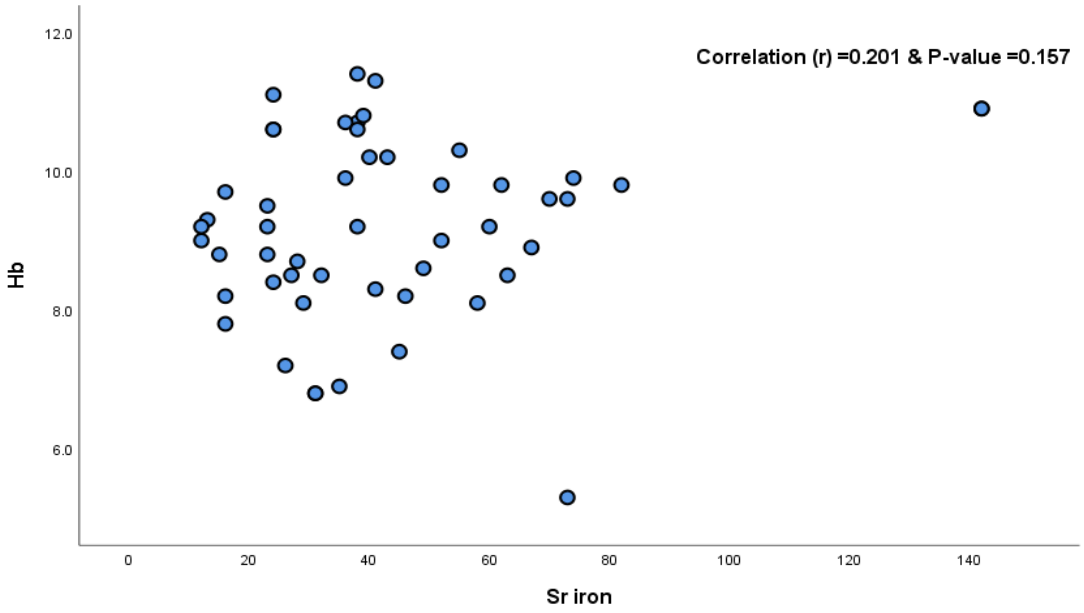
Parameters	Pearson's correlation r Value	P Value
Urinary erythropoietin	-0.116	0.419
Sr Iron	0.201	0.157
TIBC	0.045	0.755
Sr ferritin	-0.007	0.963

Figure 26: Scatterplot for Correlation Between Hb with Urinary erythropoietin



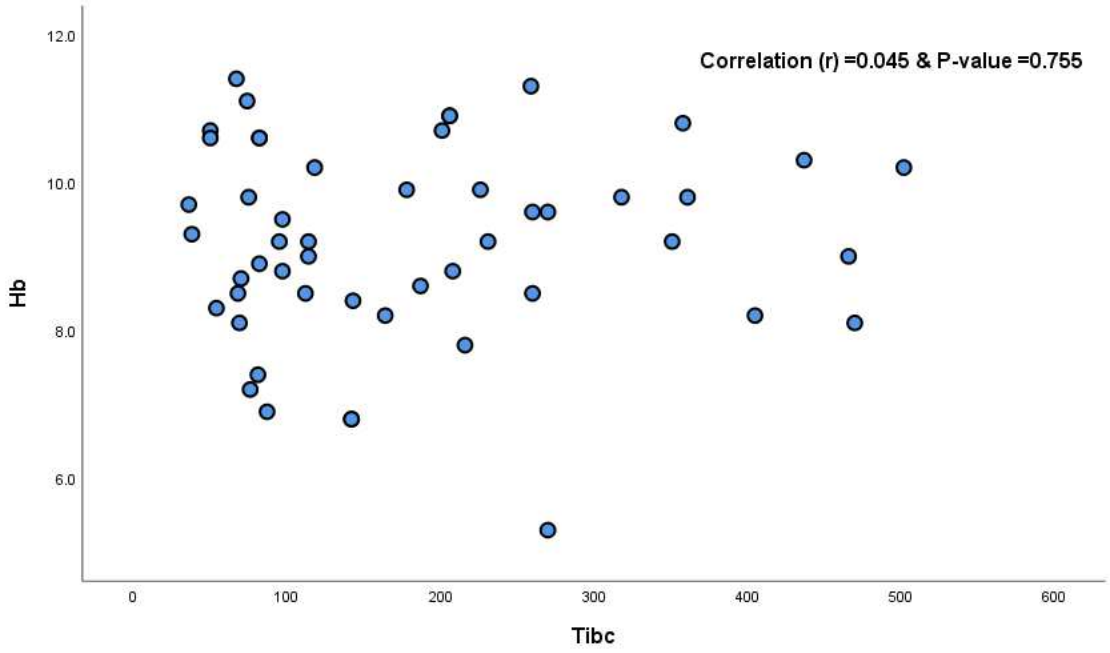
There is negative correlation between urinary erythropoietin levels and HB

Figure 27: Scatterplot for Correlation Between Hb with Sr Iron



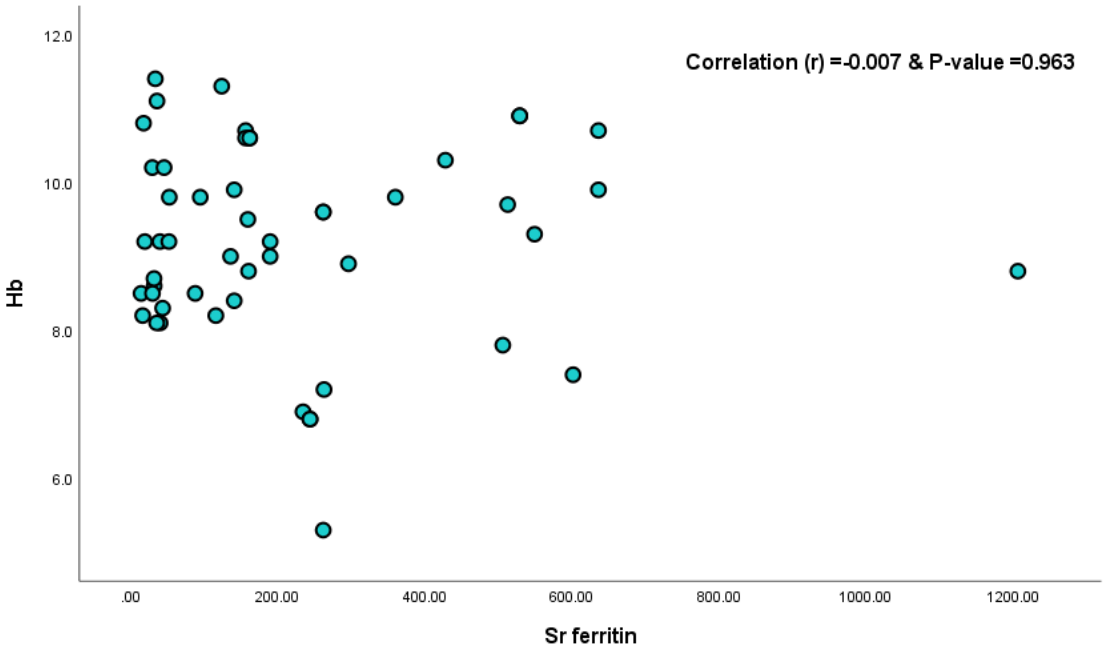
There is a positive correlation between serum Iron and HB levels.

Figure 28: Scatterplot for Correlation Between Hb with TIBC



There is a positive correlation between Total iron binding capacity and HB levels.

