Prognostic Significance of Serum Hemopexin Estimation in Children with Minimal change nephrotic syndrome

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Abstract

Background: Nephrotic syndrome (NS) is a clinical syndrome consisting of massive proteinuria (more than 40 mg/m2 per hour), hypoalbuminemia (less than 30 g/L), hyperlipidemia, edema, and other complications. Childhood nephritis syndrome (NS) consists of proteinuria (≥40 mg/m2 /h or urine protein/creatinine ratio ≥200 mg/mL), hypoalbuminemia (<25g/L), and edema. Childhood NS is reported in 4.7 (range 1.15–16.9) per 100,000 children, with substantial variability across ethnicity and geographical location. The most useful function of Hx is heme scavenging at the systemic level, (double face of heme), which is essential for life but also highly toxic. The role of Hx in the nervous system. Hemopexin molecules cannot pass the blood–brain barrier, therefore the nervous tissue cannot utilize the plasma Hx and synthesizes its protein in situ. Furthermore, there is an importance of Hx in the immune system, as iron is one of the regulators of the immune response. Another Hx functions, not related to its heme-scavenging properties, are discussed. The mechanism of onset of relapse of MCNS caused by active Hx is unclear. Various isoforms of Hx are suggested to exist. In normal conditions, the circulating Hx is inactive. Under certain circumstances Hx becomes activated as a serine protease. MCNS in relapse demonstrates altered activity of plasma Hx. There was a decreased mean titer of plasma Hx in relapsed subjects as compared with remission

Keywords: Serum Hemopexin, Minimal change nephrotic syndrome

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Nephrotic syndrome (NS) is a clinical syndrome consisting of massive proteinuria (more than 40 mg/m2 per hour), hypoalbuminemia (less than 30 g/L), hyperlipidemia, edema, and other complications (1)

Epidemiology:

Idiopathic nephrotic syndrome (INS) has a worldwide incidence of 2-7 cases per 100,000 and a prevalence of 16 cases per 100,000. Minimal change disease (MCD) is the most common form of nephrotic syndrome in children less than 10 years old (approximately 70-90% of these children) It is a primary glomerulonephritis characterized by normal glomeruli on light microscopy and podocyte effacement on electron microscopy. MCD still accounts for 50% of nephrotic syndrome in older children (2)

idiopathic nephrotic syndrome (INS) is one of the major glomerular diseases in Egyptian children and about 30% of these children are resistant to treatment by steroids. Two hundred forty-two deaths in Egyptian children are annually due to Nephrotic syndrome. This ranks Egypt as the second country in the world in this regard. The first country was Japan which tallies up to 447 deaths. The United States follows closely behind Egypt with 153 deaths (3)

Classification of childhood nephrotic syndrome (4)

Nephrotic syndrome is classified into primary or secondary depending on the underlying etiology.

Primary Causes

Differentiation between different types of glomerular diseases is usually made by clinical picture, and renal biopsy, including light microscopy, immunofluorescence, and electron microscopy [5]

Secondary Causes

Diabetes mellitus is the most common cause of secondary nephrotic syndrome in adults. A table below show the causes of nephrotic syndrome by age group [6]

Causes of childhood nephrotic syndrome*

Genetic disorders

Nephrotic-syndrome typical
Finnish-type congenital nephrotic syndrome
FSGS
Diffuse mesangial sclerosis
Denys-Drash syndrome
Schimke immuno-osseous dysplasia

Proteinuira with or without nephrotic syndrome
Nail-patella syndrome
Alport's syndrome

Multisystem syndromes with or without nephrotic syndrome
Galloway-Mowat syndrome
Charcot-Marie-Tooth disease
Jeune's syndrome
Cockayne's syndrome
Laurence-Moon-Biedl-Bardet syndrome

Metabolic disorders with or without nephrotic syndrome
Alagille syndrome
α-1 antitrypsin deficiency
Fabry disease
Glutaric acidaemia
Glycogen storage disease
Hurler's syndrome
Lipoprotein disorders
Mitochondrial cytopathies
Sickle-cell disease

Idiopathic nephrotic syndrome
MCNS
FSGS
Membranous nephropathy

Secondary causes

Infections
Hepatitis B, C
HIV-1
Malaria
Syphilis
Toxoplasmosis

Drugs
Penicillamine
Gold
Non-steroidal anti-inflammatory drugs
Pamidronate
Interferon
Mercury
Heroin
Lithium

Immunological or allergic disorders
Castleman's disease
Kimura's disease
Bee sting
Food allergens

Associated with malignant disease
Lymphoma
Leukaemia

Glomerular hyperfiltration
Oligomeganephronia
Morbid obesity
Adaptation to nephron reduction

Table.1: Causes of secondary childhood nephrotic syndrome [6]

Congenital and infantile nephrotic syndrome: -

Congenital nephrotic syndrome (CNS) has been found within the first 3 months of life, or early at birth. Infantile nephrotic syndrome (INS) occurs between 3 months and one year. Both CNS and INS are rare inherited defects in glomerular filtration involving a variety of gene mutations. CNS has a poor prognosis, usually complicated by end stage kidney disease (ESKD) in early childhood. The Finnish type is the most common form of CNS, with early genetic feature and founder mutations ([7]

Childhood nephrotic syndrome:

Childhood nephritis syndrome (NS) consists of proteinuria (≥40 mg/m2 /h or urine protein/creatinine ratio ≥200 mg/mL), hypoalbuminemia (<25g/L), and edema (1). Childhood NS is reported in 4.7 (range 1.15–16.9) per 100,000 children, with substantial variability across ethnicity and geographical location [8]

Childhood nephrotic syndromes are mostly caused by one of two idiopathic diseases: minimal-change nephrotic syndrome (MCNS) and focal segmental glomerulosclerosis (FSGS). The third distinct type is

membranous nephropathy but occurs rarely in children. Other causes of isolated nephrotic syndrome can be subdivided into two major categories: rare genetic disorders, and secondary diseases associated with drugs, infections, or neoplasia [5]

Idiopathic nephrotic syndrome

Idiopathic nephrotic syndrome remains of unknown cause, but evidence suggests it may be a primary T-cell disorder that leads to glomerular podocyte dysfunction. Genetic studies in children with familial nephrotic syndrome have identified mutations in genes that encode important podocyte proteins. Nephrotic syndrome can occur primarily or due to systemic diseases. The most common cause of nephrotic syndrome in children is minimal change disease. The most common primary causes in adults are focal segmental glomerulosclerosis (FSGS), minimal change disease, and membranous nephropathy. Approximately 30 percent of adults have secondary nephrotic syndrome due to diabetes mellitus, SLE, or amyloidosis [5]

Classification of Idiopathic NS depends on response to steroid therapy, the pattern of relapses, histopathology, or genetic etiology. While the cause remains unknown, the pathogenesis of idiopathic NS is thought to involve immune dysregulation, systemic circulating factors, or inherited structural abnormalities of the podocyte. Approximately 80%–90% of patients with childhood idiopathic NS achieve remission with steroid therapy (SSNS). However, approximately 50% [8]

Minimal change disease (MCD):

Most common pathology found in childhood nephrotic syndrome (77-85% of cases). Usually idiopathic, light microscopy of renal biopsy samples shows no change. On electron microscopy, effacement of the foot processes can be seen. Immunofluorescent staining for immune complexes is negative [9]