Figure 29: Scatterplot for Correlation Between Hb with Sr ferritin

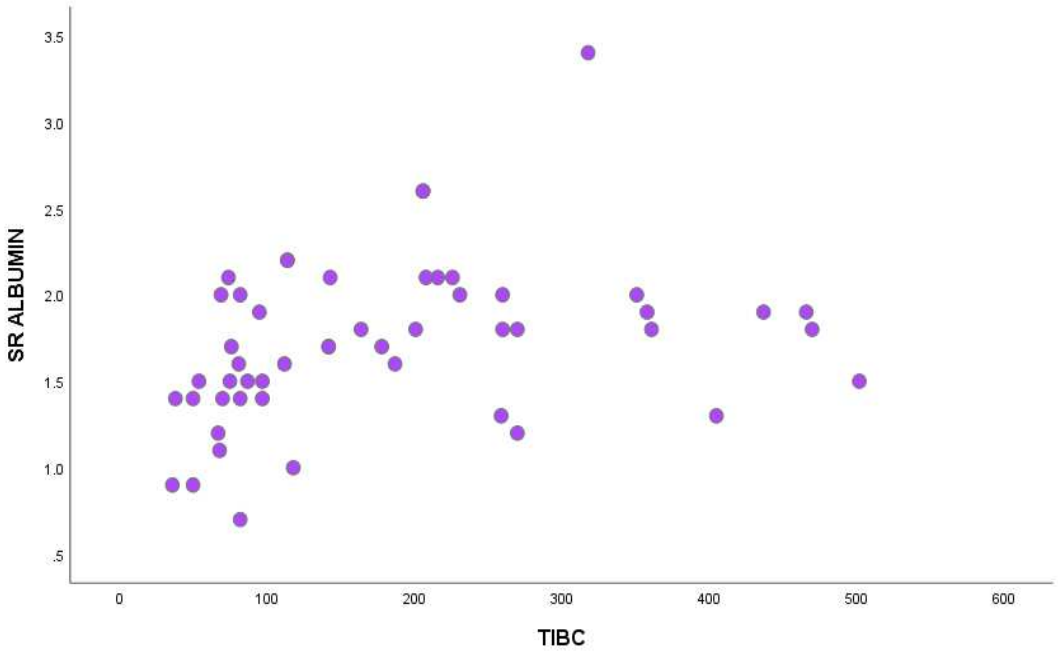


There is slight negative correlation between serum Ferritin levels and HB levels.

Table 30: Correlation between Sr Albumin and list of parameters

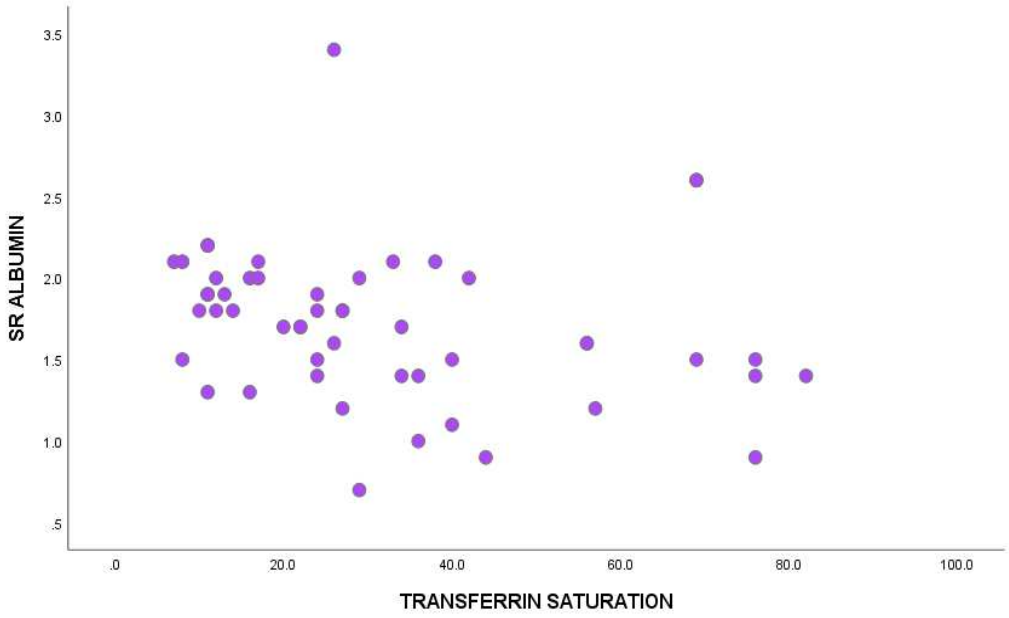
Parameters	Pearson's correlation r Value	P Value
TIBC	0.314	0.025
Transferrin saturation	-0.221	0.120
Sr Ferritin	0.215	0.129

Figure 30: Scatterplot for Correlation Between Sr Albumin with TIBC



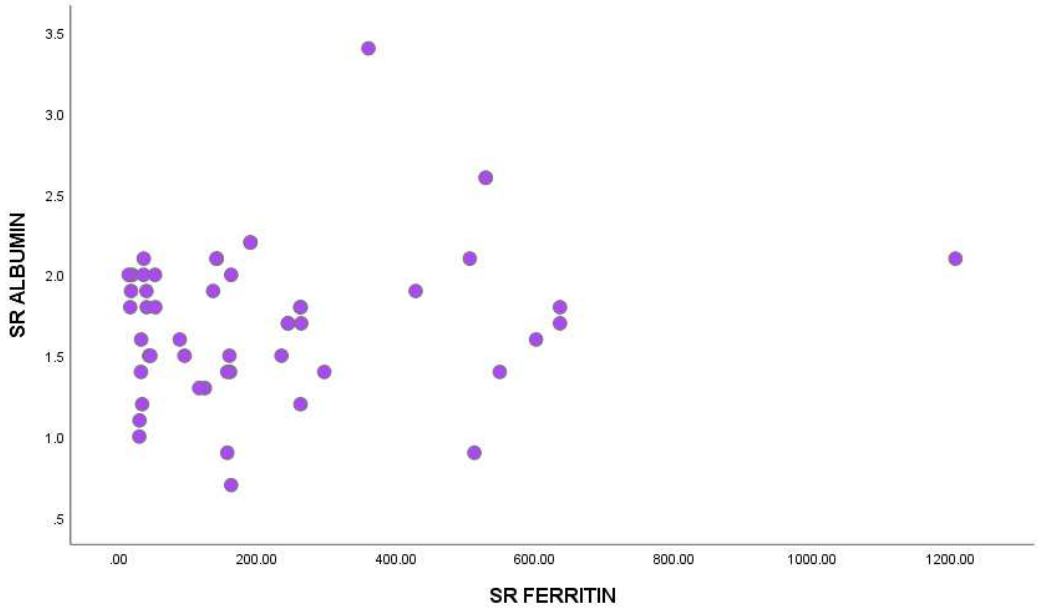
Positive correlation between serum albumin and TIBC

Figure 31: Scatterplot for Correlation Between Sr Albumin with Transferrin saturation



Negative correlation between serum albumin and transferrin saturation.

Figure 32: Scatterplot for Correlation Between Sr Albumin with Sr Ferritin



DISCUSSION

Anemia is a significant cause of mortality and morbidity in children and is a global health problem, especially in developing countries. Anemia is one of the many complications seen in patients with persistent nephrotic syndrome. The pathogenesis of anemia in nephrotic syndrome is not understood and it is complex It may be due to excessive urinary losses of iron, transferrin, erythropoietin, transcobalamin, and/or metal. This leads to a deficiency of substrates necessary for effective erythropoiesis. Requiring supplementation in order to correct the anemia. Supplementation of iron and erythropoietin alone often does not lead to correction of the anemia, suggesting other possible mechanisms which need further investigation. The treatment of anemia in patients with nephrotic syndrome is challenging and knowledge of all possible underlying pathology mechanisms is necessary for proper evaluation and successful treatment.

In our study, 170 nephrotic syndrome children were screened based on inclusion and exclusion criteria, and 51 children were included in the study fitting the criteria. All 51 children were cases of nephrotic syndrome children with anemia.