Focal segmental glomerulosclerosis (FSGS):

Focal segmental glomerulosclerosis (FSGS) is a clinical-pathological entity characterized by steroid resistant nephrotic syndrome (SRNS) and segmental sclerosis involving some but not all the glomeruli. It accounts for 7%-20% of cases of idiopathic nephrotic syndrome in the pediatric age group and is frequently associated with hematuria and hypertension, although in early stages it is indistinguishable from minimal change disease (MCD). Primary FSGS can present at any age. Children over six years have a higher incidence than younger children. The incidence of FSGS is rising worldwide. In Canada, they reported an incidence of 0.37– 0.94 new cases per 100,000 children per year [10]

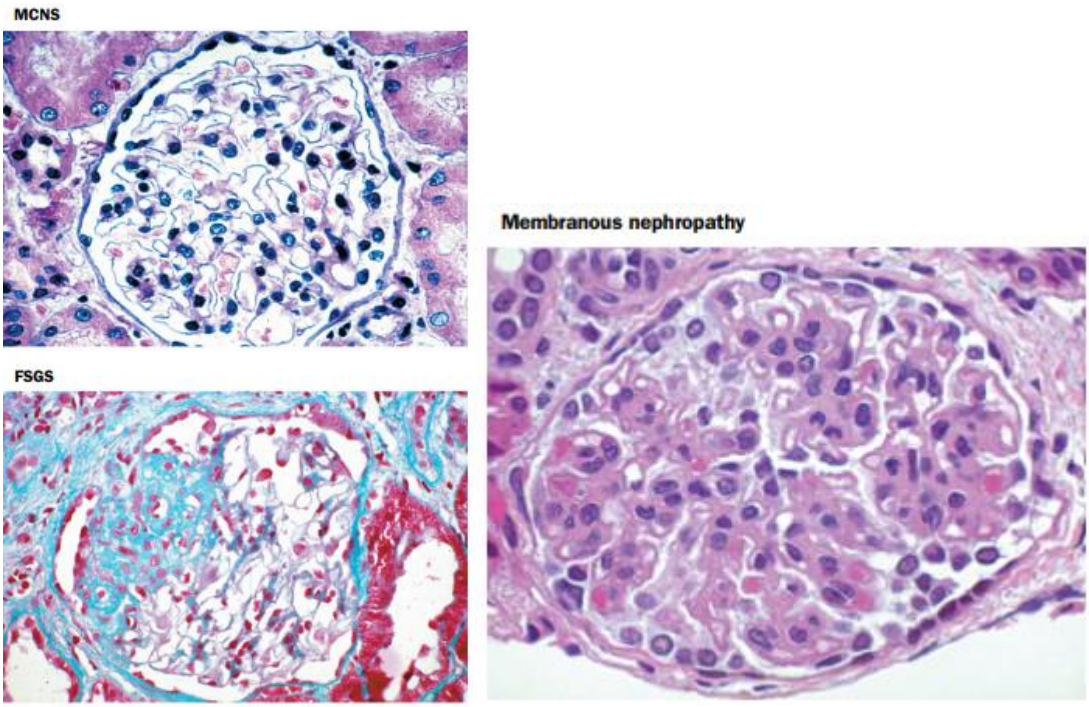
FSGS is usually steroid resistant, however 15%– 20% of FSGS patients initially respond to steroids. It is a clinical condition with a highly variable outcome; 50% reaching end stage kidney disease by the age of 15. In a multicenter study from Jordan, FSGS was the most common glomerulonephritis leading to end-stage renal disease (ESRD) in children as it was responsible for 10.2% of all cases of ESRD [11]

Membranoproliferative glomerulonephritis (MPGN):

It is more commonly presents as nephritic syndrome, involves immune complex deposition. Granular pattern seen on immunofluorescence staining, while in light microscopy, thickened basement membrane can be seen [4]

Membranous glomerulonephritis (MGN)

Accounts for just 2-4% of cases in children, it is the most common type in adults. Like membranoproliferative disease, thickened basement membrane and granular pattern on immunofluorescence can be seen. On electron microscopy, characteristic " spike and dome" appearance seen, with membrane deposition growing around subepithelial immune complex deposition, it can be a primary disease, or due to several other causes [12]



A biopsy taken from nephrotic syndrome lesions, trichrome stain [6]

Minimal change disease

Minimal change disease (MCD) is the most common type of nephrotic syndrome in children, whereas it only accounts for 10–16% cases in adults. The term MCD refers to a histological pattern characterized by the normal or near-normal appearance of glomeruli on light microscopy and immunofluorescence with podocyte foot process effacement (FPE) on electron microscopy as the sole abnormality observed in kidney biopsy. While histological findings are similar in children and adults with MCD, the clinical response to steroids, considered as first-line therapy, is different. Most children achieve resolution of proteinuria within days, whereas it can take months in adults. Therefore, a kidney biopsy is only performed in selected pediatric cases, whereas it is mandated in all the adults to rule out other forms of nephrotic syndrome including infections, malignancies, or other glomerular diseases [13]

Pathophysiology of minimal change disease

Podocyte foot process effacement is the ultrastructural hallmark in MCNS, however, the pathogenesis leading to podocyte effacement is not clear. Systemic factors, immune mediated or circulating, and local factors can contribute to podocyte effacement, but there is no single unifying hypothesis [14]

Immune dysregulation

As immunosuppression with corticosteroids is the mainstay of NS therapy, it is logical to suspect immune dysregulation as a pathogenic factor in disease development. Shalhoub [15] postulated that MCNS is a disorder of T-cell function, resulting in increased plasma levels of lymphocyte-derived permeability factor [16]

Role of cytokines

Investigators have made attempts, based on Shalhoub' s hypothesis, to identify the circulating factors released from T-cells that increase glomerular permeability to serum proteins, and some studies have confirmed that capillary permeability factor is detectable in patients with NS. Of the various factors presumed to increase glomerular permeability to serum proteins, the most likely pathogenic factors are cytokines, which are small proteins secreted by the cells of both the innate and adaptive immune systems that transfer information within the immune system [16]. Patients who suffered relapses were found to have elevated serum or urine levels of various cytokines, including interleukin (IL)-2), soluble IL-2 receptor), interferon-gamma), IL-8), IL-13), tumor necrosis factor-α), and vascular endothelial growth factor). Of the many known cytokines, IL-8 and IL-13 have been proposed to be most likely to be circulating factors. IL-8 may play a role in proteinuria by affecting the metabolism of glomerular basement membrane (GBM) components). Additionally, urinary IL-8 levels were higher in patients who suffered relapses and had a positive correlation with the degree of

proteinuria [14]. IL-13 has been revealed to stimulate intracellular podocyte protein trafficking and proteolysis [17]

Role of regulatory T-cells (Triggers) and B-cells

Historically, MCNS has been considered a T-cell disease; however, advances in the study of basic immunology have contributed to a more articulated understanding of its pathogenesis considering triggers and B-cells. Normally, cytokine release by T-cells is transient owing to the activation of triggers that interact with T effector cells to suppress cytokine production. Triggers have been suggested to constitute a second step in an MCNS cascade, of which the first remains unclear.

The induction of triggers led to a marked reduction in proteinuria in animal models, and most patients with MCNS showed decreased levels of Triggers) [18]

Unlike role of T-cells in MCNS, which has been extensively studied, the role of B-cells is currently not well understood. Clinical trials have been conducted that demonstrated MCNS remission after B-cell depletion using the anti-CD20 monoclonal antibody rituximab) [19]. The recent successful use of anti-CD20 monoclonal antibodies for the treatment of steroid sensitive NS raises the possibility of B-cells either influencing T cells or themselves being primary players in NS. CD80 is expressed by both activated B- and T- cells, and increased nitric oxide production by B cells observed in NS patients with relapse further supports the possibility of B-cell involvement). Altogether, these results revealed that not only T cells, but also B-cells or B-cell products might be implicated in the causal mechanism of MCNS via the abnormal regulation of T-cell function by circulating B-cells or by communication between B- and T-cells). However, information on the role of B-cells is currently limited [20]

Clinical picture of nephrotic syndrome

Edema:

is the dominant finding; the location of the most prominent edema can be quite variable between children. Rarely, the edema may be subtle despite massive proteinuria. The examination should include looking for findings suggestive of a syndromic condition, malignancy, or SLE or complications of nephrotic syndrome (blood clot, peritonitis, or cellulitis). Hypertension may be present, especially in patients with FSGS or MPGN. Patients typically present with insidious onset of edema, which may not be detected until significant fluid accumulation has occurred. The edema is gravity dependent, and thus periorbital edema, occasionally misdiagnosed as allergies, is more prominent in the morning. After daytime ambulation, edema becomes more noticeable in the feet and legs. Scrotal, penile, and labial edema can be particularly distressing [21]

Although the chief complaint in nephrotic syndrome is usually edema, other clinical presentations may occur: on urine analysis or when screening due to a positive family history.

Complication of nephrotic syndrome.

1- Blood clot (pulmonary embolus, deep venous thrombosis, arterial thrombosis).
2- Spontaneous bacterial peritonitis and Cellulitis.
3- Abdominal pain due to bowel wall edema or hypoperfusion [4]

Investigations:

1- **Complete blood count:** Elevated hemoglobin may occur due to hemoconcentration from intravascular volume contraction due to fluid shifting into the interstitial space. Anemia may be present if there is chronic kidney disease or a secondary cause (SLE or malignancy). Depression of white cells or platelets also raises concerns of SLE or malignancy.
2- **Electrolytes:** Hyponatremia, typically mild is a common finding due to secretion of antidiuretic hormone secondary to intravascular volume depletion. Serum total calcium is low due to the low serum albumin since ~50% of calcium normally binds to albumin. The ionized calcium is typically normal, but may be low, possibly due to urinary loss of 25- hydroxyvitamin D [22]
3- **Kidney function test** is usually normal in idiopathic nephrotic syndrome, although it may be diminished if there is significant intravascular volume depletion. Elevated serum creatinine not related to intravascular volume depletion is more common with FSGS and in secondary nephrotic syndrome [22]
4- **diagnostic investigation:**
   a- **URINE:**

proteinuria: 3 + or 4+ selective or urinary protein (mg)\urine creatinine (mg) ratio in spot sample >2. **24hr urine collection** for protein >40mg\m\hr. OR 50mg\kg\24hr

Volume oliguria (during stage of edema formation) defined as urine output that is less than 1ml/kg/h in infant, less than o.5ml/kg/h in children.

Microscopically:

Macroscopic hematuria is rare, occurring in 3of patient.

Microscopic hematuria is present 20 of cases.

Fat bodies,

Large number of hyaline casts

b- Blood

1- **Serum protein** decrease <5.5gm/dL, **serum albumin level**; <2.5gm/dL
2- **Serum cholesterol and triglycerides, high cholesterol** >200gm/dL
3- **Serum complements levels:** Low C3 is common in MPGN, C3and C4 are typically low in lupus nephritis. A low C3 is also consistent with post infectious glomerulonephritis, which may occasionally be confused with idiopathic nephrotic syndrome [(23)]

Chest radiograph: This may rarely detect evidence of malignancy. Small pleural effusions are almost universally present and do not require intervention [(24)]

Evaluations Done in Select Patients

I -Renal biopsy in nephrotic syndrome: Kidney biopsy has a cardinal role in the management of kidney diseases. Biopsy proven kidney diseases impart valuable information about incidence, distribution, and possible control of disease with effective and directed treatment. Now, the procedure in children is regarded safe and advisable. Therefore, now more biopsies are performed even in younger children as compared with the past [(25)]

The indications of renal biopsy in nephrotic syndrome (NS):

1-Childern less than 1year or more than 11year of age
2-incorporate nephrotic syndrome with nephritic syndrome
3-unexplained kidney failure with normal size kidneys
4-Macroscopic hematuria.
5-Acute kidney failure and chronic kidney disease (CKD)
6-Asymptomatic urinary abnormalities,
7-Rapidly progressive glomerulonephritis (RPGN).
8- Steroid resistant NS
9- Low serum C3 level (secondary NS)
10- Frequent relapsing NS, before considering alternative therapy
11-Presistence HTN [(26)]

ii- Antinuclear antibody: Screens for SLE in older children and adolescents or if there are signs and symptoms suggestive of SLE. Additional serologies for SLE may be indicated if suspicion is high [(27)]

iii- Viral testing (HIV, hepatitis B and C): Screen in children with CNS, older children, and adolescents or if biopsy findings suggest infection as a possible cause [(22)]

iv- Genetic testing: This is most useful in congenital and infantile nephrotic syndrome, syndromic disease, familial disease, or if there is a history of consanguinity. Genetic testing is increasingly used in children with steroid-resistant disease [(28).]

v- Histology-specific testing: There are specific additional tests that should be considered in patients diagnosed with MN and MPGN [(4)]

Steroid sensitive nephrotic syndrome in children

Definition

A rare primary glomerulopathy of unknown cause characterized by edema, nephrotic-range proteinuria and hypoalbuminemia that responds to standard prednisone treatment within 4-6 weeks [(29).]

Epidemiology

The annual incidence of idiopathic nephrotic syndrome varies by ethnicity and region ranging from 1/5,900-85,000 children, of which 70- 98% are steroid sensitive. The disease is less frequently reported in adults. It has a male predominance (approximately 2:1) in young children [30].

Clinical description

Disease onset can occur at any age, but mostly between 2 - 6 years, frequently preceded by an upper respiratory tract infection. Patients typically present with edema, mainly periorbital and of lower extremities. Anasarca may develop with pleural and pericardial effusion, ascites, abdominal pain (due to hypoperfusion) and cold extremities with hypotension. Intravascular volume depletion and oliguria are present, and concomitant factors, (sepsis, diarrhea, diuretic use) can lead to acute kidney **[31].**

Steroid resistance nephrotic syndrome in children

More than 85% of patients with idiopathic nephritic syndrome respond (i.e., complete remission of proteinuria and normal serum albumin) following treatment with prednisolone.1 Response to prednisolone is an important prognostic indicator for survival of kidney function. Although many patients with steroid-sensitive nephritic syndrome have frequent relapses or steroid dependence, the long-term outlook for kidney function is favorable. The main long-term problem in these patients is the risk of side-effects from prolonged treatment with corticosteroids and other immunosuppressive medications [32].