All the patients enrolled in the study were enrolled based on the inclusion criteria that is HB lower than the range of values occurring in healthy persons which vary substantially with age and sex provided that the child has normal kidney function tests. All the enrolled children were subjected to complete hemograms, and iron studies and urine samples were collected on the spot and stored at our research center, and processed for urinary erythropoietin levels. All the obtained values were collected and analyzed.

We analyzed 51 patients with nephrotic syndrome anemia, and the age distribution of cases ranged from 1 to 18 years. The mean age of presentation was 5.55 ± 3.86 years. The mean age was similar to that reported in other studies. In a study by sahana[62] mean age at presentation was 7.4 years. Pandya and Mehta[63] reported the mean age as 4.08 years and Kiran and Kumar[64] reported the mean age at presentation as 6.7 years. A study in Auckland[65] observed the mean age at diagnosis as 5.4 years. A single-center study done in Iran[66] reported the mean age of presentation as 4.87 years. A similar conclusion was drawn by Chahar OP et al[67] and Shastri NG et al[68].

The most common age group was 1-5years counting for 62.75% followed by 6-10years counting for 27.45%, and 11-18years counting 9.8% in our study. In a study by sahana[62], 65% of subjects belonged to 6-12years of age followed by 1-5 years[31%]. In another Indian study by Jeetendra[69], 68% of the cases belonged to the 6-10years age group followed by the 1-5years age group which accounted for 24% of the nephrotic syndrome patients. In a study done by Maiti et al[72], it was found that 47.3% of patients belonged to the age group of 13-60 months followed by 31.1% belonging to infants and 21.6% of children were of the age group above 5 years.

There were 31 males and 20 females with an M: F ratio of 1.55:1. In a study by sahana[62], found that 76% of the subjects were males while 24% were females with an M: F ratio of 3.27:1 suggesting the male preponderance. Pandya and Mehta[63][1.18:1] and Kiran and Kumar[64] also observed male predominance in their studies. In another Indian study by Jeetendra[69] 66% of the cases were males while 34% of cases were female with an M: F ratio of 1.94:1.

In this study, steroid-sensitive nephrotic syndrome accounted for a maximum number of cases -72.5%, followed by steroid-resistant cases, newly diagnosed nephrotic syndrome, and infantile nephrotic syndrome with 13.75%, 7.8%, and 5.8% respectively. Our results are in concordance with the prevalence of steroid-sensitive nephrotic syndrome in the literature and other studies. Pandya and Mehta documented steroid sensitivity of 69% and the study conducted by Safaei and Maleknajed[66] demonstrated steroid sensitivity at 66% and steroid resistance at 20.5%.

We also evaluated the patients based on their relapses. It was found that out of 51 patients enrolled 20 patients were frequent relapsers, 27 patients were infrequent relapsers and 4 patients were presenting for the first time.

64.9% of children with steroid-sensitive nephrotic syndrome had frequent relapses and 35% had infrequent relapses. All cases enrolled in our study were relapses. But in a study done by Anochie et al[78], 93.8% of children with steroid-sensitive nephrotic syndrome relapsed, while 25% had frequent relapses. While a study reported in ghana[81] 56.3% of children with steroid-sensitive nephrotic syndrome relapsed with 12.5% having frequent relapses. In this study, 13% of children had steroid resistance while in anochie et al[78] steroid resistance was found in 20% of cases whereas in a study in ghana[81] steroid resistance was observed in 25% of cases.

The mean urinary erythropoietin levels found in our study were 9.61+/-10.94. This finding was consistent with other studies As per recent studies it was found that there was no amount of urinary erythropoietin levels found in children without nephrotic syndrome and in children with nephrotic syndrome, it was found to be >10-30mU/ml[10]. There was no statistically significant difference in urinary erythropoietin levels found between gender and different age groups. Levels of urinary

erythropoietin were also compared between the types of nephrotic syndrome and no statistically significant difference was found. There was also no statistically significant difference in urinary erythropoietin levels when compared between frequent and infrequent relapsers.

Out of the enrolled cases only 20 patients underwent in the recent past. Out of these biopsied children, 9 were of MCD and FSGS each. Other etiologies were found in the remaining cases. This study in comparison with other studies showed variable histological patterns. Generally, the most common histological pattern found in nephrotic syndrome in children is MCD type whereas in adults it was FSGS type. As all the patients enrolled in our study did not undergo biopsy the prevalence of different histological variants could not be analyzed. Urinary erythropoietin levels were compared between the two common histological patterns found in our study, and no statistical significance was found.

In the total enrolled cases, hypertension was found in 47% of patients at the time of enrolment. Out of 9 cases of MCD 5 patients had normal bp and 4 patients were hypertensive. Whereas out of 9 cases of FSGS 2 patients has normal bp and 7 patients were hypertensive. This data analysis was significant and in correlation with other studies like struss et al[80] where in MCD type 20.7% of cases had hypertension and in other histological types 25.7% were hypertensive. According to nelson, hypertension is present in about 10% of cases of MCD type while in cases of other significant glomerular lesions hypertension was found in 20-35%. The urinary erythropoietin levels between normal and hypertensive patients were compared but there was no statistical significance found.

ANOVA and multivariate tests showed no significant difference for all hematological parameters between gender and different age groups. The children with anemia were characterized by severity and morphology.

The prevalence of anemia was found to be 30% of this study population. Feinstein and his group showed a prevalence of 59% in their study population, with most of the patients with anemia having steroid-resistant nephrotic syndrome[9]. Franca November and his group showed the prevalence at their study center, where approximately 28% of the population with nephrotic syndrome had anemia during the course of their disease[7]. In a study by Anirban et al[77] anemia was found in 65% of patients while in Sahana et al[62] anemia was found in 74% of patients and in anochie et al[78] 50% of cases presented with anemia. In a study done by Jeetendra et al[69], anemia was found in 60% of cases. Maiti et al[72] did a study based on the prevalence of anemia among children in a teaching hospital, where anemia was found in 42.5%, while in a similar study done by saba et al[70] anemia was found in 72.8% along with recent study done by Reddy et al[79] showed the prevalence of anemia in 43.7% but the last three studies are from the general population.

About 69% of children in this study had moderate anemia, whereas 21.6% of children had mild anemia and only 9.8% of children had severe anemia. This study's findings were similar to that reported by Saba et al[70] and Kanchana et al[71]. In a study done by maiti et al[72] 73.3% of patients had anemia of moderate severity followed by 21.3% of moderate severity and 5.4% of mild degree of anemia.

The mean hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration, and mean red cell distribution width were 9.19 ± 1.32 g/dL, 73.12 ± 12.33 (fL), 29.57 ± 3.04, and 19.71 ± 5.24 (%), respectively. Our

findings are comparable to the study by Saba et al[70] where the mean Hb and MCV were 8.5 g/dL and 75.08 fL, respectively, and Sanghavi J et al[73] where the mean Hb and MCV were 6.54 ± 1.63g/dL and 59.2 ± 5.8 fL, respectively. These findings were similar to that found in a study done by Maiti et al[72].

Anemia was further classified based on diagnostic and morphological type. Peripheral smear was done in all the patients enrolled in the study. Most of the children had microcytic hypochromic anemia-57% followed by normocytic hypochromic anemia-29% and normocytic hypochromic anemia-8%. Microcytic hypochromic anemia in our study was depicted as the most common morphological type in all the age groups. The mean MCV, MCH, and RDW showed a significant difference between normocytic normochromic and microcytic hypochromic types. The mean MCV and MCH were significantly lower in the microcytic group when compared with the normocytic group. The mean RDW is higher in the microcytic group in comparison with the normocytic group. There was no statistically significant difference in urinary erythropoietin levels found in the most common morphological types of anemia. Iron studies were compared with different morphological types of anemia but there was no statistical significance found between the two groups.There were also 3 patients with a dimorphic picture with one patient having macro-ovalocytes in the peripheral smear, which was attributable to vitamin B12 deficiency.