Patients who do not respond to prednisolone are considered to have steroid-resistant nephrotic syndrome (SRNS). The medical community has not yet reached a consensus regarding the length of time prednisolone should be given before regarding a patient as steroid resistant. Steroid resistance should be considered in patients who do not show complete remission of proteinuria despite treatment with prednisolone at a dose of 60 mg/m2 daily for 4 weeks. Others recommend treatment for 8 weeks **[33].** Several centers, including the Great Ormond Street Hospital for Children, London, UK, administer three intravenous pulses of methylprednisolone (500 mg/m2) before regarding patients as resistant. Steroid resistance most often occurs during initial treatment with prednisolone (initial resistance), but can also occur during treatment for a relapse, in a patient who had previously responded to treatment with steroids or with a second-line drug (late resistance). Steroid resistance is an important determinant of future risk for end-stage renal disease [34]

Cause of SRNS

Genetic causes

The medical community has learnt much about the genetic causes for SRNS over the past decades. Mutations in more than 70 genes encoding key podocyte proteins are recognized to cause the illness. Despite recognition of an increasing number of genetic causes, only 30% of patients with sporadic SRNS show a defined mutation **[35].**

Circulating factor

The probable cause for most patients with non-genetic SRNS is thought to be a circulating factor [36]. Circumstantial evidence exists that makes this theory quite probable, but it has been elusive to define the circulating factors responsible for SRNS [37]. A large proportion of children with non-genetic SRNS relapse quickly after kidney transplantation: these patients often respond to plasma exchange or immune adsorption. Small animals infused with patient plasma, whole or its fractions, also develop proteinuria. Other important suggestions of circulating factors include hemopexin, interleukin-13, cardiotrophin-like cytokine-1, and soluble urokinase-type plasminogen activator receptor [38].

Kidney biopsy

Renal histology is an important tool for diagnostic and prognostic categorisation. Biopsies should be examined by light, immunofluorescence, and electron microscopy to define their histological features. An adequate biopsy should have approximately 25 glomeruli, especially when evaluating lesions that are focal or segmental. Biopsies with fewer glomeruli have low diagnostic accuracy. Common histological diagnoses include focal segmental glomerulosclerosis (FSGS) in 35–55% of patients, minimal change disease in 25–40% of patients, and idiopathic mesangioproliferative glomerulonephritis in 10–15% of patients (figure 9). In about 20% of patients, the histology shows membranous nephropathy, immunoglobulin A nephropathy, or proliferative glomerulonephritis [39].

Patients with SRNS mostly require treatment with calcineurin inhibitors that can cause nephrotoxicity, which is characterised histologically by microvascular ischaemia and striped interstitial fibrosis.

Complications of Nephrotic Syndrome

1-Infections: -

Patients with NS are at increased risk for infections. Although the incidence of infections in NS has decreased in advanced countries, they are still a major problem in developing countries. Sepsis remains one of the main causes of death in children with NS. Children treated with cytotoxic drugs have a higher clinical infection rate than those treated only with prednisolone. In children with NS, Streptococcus pneumoniae is known to be the most important organism in primary peritonitis. However, other organisms such as β-hemolytic streptococci, **Haemophilus and Gram-negative bacteria** are also frequently found. Cellulitis is also the result of βhemolytic streptococci or a variety of Gram-negative bacteria.

Nephrotic patients are liable to infection because: -

A- loss of immunoglobins in urine and an impaired synthesis.
B- B- ImpairedT lymphocyte function
C- C-The edema fluid act as a culture medium
D- D-Use corticosteroid and immunosuppressive agent E- Malnutrition [(40)]

Pneumococcal vaccines against capsular antigens are recommended for all children with NS, but vaccination should be administered when the treatment with high doses of CS or with cytotoxic therapy is discontinued. Nephrotic children taking high-dose CS or other immunosuppressive agents within three months of their use are at risk of varicella infection, requiring varicella zoster immunoglobulin treatment within 72 hours of exposure and intravenous acyclovir during active varicella zoster infection [(22)]

Treatment of nephrotic syndrome

1- Control Diet in nephrotic syndrome.

Protein

The metabolic disorder in the nephrotic syndrome includes discharge of protein reserves of the plasma and tissues. A review of the literature shows that high-protein diets (2-3 mg/kg/day) cannot increase the plasma level of albumin and only increase the urine protein level. Moreover, a high-protein diet can cause glomerular hypertrophy and hyperfiltration, resulting in more glomerular damage [(41).] However, a low-protein diet (e.g. 0.8 mg/kg/day) may cause a significant decrease in the urine protein level and a significant increase in the serum albumin level, which is the reason why recommend a low protein diet (e.g. 0.6-0.8 mg/kg/day). In addition to the amount of protein, its source and compound are important, too. In fact, plant proteins have less effect on glomerular permeability and hemodynamics than animal proteins. Reviews show that soybean may significantly decrease proteinuria and improve blood lipids [(41).] Protein at a dose of 0.8 mg/kg/day is the most effective diet in nephrotic patients [(41).]

Lipid

In a patient suffering from nephrotic syndrome, hyperlipidemia occurs due to excessive lipid synthesis and disrupted lipid metabolism. Nephrotic syndrome has a marked effect on triglyceride and cholesterol. The level of LDL and VLDL usually increases while the level of HDL remained unchanged or decreases. The severity of these disturbances depends on the severity of proteinuria. Hyperlipidemia may accelerate kidney damage in addition to increasing the risk of cardiovascular diseases. For this reason, correction of hyperlipidemia is one of the treatment goals in nephrotic syndrome. Many studies have shown that low-fat diets (calorie intake<30%, and cholesterol<200mg/day) can improve hyperlipidemia by 25% in adults, but there is not enough evidence in children. In addition to the amount, the quality and type of the lipid are very important, too. For example, fish oil omega 3 has a beneficial effect on the cardiovascular system and can decrease systolic blood pressure, [(42).]

Fluid and salts

Edema is an important clinical manifestation in children with nephrotic syndrome. There are two theories of overfilling and underfilling about the pathophysiology of edema neither of which has been proven definitely. The involvement of each of these two theories seems to depend on the edema severity. Edema occurs due to water and salt retention and water and salts restriction is the main treatment method. The amount of salt

should be restricted in a range of less than 2 grams/day and water consumption should be limited to less than 1-1.5 liters/day. In case of no recovery, diuretic therapy should be considered. It is recommended that weight loss should not exceed 0.5 to 1 kg/day because of the risk of thromboembolism [43].

Minerals and vitamins

Nephrotic syndrome patients have iron, copper, zinc, and calcium deficiency due to increased urine protein excretion or metabolism disorders. For example, increased excretion of ferritin as an iron transportermay cause iron deficiency anemia. On the other hand, released iron from transferrin can produce free oxygen radicals that can harm the tubulointerstitium. For these reasons, iron should be administrated carefully. Urine excretion of erythropoietin can exacerbate the patient' s anemia Almost 95% of copper is carried by ceruloplasmin in the serum. For this reason, any increase in the urine excretion of ceruloplasmin can cause copper deficiency. Copper deficiency has no clinical manifestations in most of the nephrogenic syndrome cases and does not require treatment. Zinc deficiency in nephrotic syndrome is due to hypoalbuminemia, excessive excretion, and intestinal absorption disorders. Clinical manifestations of zinc deficiency in nephrotic syndrome are not clear yet, but studies have shown that administration of copper at a dose of 10 mg/day reduces the recurrence rate of nephrotic syndrome. Vitamin-D deficiency in nephrotic syndrome is common and causes hypocalcemia, hyperparathyroidism, and decreased bone density. Bone density is even worsened due to the use of corticosteroids in nephrotic syndrome. For this reason, it is recommended that patients on corticosteroid therapy receive 1000 IU vitamin D and 500 mg/day calcium for 12 weeks [42].

Foods to eat on a nephrotic syndrome diet.

Lean meats (poultry, fish, pork, lamb, veal, shellfish, egg), dried beans (azuki, lentils, mungo, navy, pinto, soy, split peas), soy products (natto, tempeh, tofu), nuts, fresh or frozen fruit (apples, blueberries, pears, pineapple, peaches, strawberries, mandarin orange, passion fruit), fresh or frozen vegetables (green beans, lettuce, bean sprouts, green onion, cucumber, cabbage, Chinese peas, asparagus, green peppers, corn, cauliflower), low-sodium canned vegetables, potatoes, rice, tortilla, low sodium bread, pasta, unsalted snacks (nuts, popcorn), low salt cottage cheese, low fat milk, heart healthy oils (olive, canola, safflower), ketchup, herbs and spices, vinegar, lemon juice, and no- or low-sodium seasoning blends. If a recipe calls for garlic salt, substitute with fresh garlic or garlic powder [44].

Foods to avoid on a nephrotic syndrome diet.

Cheese, high-sodium, or processed meats (SPAM, Vienna sausage, bologna, ham, bacon, Portuguese sausage, hot dogs), frozen dinners, canned meats, or fish, dried or canned soups, pickled vegetables, lomi salmon, salted potato chips, popcorn and nuts, salted bread. Keep in mind that certain seasonings and condiments are high in sodium, such as MSG, Worcestershire sauce, bouillon cubes, olives, pickles, mustard, oyster sauce, patis, bagoong, and soy sauce. High-fat foods such as fried food, excess oil, mayonnaise [44]

2. Albumin:

Is indicated in clinical hypovolemia and symptomatic edema. A low serum albumin is not an indication for intravenous albumin. If there is evidence of hypovolemia, give 1 gm/kg 20% albumin (5 ml/kg) over 4 to 6 hours. Give 2 mg/kg of IV furosemide mid-infusion. If clinically shocked, give 10 ml/kg 4.5% albumin. Children should be closely monitored during albumin infusions, and where possible should be administered during working hours [45].

2- **Penicillin prophylaxis:**

Penicillin V can be given during proteinuria and discontinued when the child goes into remission. Grossly edematous children are at risk of cellulitis and may benefit from antibiotic prophylaxis [46].

Dose: Under 5 years—125 mg bid and for above 5 years 250 mg bid.

4-**Vaccination:** Pneumococcal vaccination is recommended for children with ephrotic syndrome. Varicella vaccination is only available on a named patient basis [47].

5- steroid: Should be restarted daily (60mg/m2) (maximum80mg)until the urine is negative or trace for 3days,then 40mg/m2/on alternate day for 4 weeks (maximum60mg)then stop or taper the dose over4to 8 weeks Treatment of Relapse Nephrotic Syndrome Up to 60 to 70% of children with nephrotic syndrome may have one or more relapse. These are diagnosed if there is +++ or ++++ proteinuria for 3 or more days. Urine

should be checked initially twice weekly, then weekly after the first episode, and the families should be instructed to get in contact in case a relapse of proteinuria occur, or if there is ++ for more than 1 week [48].

(**A) Frequent relapse: -**Two or more relapses in initial 6months or more than four in year.

(B) Steroid dependency: -Two consecutive relapses when on alternate day steroids or within 14 days of its discontinuation

(C) Steroid resistance: - Failure to attain remission despite 4 weeks of therapy with daily prednisolone at 60mg/m2 [48]

Treatment of Frequent relapses and steroid dependence

Patients with frequent relapses or steroid dependence should be managed in consultation with a pediatric nephrologist. It is usually not necessary to perform a renal biopsy in these cases. Following treatment of a relapse, prednisolone is gradually tapered to maintain the patient in remission on alternate day dose of 0.5-0.7 mg/kg, which is administered for 9-18 months. A close monitoring of growth and blood pressure, and evaluation for features of steroid toxicity is essential [49].

Side effect of steroid (Steroid toxicity): -

1- Hyperglycemia 2- Hypertension 3-Hyperlipidemia 4-cushingoid changes 5-growth impairment 6-osteoporosis 7-Psychosis 8- Osteonecrosis 9- Opportunistic infection If the prednisolone threshold, to maintain remission, is higher than 0.5 mg/kg on alternate days or if features of corticosteroid toxicity are seen, additional use of the following immunomodulators is suggested [50]

(a) Levamisole

It is administered at a dose of 2-2.5 mg/kg on alternate days for 12- 24 months. Cotreatment with prednisolone at a dose of 1.5 mg/kg on alternate days is given for 2-4 weeks; the dose is gradually reduced by 0.15-0.25 mg/kg every 4 weeks to a maintenance dose of 0.25-0.5 mg/kg that is continued for six or more months. Occasionally, it might be possible to discontinue treatment with corticosteroids. The chief side effect of levamisole is leukopenia; flu-like symptoms, liver toxicity, convulsions. and skin rash are rare. The total leukocyte count should be monitored every 12-16 weeks [51].

(b)Alkylating agent

Treatment of Frequent relapses and steroid dependence

Cyclophosphamide (CYC)

It is administered at a dose of 2-2.5 mg/kg/day for 12 weeks. Prednisolone is co-administered at a dose of 1.5 mg/kg on alternate days for 4 weeks, followed by 1 mg/kg for the next 8 weeks; steroid therapy is tapered and stopped over the next 2-3 months. Therapy with cyclophosphamide should be instituted preferably following remission of proteinuria. Total leukocyte counts are monitored every 2 weeks; treatment with cyclophosphamide is temporarily discontinued if the count falls below 4000/mm3. An increased oral fluid intake and frequent voiding prevents the complication of hemorrhagic cystitis; other side effects are alopecia, nausea, and vomiting. The risk of gonadal toxicity is limited with a single (12 weeks) course of cyclophosphamide. The use of more than one course of this agent should preferably be avoided. In view of its toxicity, the use of chlorambucil, unless under close supervision, is not recommended [52].

Chlorambucil (CHL):

Is indicated at a dose of 0.1-0.2 mg/kg/day for 8 weeks with a maximum cumulative dose of 11.2 mg/kg [53].

Calcineurin inhibitors (CNI):

There are two mechanisms of the antiproteinuric effect exerted by calcineurin inhibitors (CNI):

(1) through inhibition of T-cell signalling in lymphocytes.