The diagnosis of anemia is simply based on the WHO definition as a reduction of the hemoglobin concentration or red blood cell (RBC) volume below the range of values for age and sex. However, confirming that iron deficiency is the origin of anemia is not always easy. Sometimes the simple blood cell count strongly suggests this origin, the typical pattern being microcytosis, hypochromia (perhaps the most

important, even more than the microcytosis), and the elevation of red cells distribution width. However, up to 40% of pure iron deficiency anemia cases are normocytic. Therefore, a normal mean corpuscular volume does not exclude iron deficiency from being the cause of the anemia. Moreover, the presence of microcytosis does not necessarily imply iron deficiency and can be produced by other anemias like chronic processes like nephrotic syndrome. Serum ferritin in the absence of inflammation reflects total iron deposits. Thus low serum ferritin (<30ng/ml) unequivocally means iron deficiency, whether accompanied by anemia or not. However, as serum ferritin is an acute phase reactant, normal or even elevated ferritinemia does not exclude the presence of iron deficiency. Thus, in the presence of an inflammatory process, iron deficiency could exist even with levels of ferritin up to 100ng/ml. Another parameter of normal iron metabolism, especially useful when the determination of ferritin is equivocal, is the transferrin saturation index. This shows the percentage of transferrin that transports iron and thus decreases (<16%) implying iron deficiency anemia is either absolute or functional.

All the children enrolled in the study were further tested for iron deficiency anemia. Serum iron, serum ferritin, and total iron binding capacity were determined in all the patients. Children were diagnosed to have IDA based on serum iron, ferritin, TIBC, and transferrin saturation (at least 2 of 4 positive tests). In this study, 51% of children had iron deficiency anemia followed by 47% of children who had non-nutritional anemia with one child having iron deficiency anemia with vitamin b12 deficiency. This finding in our study was similar in accordance with the WHO global database on anemia[74] and Sanghvi j et al[73] study where 61% had IDA. IDA was documented in close to 72.4% of children with microcytic hypochromic anemia. Different hematological parameters were compared between two etiological types of

anemia along with iron studies. Mean MCV and serum iron levels were showing statistically significant data. Other iron studies parameters were not statistically significantly different, mainly due to significant losses of transferrin and deviating of parameters from normal iron deficiency anemia. Mean erythropoietin levels were also compared between iron deficiency anemia and non-nutritional anemia but there was no statistically significant difference found.

The mean levels of serum iron were found to be 43.08 ± 27.18 with a median value of 38mcg/dl, ferritin was found to be 221.78 ± 232.23 with a median value of 156ng/ml, TIBC was found to be 182.84 ± 126.95 with a median value of 142, and transferrin saturation was found to be 31.75 ± 21.13 with a median value of 26%. Ideally in iron deficiency anemia low serum iron, low serum ferritin, an increase in total iron binding capacity, and low transferrin saturation can be found. But in our study, there was significant high transferrin saturation along with high serum ferritin levels. This was explained in a study by Le Van An et al[75], where a study was done on the concentrations of iron, transferrin, and ferritin in serum, and the relationship with serum albumin in nephrotic syndrome patients was investigated. It was concluded in this study that the average concentration of serum iron and transferrin is lower than normal, especially transferrin concentrations, leading to decreased total iron binding capacity and increased transferrin saturation. This study also found that serum ferritin concentrations were often elevated in nephrotic syndrome patients[76]. These findings were also concluded in our study. There was a negative correlation between hemoglobin and serum ferritin, which was indicative of elevated ferritin concentrations in nephrotic syndrome.

Hypotransferrinemia in the nephrotic syndrome may induce low plasma iron concentrations that eventually result in microcytic anemia, although the urinary loss of erythropoietin may also be significant as a cause of anemia in these patients. The pathophysiologic mechanism responsible for the decreased plasma transferrin concentration in the nephrotic syndrome is poorly understood. Transferrin synthesis may be modulated as a component of the acute phase cascade or a consequence of iron deficiency. Furthermore, malnutrition causes downregulation of hepatic transferrin gene expression, resulting in decreased synthesis. Inflammation, iron deficiency, and malnutrition are often found in nephrotic syndrome leading to decreased concentration of transferrin and decreased total iron binding capacity.

In a study done by Monique et al[37] it was found that there was no significant relationship between the levels of serum iron and c reactive protein values with serum transferrin levels when compared individually. It was indicated that it was very unlikely that either serum iron or inflammation are the main causes for increased production of transferrin. But in this study, it was found that there was a significant relationship between serum albumin levels along with transferrin synthesis indicating that synthesis of transferrin is none other than a general response in a nephrotic syndrome where there is a general increase in the production of proteins by the liver. We came to the same conclusion as there was a positive correlation between serum albumin and TIBC. There was also a negative correlation between transferrin saturation and serum albumin.

In a study done by Sreekanth et al[82]**,** it was concluded that iron deficiency is common in nephrotic syndrome especially those with steroid-resistant cases. But our incidence of iron deficiency anemia is equally distributed among steroid-sensitive and

steroid-dependent cases. Urinary loss of iron, transferrin, soluble transferrin receptor and erythropoietin have been hypothesized to cause anemia in persistent nephrotic syndrome. Although we did not examine the urinary losses of iron and transferrin in our study, we found a negative correlation between the urinary erythropoietin levels and hemoglobin levels, which is suggestive that urinary erythropoietin loss in turn leads to anemia in nephrotic syndrome.

CONCLUSION

Anemia is a major cause of mortality and morbidity in children and a universal health concern, particularly in underdeveloped nations. In cases of persistent nephrotic syndrome, anemia was found to be one of the many complications. Iron, transferrin, erythropoietin, transcobalamin, and metal can all be significantly lost in the urine and be the root of anemia. As a result, there are not enough of the substrates needed for successful erythropoiesis.

This study was conducted with the objective to estimate the urinary erythropoietin levels in nephrotic syndrome children with anemia.

The mean urinary erythropoietin levels found in our study were 9.61+/-10.94. There was a negative correlation between the urinary erythropoietin levels and hemoglobin levels, which is suggestive that urinary erythropoietin loss in turn leads to Anemia in Nephrotic Syndrome. It requires further studies as our sample size is small.

The prevalence of anemia in our study is 30%. Iron deficiency anemia was found to be the most common cause of anemia. The incidence of iron deficiency anemia is equally distributed among steroid-sensitive and steroid-dependent cases. Urinary erythropoietin levels were found in all the cases with no significant difference between the different groups.

SUMMARY

In our study, we did urinary erythropoietin levels to understand the effectiveness of erythropoietin losses in the urine leading to anemia in nephrotic syndrome.

The mean urinary erythropoietin levels found in our study were 9.61+/-10.94. This finding was consistent with other studies. As per recent studies, it was found that there was no amount of urinary erythropoietin levels found in children without the nephrotic syndrome, and in children with nephrotic syndrome, was found to be >10-30mU/ml[10].

Urinary erythropoietin levels were compared with different parameters like age groups and gender groups along with different diagnostic types. But no significance was found. But there was a negative relationship between hemoglobin levels and urinary erythropoietin levels suggesting that urinary losses of erythropoietin contribute to the development of anemia along with other factors.

In recent studies, it was found that frequently relapsing or persistent nephrotic syndrome was mostly associated with the development of anemia in Nephrotic Syndrome children. But in our study, it was similar when compared between different diagnostic groups of nephrotic syndrome, be it frequent or infrequent relapsers. Out of the biopsied patients, urinary erythropoietin levels were compared between different histological types but it was not significant.

The prevalence of anemia in this is 30% . Various histological parameters were analyzed in this study. Microcytic hypochromic anemia was the most prevalent morphological variety discovered, followed by normocytic normochromic anemia. Between normocytic normochromic and microcytic hypochromic types, the mean MCV, MCH, and RDW indicated a significant relationship. The mean MCV and

MCH were significantly lower in the microcytic group when compared with the normocytic group. One child had macro-ovalocytes in peripheral with dimorphic picture.