(2) direct non-immune effects on the podocyte actin cytoskeleton there many types: -

Cyclosporin (CsA): - is given at a dose of 4-5 mg/kg daily for 12-24 months. Prednisolone is co-administered at a dose of 1.5 mg/kg on alternate days for 2-4 weeks; its dose is gradually reduced by 0.15-0.25 mg/kg every 4 weeks to a maintenance dose of 0.25-0.5 mg/kg that is continued for 6 or more months. Occasionally, treatment with corticosteroids may be discontinued. Estimation of trough blood levels of CsA is required in patients with suspected noncompliance, unsatisfactory response, or nephrotoxicity (increase in serum creatinine by 30% or more from the baseline). Trough (12-h) CsA levels should be kept between 80 and 120 ng/ml. Side effects of CsA therapy include hypertension, cosmetic symptoms (gum hypertrophy, hirsutism),

and nephrotoxicity; hypercholesterolemia, and elevated transaminases may occur. Estimation of serum creatinine is required every 2-3 months and a lipid profile annually. A repeat kidney biopsy, to examine for histological evidence of nephrotoxicity, should be done if therapy with calcineurin inhibitors is extended beyond 2 years [(54).]

Tacrolimus: - is an alternative agent, administered at a dose of 0.1-0.2 mg/kg daily for 12-24 months. Side effects include hyperglycemia, diarrhea, and rarely neurotoxicity (headache, seizures). The use of tacrolimus is preferred especially in adolescents, because of lack of cosmetic side effects. Blood levels of creatinine and glucose should be estimated every 2-3 months [(55).]

Mycophenolate mofetil (MMF)

Mycophenolate mofetil (MMF) can inhibit the de novo pathway of guanosine nucleotide synthesis which is important in the proliferation of Tand B-lymphocytes. Mycophenolate mofetil is given at a dose of 800-1200 mg/m2 along with tapering doses of prednisolone for 12-24 months. The principal side effects include gastrointestinal discomfort, diarrhea, and leukopenia. Leukocyte counts should be monitored every 1-2 months; treatment is withheld if count falls below 4000/mm3. [(56)]

Choice of agent

The advantages of using these drugs should be balanced against their potential toxicity. There are few studies comparing one agent with another, but evidence for efficacy is strongest for cyclophosphamide and CsA.

Levamisole has a modest steroid sparing effect and is a satisfactory initial choice for patients with frequent relapses or steroid dependence. Treatment with cyclophosphamide is preferred in patients showing:

(i)significant steroid toxicity.

(ii) severe relapses with episodes of hypovolemia or thrombosis.

(iii) poor compliance or difficult follow-up, where 12 weeks therapy might be possible to ensure than long-term compliance.

Treatment with CsA or tacrolimus is recommended for patients who continue to show steroid dependence or frequent relapses despite treatment with the above medications. Either of these agents is effective in maintaining remission in most patients with steroid-sensitive nephrotic syndrome.

The chief concern with their use is nephrotoxicity, but with careful assessment of renal function, minimizing the maintenance dose and utilizing renal biopsies in those receiving prolonged therapy, this risk can be minimized. Recent case series and a controlled trial support the use of MMF as a steroid sparing agent. The lack of renal, hemodynamic and metabolic toxicity with this agent makes it an attractive alternative to calcineurin inhibitors. In some patients receiving therapy with levamisole, MMF and calcineurin inhibitors, treatment with prednisolone might be tapered and discontinued after 6-12 months. Some patients who respond to therapy with levamisole, MMF, and calcineurin inhibitors may relapse once these medications are discontinued. Relapses during or following therapy with these agents are treated with prednisolone as described above [(48)]

Treatment of steroid resistance Nephrotic Syndrome

Prolonged steroid treatment

The initial response to conventional doses of glucocorticoid therapy for idiopathic FSGS is poor in contrast to that of minimal change glomerulopathy. In most studies published, the response rate has been less than 30%. Using a more prolonged course of prednisolone therapy, 44% of children with idiopathic FSGS entered complete remission. The available evidence on the efficacy of such treatment is inconsistent and therefore it is more appropriate, effective and gentler to the patient to introduce other immunosuppressive drugs at this juncture [(57).]

High-dose intravenous methylprednisolone

Methylprednisolone administered intravenously either daily or on alternate days at a dosage of 1 g/1.73 m2 body surface areas to a total of 3- 6 doses is effective in the treatment of renal allograft rejection and some forms of rapidly progressive glomerulonephritis. It has succeeded in a small number of cases in inducing remission in children with NS who have not responded to a conventional course of oral steroids. This treatment is usually well tolerated, especially if given on alternate days, and some prefer to try this approach before exposing patients to the multiple toxic side-effects of the other drugs discussed below [(39).]

Alkylating agent: - include Cyclophosphamide (CYC); chlorambucil (CHL):-

Evidence suggests that steroid-resistant children with MCD are more likely to respond than those with FSGS. Because of the potentially serious consequences of failure to induce remission of proteinuria, it is worth a trial of a standard course of cyclophosphamide (CYC) before abandoning a child to the prospect of renal replacement therapy. Additional strength is given to this argument because children with FSGS who receive transplants have a high incidence of early recurrence of their original disease in the graft, often leading to graft loss. One study group has treated children with SRNS with a prolonged course of intravenous methylprednisolone, combined with chlorambucil (CHL) or CYC if the steroid alone failed to induce a remission after 10 weeks of treatment [(57).]

Levamisole

Levamisole-induced changes in the immune-mediated response may cause reduction of relapses in predominantly immune mediated diseases. This may be one explanation for its ineffectiveness in cases of steroidresistant nephrotic syndrome [(58)]

Calcineurin inhibitors (CNI): -

Cyclosporin A(CsA): A has successfully induced remission in children with SRNS due to FSGS, in whom previous attempts to control the disease with alkylating agents had failed. Increasingly CsA has been used as second-line treatment for corticosteroid-resistant FSGS. Higher proportion of sustained remissions have been achieved with CsA administered in conjunction with alternate day corticosteroids than CsA alone. CsA was given in a starting dose of 150 mg/m2/day or 3-5 mg/kg in two divided doses, adjusted to achieve a trough plasma CsA concentration of 100-200 ng/ml. Prednisone is given at 30 mg/m2/day, also in two divided doses, for the first month, followed by 30 mg/m2 on alternate days for 3-6 months. However, many children relapse following discontinuation of CsA therapy, making them CsA dependant or increasing steroid sensitivity in steroid-resistant children [(59)]

Tacrolimus: - use in patients who were resistant to both steroids and cyclosporine [(57).]

Vincristine

A handful of children with steroid and alkylating agent-resistant nephrotic syndrome have lost their proteinuria following treatment with the antibiotic alkaloid vincristine. In most cases the drug was given with steroids and a second or third course of an alkylating agent. It is therefore impossible to be certain whether the success of the treatment was due to vincristine or the other simultaneously administered drugs. Vincristine is neurotoxic and must be given intravenously, its effect on tissues being like that of mustine if extravasation occurs. It cannot be said to have an established place in the management of SRNS but encouraging results are emerging **[(60)]**

Rituximab The chimeric anti-CD20 monoclonal antibody Rituximab is used for B cell lymphomas. The mechanism of action of rituximab in NS is not yetclearly illustrated; however, one hypothesis says that its effect on T cells causes a lasting effect to reduce proteinuria. Several studies reported their results for rituximab use in steroid-dependent (SDNS), frequently-relapsing (FRNS) and steroid-resistant NS. From these, results for SDNS are the most successful with 12-16 months sustained remission whereas they are less effective but still encouraging in SRNS patients. rituximab gives no benefit when used as add-on therapy to steroids and calcenurin inhibitors in SRNS. Though rituximab seems to be a potent drug in NS, further randomized studies would shed more light on its use in SRNS. [(61)]

Mycophenol atemofetil

Since MMF is neither nephrotoxic nor gonadotoxic it might seem a good option to treat children who show these adverse effects due to prolonged treatment. Though not many studies have been done to investigate MMF' s efficacy in SRNS, reduction of proteinuria by MMF better than cyclosporine A in a small group of SRNS patients with major steroid-sparing effects and no toxicity [(62).]