In this study, 51% of children had iron deficiency anemia followed by 47% of children who had non-nutritional anemia with one child having iron deficiency anemia with vitamin b12 deficiency. Nearly 72.4% of children with microcytic hypochromic anemia had evidence of IDA.

Ideally in IDA low serum iron, low serum ferritin, an increase in total iron binding capacity, and low transferrin saturation can be found. But in our study, there was significant high transferrin saturation and low total iron binding capacity, which can be due to urinary losses of transferrin. But in this study, it was found that there was a significant relationship between serum albumin levels along with transferrin synthesis indicating that synthesis of transferrin is none other than a general response in a nephrotic syndrome where there is a general increase in the production of proteins by the liver.

The incidence of iron deficiency anemia is equally distributed among steroid-sensitive and steroid-dependent cases. Anemia in chronic nephrotic syndrome has been linked to urine losses of erythropoietin, transferrin, soluble transferrin receptor, and iron. While we did not analyze the urine losses of iron and transferrin in this investigation, we did discover a negative association between urinary erythropoietin levels and hemoglobin levels, which may indicate that urinary erythropoietin loss in the nephrotic syndrome is a causative factor of anemia.

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ANNEXURE I – CONSENT FORM

CONSENT FOR PARTICIPATION IN RESEARCH

"URINARY ERYTHROPOIETIN LEVELS IN NEPHROTIC SYNDROME CHILD WITH ANEMIA"

Principal Investigator: REG NO. BM0120003

Co – investigator: DR _____

Introduction: You are being invited to participate in this study to find out **URINARY ERYTHROPOIETIN LEVELS IN NEPHROTIC SYNDROME CHILD WITH ANEMIA**. Participation of your child will help us to know the urinary erythropoietin levels in nephrotic syndrome child with anemia and determining the cause of anemia. Nephrotic syndrome is characterized by proteinuria due to permeability at the glomerular capillary membrane, resulting in its ability to restrict the urinary loss of protein. In addition to albumin, many other proteins are also lost in urine. Excessive urinary losses of iron, transferrin, erythropoietin, etc. This leads to a deficiency of substrate necessary for effective erythropoiesis. The aim of this study is to estimate urinary levels of erythropoietin that can be potential cause of anemia in nephrotic syndrome. So that intervention can be done as early possible to avoid the condition leading to anemia

VOLUNTARY PARTICIPATION: You/your child participation in this study in your voluntary decision. Whether to participate or not to participate will not affect your current or future relationship with the KLES DR. PRABHAKAR KORE Hospital and medical research centre, Belgaum. You are free to discontinue the participation in the study at any time for any reasons and you will not be paid any reimbursement for participation in the research

Possible Benefits: To know the urinary levels of erythropoietin that can be potential cause of anemia in nephrotic syndrome

Possible Risks: There is no risk involved in this study.

Benefits from the study: We will know the levels of urinary erythropoietin levels in nephrotic syndrome child that can cause anemia so that EPO supplementation can be given.

Confidentiality: All the data collected will remain confidential and only aggregated data will be published. Your personal identity will not be revealed.

Withdrawal: Your participation in this study is purely voluntary. You may decide to participate or not. Even though you decide not to participate, you will not be deprived of the benefits of this study.

Costs of Participation: The cost of the study will be borne by the subjects if they can afford. If not, it will be borne by the researcher. It involves the cost of ELISA kits and the cost of iron studies. There will be no additional cost to you for participating in this study.

Payment of Participation: There will be no incentives to you for participating in this study.

Questions: If you have any questions regarding the study, you should contact Principal Investigator **REG NO. BM0120003 admission batch,** Department of Paediatrics. J. N. Medical College, Belagavi, 590010, Ph. No- 8019791275

Guide: Dr. _____, Professor Department of Paediatrics, J. N. Medical College, Belagavi, 590010.

If you have any questions about your rights as a study participant, you may contact **Dr.Harsha Hegde**, Chairman, Institutional Ethics Committee on Human Subjects' Research, J.N. Medical College, Belagavi - 590010, Ph. No 9480422500, Extn 4052, 4057.

Legal Rights: By signing this consent form; you are not waiving any of your Legal rights.

Consent statement:

"I volunteer and consent to participate in the study. I have read (or it has been read to me in the language known to me) the information sheet thoroughly. Full opportunity was given to me to ask questions. I am fully satisfied with the answers to the questions I wanted to ask. I hereby voluntarily agree to participate in this research project".

Name of the Participant	Signature of the participant or Left-Hand Thumb impression
Name of Investigator	Signature of investigator
Name of Witness	Signature of Witness

Date: _____ Place: _____

Assent (<18 years)

I have read the information in this form. After understanding all details about the study, I agree to give assent to be included as a volunteer in the study titled "**URINARY ERYTHROPOIETIN LEVELS IN NEPHROTIC SYNDROME CHILDREN WITH ANEMIA"**

Name of the Participant	Signature of the participant or Left-Hand Thumb impression
Name of the Parent	Signature of the parent
Name of Investigator	Signature of investigator
Name of Witness	Signature of Witness

Date: _____ Place: _____

ANNEXURE III – PROFORMA

"URINARY ERYTHROPOIETIN LEVELS IN CHILDREN WITH NEPHROTIC SYNDROME"

1)NAME 2)AGE 3)GENDER
4)DATE

5)HISTORY OF PRESENT ILLNSS:

6)PAST HISTORY:

:

7)ANTHROPMETRY:

8)VITALS:

9)BP CENTILE CHART:

10)INVESTIGATIONS:

URINE ALBUMIN	
SERUM CREATININE	
SERUM ALBUMIN	
SERUM CHOLESTEROL	

11)RENAL BIOPSY REPORT:
(IF DONE)

HEMOGLOBIN	
PACKED CELL VOLUME	
RBC COUNT	
MCV	
MCH	
MCHC	
RED CELL DISTRIBUTION	
PLATELET COUNT	
TOTAL LEUKOCYTE COUNT	
SEGMENTED NEUTROPHILS	
LYMPHOCYTES	
MONOCYTES	
EOSINOPHILS	
BASOPHILS	
SERUM IRON	
TOTAL IRON BINDING CAPACITY	
TRANSFERRIN SATURATION	
SERUM FERRITIN	
URINARY ERYTHROPOIETIN	

12)PERIPHERAL SMEAR REPORT-

13)Egfr-

14)GENERAL EXAMINATION:

15)SYSTEMIC EXAMINATION:

FINAL DIADNOSIS:

ANNEXURE IV

KEY TO MASTERCHART

BP Blood Pressure

SR Serum

HB Hemoglobin

PCV Packed Cell Volume

RBC Red blood cells

MCV Mean corpuscular volume

MCH Mean corpuscular hemoglobin

MCHC Mean corpuscular hemoglobin concentration

RDW Red cell distribution width

TLC Total leukocyte count

N Neutrophils

L Lymphocytes

B Basophils

E Eosinophils

M Monocytes

TIBC Total iron binding capacity

eGFR Estimated glomerular filtration rate

NO	AGE	SEX	STERIOD DEPENDENT/RESISTANT	FREQUENT/INFREQUENT RELAPSES	RELAPSE SPONTANEOUS/SEC TO	WEIGHT	HEIGHT	BP NORMAL/HYPERTENSIVE	URINE ALBUMIN	SR CREATININE	SR ALBUMIN	SR CHOLESTEROL	HB	ANEMIA SEVERITY	PCV	RBC	MCV	MCH	MCHC
1	3YR	MALE	STERIOD SENSITIVE	INFREQUENT	SPONTANEOUS	15KG	89CM	NORMAL	3+	0.34	1	383	10.2	MILD	32.8	5.53	59.4	18.4	31
2	10YR	MALE	STERIOD SENSITIVE	INFREQUENT	SEC TO PERITONITIS	30KG	136CM	HYPERTENSIVE	4+	0.6	0.9	329	9.7	MODERATE	28.7	3.49	82.7	27.8	33.7
3	16YR	MALE	STEROID RESISTANT	FREQUENT	SPONTANEOUS	30.5KG	136CM	HYPERTENSIVE	3+	0.5	1.5	510	9.5	MODERATE	29.1	4.68	79	25.7	32.5
4	3YR	MALE	STERIOD SENSITIVE	INFREQUENT	SEC TO LRTI	17.8KG	89CM	HYPERTENSIVE	4+	0.34	1.4	270	9.3	MODERATE	28.7	3.65	81.6	26.3	32.3
5	15YR	MALE	STEROID RESISTANT	INFREQUENT	SPONTANEOUS	33.5KG	131.5CM	NORMAL	3+	0.57	2.2	287	9	MODERATE	28.1	3.82	79.5	25.5	32
6	2YR	FEMALE	STERIOD SENSITIVE	INFREQUENT	SPONTANEOUS	10.5KG	84CM	NORMAL	3+	0.32	1.2	586	11.4	MILD	38.2	5.11	34.8	22.5	30.1
7	1YR	MALE	INFANTILE NEPHROTIC SYNDROME	FREQUENT	SEC TO PERITONITIS	10.9KG	66CM	HYPERTENSIVE	4+	0.15	1.8	237	8.2	MODERATE	30.5	5.6	54.5	14.6	26.8
8	3YR	MALE	STERIOD SENSITIVE	INFREQUENT	SEC TO PERITONITIS	13.5KG	85CM	HYPERTENSIVE	4+	0.31	1.5	627	9.8	MODERATE	32.5	5.42	60	18	30
9	3YR	FEMALE	STERIOD SENSITIVE	INFREQUENT	SPONTANEOUS	13.7KG	91CM	NORMAL	3+	0.26	0.9	531	10.7	MILD	33.8	4.47	75.7	24	31.7
10	3YR	MALE	STERIOD SENSITIVE	FREQUENT	SPONTANEOUS	13.2KG	82CM	HYPERTENSIVE	4+	0.3	1.5	564	8.3	MODERATE	28.4	4.41	64.2	18.8	29.3
11	5YR	MALE	NEWLY DIAGNOSED	1ST EPISODE	SPONTANEOUS	28KG	141CM	HYPERTENSIVE	4+	0.5	2	245	9.2	MODERATE	31.5	4.95	63.5	18.7	29.4
12	15YR	MALE	STERIOD SENSITIVE	FREQUENT	SPONTANEOUS	52KG	157CM	NORMAL	3+	0.45	2.1	281	8.4	MODERATE	29.2	4.53	64.5	18.6	28.9
13	3YR	FEMALE	STERIOD SENSITIVE	FREQUENT	SEC TO PERITONITIS	10.7KG	82CM	NORMAL	3+	0.2	1.6	309	7.4	MODERATE	26.6	3.71	71.8	19.8	27.6
14	10YR	MALE	STERIOD SENSITIVE	FREQUENT	SPONTANEOUS	29KG	136CM	NORMAL	4+	0.4	2.1	358	9.9	MODERATE	41	5.55	73.8	17.8	24.1
15	5YR	MALE	STERIOD SENSITIVE	INFREQUENT	SEC TO LRTI	20.8KG	103CM	HYPERTENSIVE	4+	0.29	1.5	661	10.2	MILD	34.9	5.06	69	20.3	29.3
16	6YR	MALE	STERIOD SENSITIVE	FREQUENT	SEC TO PERITONITIS	20.7KG	101CM	HYPERTENSIVE	3+	0.22	2	442	8.5	MODERATE	26.6	3.55	74.9	24.1	32.2
17	7YR	MALE	STERIOD SENSITIVE	INFREQUENT	SEC TO PERITONITIS	26.7KG	127CM	NORMAL	4+	0.45	2.1	500	8.8	MODERATE	29.1	4.32	69.1	20.2	30.1
18	6YR	MALE	STERIOD SENSITIVE	FREQUENT	SEC TO PERITONITIS	20.3KG	117CM	HYPERTENSIVE	3+	0.34	1.3	293	11.3	MILD	37.7	6.34	59.4	17.8	30
19	2YR	MALE	INFANTILE NEPHROTIC SYNDROME	FREQUENT	SEC TO PERITONITIS	8.7KG	71CM	NORMAL	4+	0.2	1.5	360	6.9	SEVERE	21.9	3.15	69.6	21.9	31.4
20	3YR	FEMALE	STERIOD SENSITIVE	FREQUENT	SPONTANEOUS	13.5KG	86CM	NORMAL	4+	0.23	1.7	495	7.2	MODERATE	23	3.22	71.3	22.3	31.3
21	7R4MONT	MALE	STERIOD SENSITIVE	INFREQUENT	SEC TO LRTI	16KG	105CM	NORMAL	4+	0.4	1.7	406	6.8	SEVERE	21.9	3.11	70.4	21.9	31.2
22	10YR	FEMALE	STERIOD SENSITIVE	INFREQUENT	SEC TO LRTI	23.9KG	124CM	HYPERTENSIVE	3+	0.4	1.4	502	8.9	MODERATE	28.9	3.25	88.8	27.4	30.9
23	7R5MONT	MALE	STERIOD SENSITIVE	FREQUENT	SEC TO LRTI	13KG	86CM	HYPERTENSIVE	4+	0.35	1.6	554	8.5	MODERATE	29	3.23	89.8	26.4	29.4