Renal transplant

Kidney transplantation is the most ideal renal replacement therapy for children with SRNS. Children with SRNS run the risk of recurrence of nephrotic syndrome following transplantation. Recurrence of nephrotic syndrome, if not controlled, can cause delayed graft function, acute rejection, and diminished allograft

survival. Most of the information regarding post-transplant outcomes comes from pediatric patients with FSGS, the most common histopathologic finding in patients with SRNS.

The risk of recurrence of FSGS

Post-transplant is ~30%. Risk factors for recurrence of FSGS post-transplant include childhood-onset SRNS, rapid progression to End-Stage Renal Disease (ESRD) and recurrence following a previous allograft. The concept of a circulating permeability factor as the pathogenic mechanism of SRNS arose following the frequent observation of SRNS recurrence following renal transplantation. Some investigators have found that initiation of plasmapheresis may induce remission in 50%– 90% of recurrent cases. The favorable outcomes reported with plasmapheresis in patients with recurrent SRNS have resulted in the use of this treatment in primary SRNS pre transplant. Although. Dependent on the local practices of renal transplantation programs, plasmapheresis may be part of the pre- or postoperative protocol in patients with SRNS [(63).]

Treatment of Congenital Nephrotic Syndrome

Goals of treatment are to control edema with a combination of albumin infusions and diuretics and to prevent and treat complications such as infections and thromboembolism. Nutritional support and thyroxine and cholecalciferol to replace urinary losses are important to promote growth and development. Children eventually need kidney transplantation, which is commonly preceded by nephrectomies to address hypercoagulability and hyperlipidemia if the patient remains nephritic [(4).]

New advance treatment: -

Stem cells (SCs) are described by their self-renewal abilities and the capability to develop into various functional cells under certain conditions. Based on the advantages of plasticity, infinite amplification, and ease of genetic manipulation, stem cell therapy opens new avenues for almost all human diseases. The application of SC therapy in treating a variety of diseases such as immunological, vascular, cardiac, and renal diseases has been extensively explored. The use of SCs is a promising therapeutic strategy for kidney diseases as well. Increasing results obtained in models of acute kidney injury (AKI) and chronic kidney disease (CKD) document that SCs have therapeutic potential in repair of renal injury, [(64)]

Prognosis of nephrotic syndrome

The prognosis of children with NS depends on underlying histopathology but can be better by response to steroid treatment Steroid sensitivity is seen in 93-98% patient with MCNS and 17-30% of patient with FSGS with an average 80% children with primary NS responding to corticosteroid. Steroids resistance is seen in about 20% of NS patients. They are more prone to complication with high risk of having deterioration of kidney function and progression to end stage kidney disease needing renal replacement therapy [(65).]

For patients with minimal change pathology, the prognosis is very good, with most patients going into remission following corticosteroid treatment. 85 to 90 % of patients are steroid-responsive and may relapse placing them at risk for steroid toxicity, systemic infections, and other complications. For patients with focal-segmental glomerulosclerosis, the prognosis is grave. Generally, will progress to an end-stage renal disease requiring dialysis and kidney transplant [(1).]

The prognosis for nephrotic syndrome under treatment is generally good although this depends on the underlying cause, the age of the person and their response to treatment. It is usually good in children, because minimal change disease responds very well to steroids and does not cause chronic kidney failure. Any relapses that occur become less frequent over time; the opposite occurs with mesangiocapillary glomerulonephritis, in which the kidney fails within three years of the disease developing, making dialysis necessary and subsequent kidney transplant. In addition, children under the age of 5 generally have a poorer prognosis than prepubescents, as do adults older than 30 years of age as they have a greater risk of kidney failure [(66)]

Hemopexin

Structure of Hemopexin

Hemopexin (Hx) is a plasma glycoprotein with a molecular weight of 60-kDa and composed of a single long peptide chain formed of 439 amino acids. It is encoded by a gene formed of 11-Kb and located on human chromosome 11 (chromosome 7 in mice) and is expressed in many tissues but mainly in liver. Other sites of Hx synthesis include the nervous system, skeletal muscle, retina, and kidney. The normal concentration of

Hx in plasma is 0.5–1 mg/ml. However, this concentration increase with inflammatory conditions , as Hx is one of an acute-phase proteins. Production of Hx is controlled by the cytokines interleukin (IL)-6, IL-11, IL-1β, leukaemia inhibitory factor, oncostatin M, and tumor necrosis factor (TNF)-α (50).Hx has the highest binding affinity to heme (Kd less than pM), and, then bind to heme forming the (heme–Hx complex), then internalize in liver cells through the process of endocytosis. The only known receptor for the heme–Hx complex is called the (scavenger receptor), is related to LDL receptor and therefore, called (LRP)1. LRP1 is expressed in several cell types, including hepatocytes, macrophages, neurons, and syncytiotrophoblasts, thus indicating multiple sites of heme–Hx catabolism [67]

Functions of Hemopexin

The most useful function of Hx is heme scavenging at the systemic level, (double face of heme), which is essential for life but also highly toxic. The role of Hx in the nervous system. Hemopexin molecules cannot pass the blood–brain barrier, therefore the nervous tissue cannot utilize the plasma Hx and synthesizes its protein in situ. Furthermore, there is an importance of Hx in the immune system, as iron is one of the regulators of the immune response. Another Hx functions, not related to its heme-scavenging properties, are discussed [68]

Heme as a Double-Faced Molecule

The Heme structure consist of a ring of tetrapyrrole, with a central iron ion It is synthesized in all cells; in a series of reactions occurring in parts within the mitochondrion and in parts within the cytoplasm. The most rapid rates of heme synthesis take place in the erythroid cells of the bone marrow and in hepatocytes. In human erythroid cells, ∼300 mg of heme, which accounts for 75% of total body heme, is synthesized daily to support hemoglobin production. In hepatocytes, ∼50 mg of heme is synthesized per day and contribute to cytochrome P450, catalases, reticuloendoplasmic cytochrome B5, and mitochondrial cytochromes [69, 70]

The heme has a multitude of biologic functions, including the action as a prosthetic group in hemoproteins and as a crucial factor in the regulation of the expression of numerous proteins.

In addition to its role as a prosthetic group in the molecule of hemoglobin, heme can interact with many different apo-hemoproteins to give 306rise, for example, to hemoglobin, myoglobin, cytochromes, catalases, peroxidases, and other enzyme systems.