24	12YR	MALE	STERIOD SENSITIVE	FREQUENT	SEC TO URTI	37.3KG	137CM	NORMAL	3+	0.58	1.6	368	8.6	MODERATE	28.9	3.24	89	26.6	29.8
25	7R3MONT	FEMALE	INFANTILE NEPHROTIC SYNDROME	INFREQUENT	SPONTANEOUS	8.5KG	74CM	NORMAL	4+	0.23	1.9	294	9.2	MODERATE	28.8	4.28	67.3	21.4	31.8
26	9YR	MALE	STERIOD SENSITIVE	INFREQUENT	SEC TO LRTI	22.6KG	117CM	NORMAL	4+	0.27	1.9	297	10.3	MODERATE	34.9	3.73	93.5	27.7	29.6
27	10YR	MALE	STERIOD SENSITIVE	FREQUENT	SPONTANEOUS	40.6KG	124CM	HYPERTENSIVE	4+	0.42	3.4	368	9.8	MODERATE	30.9	3.14	98.3	31.4	31.9
28	4YR	MALE	STERIOD SENSITIVE	INFREQUENT	SPONTANEOUS	23.2KG	91CM	NORMAL	4+	0.28	2	360	9.2	MODERATE	31.6	3.6	87.8	25.7	29.2
29	7R6MONT	FEMALE	STERIOD SENSITIVE	INFREQUENT	SPONTANEOUS	14.4KG	94CM	NORMAL	3+	0.38	1.9	420	10.8	MILD	36.9	4.8	77	24.7	32
30	5YR	MALE	STERIOD SENSITIVE	INFREQUENT	SEC TO PERITONITIS	17.3KG	105CM	HYPERTENSIVE	4+	0.32	1.9	274	9	MODERATE	24.4	3.82	64	13	20.3
31	2YR	FEMALE	STERIOD SENSITIVE	INFREQUENT	SPONTANEOUS	13.1KG	90CM	HYPERTENSIVE	4+	0.23	1.3	386	8.2	MODERATE	29.6	4.08	72.6	20	27.6
32	8YR	FEMALE	STERIOD SENSITIVE	INFREQUENT	SPONTANEOUS	25.9KG	121CM	NORMAL	3+	0.45	1.8	470	8.1	MODERATE	29.9	4.64	64.6	17.5	27.1
33	4YR	FEMALE	STERIOD SENSITIVE	INFREQUENT	SEC TO URTI	15KG	105CM	HYPERTENSIVE	4+	0.17	2.6	203	10.9	MILD	41.3	4.55	90.6	24	26.5
34	5YR	FEMALE	STERIOD SENSITIVE	FREQUENT	SPONTANEOUS	19KG	101CM	NORMAL	4+	0.45	1.8	418	9.6	MODERATE	22.3	3.8	58.7	13.4	22.8
35	4YR	FEMALE	STERIOD SENSITIVE	FREQUENT	SEC TO PERITONITIS	13.3KG	98CM	HYPERTENSIVE	4+	0.4	2	468	8.1	MODERATE	31.3	3.92	79.8	20.7	25.9
36	7YR	MALE	STERIOD SENSITIVE	FREQUENT	SEC TO LRTI	20.19KG	112CM	HYPERTENSIVE	4+	0.5	1.7	705	9.9	MODERATE	31.7	3.73	85	26.5	31.2
37	5YR	MALE	STERIOD SENSITIVE	INFREQUENT	SPONTANEOUS	14KG	106CM	HYPERTENSIVE	3+	0.45	1.8	418	9.6	MODERATE	22.3	3.8	58.7	13.4	22.8
38	6YR	MALE	STERIOD SENSITIVE	INFREQUENT	SEC TO PERITONITIS	18.11KG	101CM	HYPERTENSIVE	4+	0.32	0.7	541	10.6	MODERATE	31	3.91	79.3	27.1	34.2
39	7R4MONT	MALE	NEWLY DIAGNOSED	1ST EPISODE	SPONTANEOUS	10.4KG	78CM	NORMAL	4+	0.28	1.1	263	8.5	MODERATE	27	4.41	61.1	18.4	30.2
40	2YR	FEMALE	NEWLY DIAGNOSED	1ST EPISODE	SEC TO LRTI	10.6KG	81CM	HYPERTENSIVE	3+	0.32	1.4	599	8.7	MODERATE	29.4	4.84	60.8	18	29.6
41	3YR	FEMALE	NEWLY DIAGNOSED	1ST EPISODE	SPONTANEOUS	13.9KG	95CM	HYPERTENSIVE	3+	0.2	2.1	466	11.1	MILD	37.5	5.7	65.8	19.5	29.6
42	2YR	MALE	STEROID RESISTANT	INFREQUENT	SPONTANEOUS	15KG	86CM	NORMAL	3+	0.23	1.8	608	10.7	MILD	30.8	3.83	85	26.5	31.2
43	5YR	FEMALE	STERIOD SENSITIVE	INFREQUENT	SPONTANEOUS	14.7KG	95CM	NORMAL	3+	0.35	1.8	388	9.8	MODERATE	30.2	3.8	88.8	25.9	28.2
44	6YR	FEMALE	STEROID RESISTANT	FREQUENT	SPONTANEOUS	14.8KG	103CM	HYPERTENSIVE	4+	0.4	2.1	430	7.8	SEVERE	25.6	4.12	62.1	19	30.5
45	2YR	FEMALE	STEROID RESISTANT	FREQUENT	SPONTANEOUS	14.6KG	85CM	NORMAL	4+	0.35	1.4	486	10.6	MILD	32.8	4.48	75.6	24.2	31.8
46	4YR	MALE	STEROID RESISTANT	FREQUENT	SPONTANEOUS	12.8KG	92CM	HYPERTENSIVE	4+	0.37	2.2	287	9.2	MODERATE	28.2	3.58	78.5	26.5	32.1
47	15YR	FEMALE	STERIOD SENSITIVE	INFREQUENT	SPONTANEOUS	40.7KG	149.5CM	NORMAL	3+	0.5	1.2	333	5.3	SEVERE	20.6	3.86	58.7	13.4	22.8
48	4YR	MALE	STEROID RESISTANT	FREQUENT	SPONTANEOUS	11.7KG	90CM	NORMAL	3+	0.5	1.4	420	8.8	MODERATE	31.1	5.49	79	25.7	32.5
49	6YR	MALE	STERIOD SENSITIVE	INFREQUENT	SEC TO PERITONITIS	21.5KG	116CM	NORMAL	3+	0.3	2	460	10.6	MODERATE	31	3.91	79.3	27.1	34.2
50	4YR	FEMALE	STERIOD SENSITIVE	INFREQUENT	SEC TO URTI	17KG	110CM	NORMAL	4+	0.17	2.6	203	10.9	MILD	41.3	4.55	90.6	24	26.5
51	5YR	FEMALE	STERIOD SENSITIVE	INFREQUENT	SPONTANEOUS	16KG	105CM	NORMAL	4+	0.4	1.7	406	6.8	SEVERE	21.9	3.11	70.4	21.9	31.2

RDW	PLATELET	TLC	N	L	M	E	B	SR IRON	TIBC	TRANSFERRIN SATURATION	SR FERRITIN	VIT B12	ETIOLOGY OF ANEMIA	URINARY ERYTHROPOIETIN	PERIPHERAL SMEAR	EGFR(ml/min/1.73m2)	BIOPSY REPORT IF DONE	
21.9	484K	8100	70	27	1	2	0	43	118	36	29.69		NON NUTRITIONAL ANAEMIA	0	MICROCYTIC HYPOCROMIC ANEMIA	108	MCD	
16.8	304K	9100	77	15	0	8	0	16	36	44	512.6		NON NUTRITIONAL ANAEMIA	5.258	NORMOCYTIC NORMOCHROMIC ANEMIA	94	NOT DONE	
13.1	463K	17200	77	16	0	7	0	23	97	24	159.5		NON NUTRITIONAL ANAEMIA	0.713	MICROCYTIC HYPOCROMIC ANEMIA	112	FSGS	
16.6	188K	8200	81	9	0	10	0	13	38	34	549.2		NON NUTRITIONAL ANAEMIA	10.668	NORMOCYTIC NORMOCHROMIC ANEMIA	108	FSGS	
15.8	360K	12200	60	31	1	6	2	12	114	11	189.8		IRON DEFICIENCY ANAEMIA	0.115	MICROCYTIC HYPOCROMIC ANEMIA	94	FSGS	
18.4	693K	18000	41	44	7	8	0	38	67	57	33.74		IRON DEFICIENCY ANAEMIA	0.713	MICROCYTIC HYPOCHROMIC ANEMIA	108	NOT DONE	
26.4	555K	12000	29	69	2	5	0	16	164	10	16.6	103.70pg/ml	IRON DEFICIENCY ANAEMIA AND VITAMIN B12 DEFICIENCY	3.529	CYTIC HYPOCHROMIC, NORMOCYTIC HYPOCHR	181	NOT DONE	
21.1	1055K	17700	53	38	0	9	0	52	75	69	94.83		NON NUTRITIONAL ANAEMIA	6.101	MICROCYTIC HYPOCHROMIC ANEMIA	113	NOT DONE	
13.9	723K	13000	36	51	8	5	0	38	50	76	156.5		NON NUTRITIONAL ANAEMIA	3.928	NORMOCYTIC NORMOCHROMIC ANEMIA	144	NOT DONE	
25.7	595K	29200	84	14	1	1	0	41	54	76	43.73		IRON DEFICIENCY ANAEMIA	2.509	MICROCYTIC HYPOCHROMIC ANEMIA	112	NOT DONE	
22.5	570K	9100	41	46	6	7	0	38	231	16	19.38		IRON DEFICIENCY ANEMIA	3.063	MICROCYTIC HYPOCHROMIC ANEMIA	116	ENTRIC GLOMERULONEPHRITIS	
25.4	619K	26400	78	13	0	9	0	24	143	17	141		IRON DEFICIENCY ANAEMIA	2.642	MICROCYTIC HYPOCHROMIC ANEMIA	144	MCD	
26.2	240K	46000	79	11	0	2	0	45	81	56	601.5		IRON DEFICIENCY ANAEMIA	13.661	MICROCYTIC HYPOCHROMIC ANEMIA	169	NOT DONE	
19	418K	7400	60	35	2	3	0	74	226	33	141		NON NUTRITIONAL ANAEMIA	6.899	MICROCYTIC HYPOCHROMIC ANEMIA	140	NOT DONE	
31.1	367K	18300	80	20	0	0	0	40	502	8	45.64		IRON DEFICIENCY ANAEMIA	13.971	MICROCYTIC HYPOCHROMIC ANEMIA	146	MCD	
30	119K	6500	27	65	0	8	0	32	260	12	14.45		IRON DEFICIENCY ANAEMIA	1.134	MICROCYTIC HYPOCHROMIC ANEMIA	189	NOT DONE	
26	319K	6600	49	39	4	8	0	15	208	7	1206		IRON DEFICIENCY ANAEMIA	4.904	ROCYTIC HYPOCHROMIC ANEMIA AND NORMO	118	NOT DONE	
17	532K	23400	96	3	0	1	0	41	259	16	124		IRON DEFICIENCY ANAEMIA	0	MICROCYTIC HYPOCHROMIC ANEMIA	142	NOT DONE	
19.9	733K	11500	83	13	0	4	0	35	87	40	234.5		IRON DEFICIENCY ANAEMIA	3.884	MICROCYTIC HYPOCHROMIC ANEMIA	146	FSGS	
20.4	723K	11500	82	14	0	4	0	26	76	34	263		IRON DEFICIENCY ANAEMIA	0.492	MICROCYTIC HYPOCHROMIC ANEMIA	154	NOT DONE	
20.1	730K	11100	83	13	0	4	0	31	142	22	244		NON NUTRITIONAL ANAEMIA	9.692	MICROCYTIC HYPOCHROMIC ANEMIA	108	NOT DONE	
16.3	376K	18400	79	17	0	4	0	67	82	82	296.3		NON NUTRITIONAL ANAEMIA	11.754	NORMOCYTIC NORMOCHROMIC ANEMIA	128	FSGS	
14.6	672K	8900	52	48	0	0	0	63	112	56	87.92		NON NUTRITIONAL ANAEMIA	55.897	NORMOCYTIC NORMOCHROMIC ANEMIA	101	FSGS	
14.6	689K	8800	79	8	1	2	0	49	187	26	32.29		NON NUTRITIONAL ANAEMIA	6.278	NORMOCYTIC NORMOCHROMIC ANEMIA	97	NOT DONE	
22.9	900K	13400	62	33	1	4	0	23	95	24	40		IRON DEFICIENCY ANAEMIA	1.866	MICROCYTIC HYPOCHROMIC ANEMIA	133	NOT DONE	
18.5	234K	23500	91	7	1	1	0	55	437	13	428		NON NUTRITIONAL ANAEMIA	4.371	NORMOCYTIC NORMOCHROMIC ANEMIA	178	MCD	
12.7	110K	7500	43	46	1	10	0	82	318	26	360		NON NUTRITIONAL ANAEMIA	5.458	NORMOCYTIC NORMOCHROMIC ANEMIA	121	FSGS	
14	409K	11500	52	40	1	7	0	60	351	17	52.27		NON NUTRITIONAL ANAEMIA	7.941	NORMOCYTIC NORMOCHROMIC ANEMIA	138	NOT DONE	
15.3	446K	12200	40	50	3	7	0	39	358	11	17.8		NON NUTRITIONAL ANAEMIA	1.245	NORMOCYTIC NORMOCHROMIC ANEMIA	102	NOT DONE	
24.6	421K	18600	64	28	3	5	0	52	466	11	136		IRON DEFICIENCY ANAEMIA	13.883	MICROCYTIC HYOCHROMIC ANEMIA	136	NOT DONE	
17.8	241K	6600	36	56	0	8	0	46	405	11	116		IRON DEFICIENCY ANAEMIA	10.772	MICROCYTIC HYOCHROMIC ANEMIA	161	IGA NEPHROPATHY	
22.1	763K	17800	75	17	0	8	0	58	470	12	40.35		IRON DEFICIENCY ANAEMIA	8.185	MICROCYTIC HYOCHROMIC ANEMIA	111	NOT DONE	
23.7	645K	6800	62	20	11	7	0	142	206	69	529		NON NUTRITIONAL ANAEMIA	19.226	NORMOCYTIC HYPOCHROMIC ANEMIA	255	MCD	
29.2	488K	13700	68	26	3	3	0	73	270	27	262		NON NUTRITIONAL ANAEMIA	7.365	MICROCYTIC HYOCHROMIC ANEMIA	93	NOT DONE	
23	660K	16500	50	43	1	6	0	29	69	42	35.7		IRON DEFICIENCY ANAEMIA	46.319	MICROCYTIC HYOCHROMIC ANEMIA	101	MCD	
14.3	549K	21210	90	8	1	1	0	36	178	20	636		NON NUTRITIONAL ANAEMIA	34.612	NORMOCYTIC NORMOCHROMIC ANEMIA	92	FSGS	
29.2	488K	13700	68	26	3	3	0	70	260	27	262		NON NUTRITIONAL ANAEMIA	22.64	MICROCYTIC HYOCHROMIC ANEMIA	95	NOT DONE	
12.9	271K	9140	38	54	3	5	0	24	82	29	162		IRON DEFICIENCY ANAEMIA	0	NORMOCYTIC HYPOCHROMIC ANEMIA	130	NOT DONE	
16.7	985K	16830	50	40	2	8	0	27	68	40	30		IRON DEFICIENCY ANAEMIA	16.61	MICROCYTIC HYPOCHROMIC ANEMIA	115	NOT DONE	
19.2	484K	10400	65	32	1	2	0	28	70	36	32		IRON DEFICIENCY ANAEMIA	7.298	MICROCYTIC HYPOCHROMIC ANEMIA	104	NOT DONE	
21.3	462K	17470	41	52	0	7	0	24	74	38	36		IRON DEFICIENCY ANAEMIA	10.646	MICROCYTIC HYPOCHROMIC ANEMIA	196	MCD	
15.6	323K	16000	74	22	0	4	0	36	201	24	636		NON NUTRITIONAL ANAEMIA	3.174	NORMOCYTIC NORMOCHROMIC ANEMIA	154	MCD	
14	402K	12600	52	40	1	7	0	62	361	14	52.78		NON NUTRITIONAL ANAEMIA	5.569	NORMOCYTIC NORMOCHROMIC ANEMIA	112	NOT DONE	
16.3	685K	18090	92	6	1	1	0	16	216	8	506		IRON DEFICIENCY ANAEMIA	4.482	OCYTIC HYOCHROMIC ANEMIA WITH NORMOC	106	FSGS	
14	623K	14000	36	51	8	5	0	38	50	76	156.5		NON NUTRITIONAL ANAEMIA	7.741	NORMOCYTIC NORMOCHROMIC ANEMIA	100	MCD	
16	480K	12400	60	31	0	8	0	12	114	11	189.8		IRON DEFICIENCY ANAEMIA	6.522	NORMOCYTIC NORMOCHROMIC ANEMIA	102	NOT DONE	
29.2	488K	13700	68	26	3	3	0	73	270	27	262		NON NUTRITIONAL ANAEMIA	10.491	MICROCYTIC HYPOCHROMIC ANEMIA	123	NOT DONE	
13.1	303K	10000	88	9	0	3	0	23	97	24	160.5		IRON DEFICIENCY ANAEMIA	19.226	MICROCYTIC HYOCHROMIC ANEMIA	112	NOT DONE	
12.9	271K	9140	38	54	3	5	0	24	82	29	162		IRON DEFICIENCY ANAEMIA	13.971	NORMOCYTIC HYPOCHROMIC ANEMIA	159	NOT DONE	
23.7	645K	6800	62	20	11	7	0	142	206	69	529		NON NUTRITIONAL ANAEMIA	19.004	NORMOCYTIC HYPOCHROMIC ANEMIA	267	NOT DONE	
20.1	730K	11100	83	13	0	4	0	31	142	22	244		IRON DEFICIENCY ANAEMIA	13.661	MICROCYTIC HYPOCHROMIC ANEMIA	108	NOT DONE	