Heme has a role in the regulation of gene expression. Heme binds to the transcriptional repressor Bach1. Bach1 forms bind to proteins of the Maf-related oncoprotein family (MafK, MafF, MafG). The Bach1–Maf heterodimers bind to the Maf recognition element (MARE) in the regulatory region of their target genes. MAREs are found in the regulatory regions of genes involved in heme metabolism, such as oxidative stress–response genes, globin genes, heme oxygenase (HO)-1, and heme biosynthetic enzymes. Under normal conditions, when expressed with small Maf proteins, Bach1 is in the nucleus and represses transcription. However, under conditions with increased heme levels, Bach1 is exported from the nucleus to the cytoplasm, thus allowing gene expression [71]

Finally, some authors suggested that heme controls the rate of its own transport across the plasma membrane. Heme might regulate proteins like the heme. The folate importer is the heme carrier protein (HCP)1. (HCP) distribution from the cytoplasm to the plasma membrane is regulated by iron. The heme exporter includes feline leukemia virus subgroup C receptor (FLVCR). (FLVCR) expression is regulated during the differentiation of erythroid precursors according to the heme requirement, thus sense heme [72].

The toxic properties of heme are derived from its hydrophobic nature and from the iron atom contained in the porphyrin ring. Heme has lipophilic nature that allow it to intercalate into biologic membranes. Then, perturbing lipid bilayers, promoting the conversion of low-density lipoprotein to cytotoxic oxidized products. Heme also, favors iron (Fe2+), participating in Fenton reaction, a process included in production of reactive oxygen species (ROS) **[73].**

The heme overload occurs in the bloodstream under pathologic conditions. The circulating heme firstly, injure endothelial cells. Heme entraps into red blood cell membranes and shortens the erythrocyte life span, thus enhancing hemolysis. Moreover, heme activates endothelial cells by increasing the surface expression of specific adhesion molecules, as intercellular adhesion molecule (ICAM)1, vascular cell adhesion molecule (VCAM)1, and selectins, required to promote recruitment of circulating activated leukocytes at the site of

inflammation. Heme also acts as a proinflammatory molecule and starts the inflammatory cascades. Finally, free heme causes vasopermeabilization, due to partial retraction of endothelial cells of venules in the vicinity of inflammation, leaving a small intercellular gap. Vascular leakage results in slower blood flow by allowing the passage of water, salts, and small proteins from the plasma into the damaged area. By binding heme with high affinity, Hx participates both in heme recycling, thus preventing iron loss, and in heme detoxification (74)

Hemopexin in Heme Recycling

The ex-novo synthesis of heme is supported by the fact that; life forms have evolved efficient mechanisms to support a constant availability of iron to cells. Iron circulating in the human body corresponds to ~4–5 g and is derived mainly from continuous recycling instead of from new intake from external sources. In humans, only ~1–2 mg of iron is absorbed daily by the intestine, and, at the same time, an approximately equal amount is lost by epithelial shedding in the gastrointestinal tract and skin and through blood loss in menstruating women (75).

Among the wide range of events that account for heme recovery, erythrophagocytosis plays the major role.

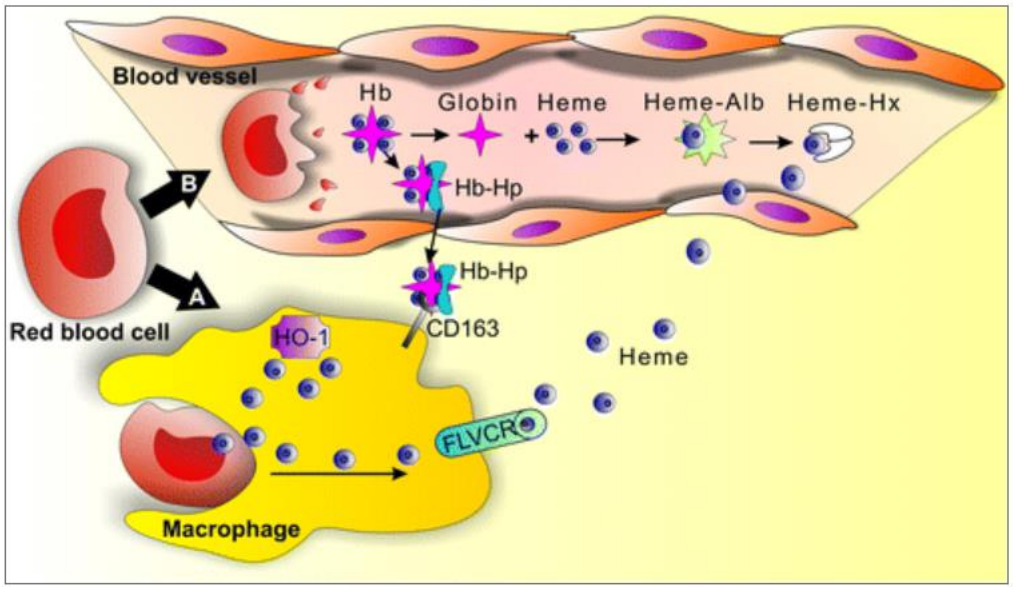


Figure: (4): Role of hemopexin in recycling of heme (68)

The term erythrophagocytosis indicates the process through which spleen macrophages, as well as bone marrow macrophages and liver Kupffer cells, normally eliminate 90% of senescent erythrocytes. Senescent red blood cells are recognized by macrophages because of changes in the erythrocyte shape and because of a series of biochemical modifications that accumulate at the red blood cell membrane during aging. After recognition, the erythrocytes undergo a process of phagocytosis and subsequent degradation of their constituents. During these events, a large amount of heme is released in the macrophage, where it is catabolyzed by the enzyme heme oxygenase (HO), anchored to the endoplasmic reticulum membrane. Moreover, it was recently reported that not all heme derived from erythrophagocytosis is degraded by HO but is in part exported to plasma. Furthermore, circulating heme can be generated by different processes, such as enucleation of mature erythroblasts, intravascular oxidation of free haemoglobin, and dietary heme absorption. Besides these physiological events, many pathologic conditions can account for the liberation of heme in the organism, including trauma, hemoglobinopathies, hemorrhages, malaria, bacterial infections, and other diseases, all associated with intravascular hemolysis (76)

In intravascular hemolysis, erythrocytes release a high amount of hemoglobin. Hemoglobin first, stimulates the formation of stable complexes with the acute-phase protein haptoglobin (hp). The haptoglobin–hemoglobin complexes are subsequently delivered to hepatocytes and macrophages of the reticuloendothelial system and internalized through CD163 receptor–mediated endocytosis. This function carried out by haptoglobin is fundamental. Haptoglobin limits the loss of hemoglobin through renal filtration, thus preventing renal iron loading (77).

After exhaustion of haptoglobin, hemoglobin undergoes a rapid conversion to methemoglobin, liberating heme. Ferriheme then binds to albumin (Kd ∼10 nM) to form methemalbumin and is subsequently transferred to Hx . Heme is initially associated with albumin because the higher molar concentration of albumin (albumin, 650 μM; Hx, 10–20 μM). However, methemalbumin is an abnormal component of plasma and has been found only when Hx-binding capacity is exceeded in diseases associated with massive hemolysis [(78).]

A role for Hx in heme catabolism was originally proposed based on two observations: (a) injection of hematin increased the catabolic rate of Hx and the plasma clearance of the protein; and (b) in human hemolytic disorders, Hx concentrations decreased, and its catabolism increased. Subsequently, several evidence supported the conclusion that heme is delivered to the liver by Hx. After intravenous injection of 55Fe-heme–125I-Hx, nearly 90% of the administered heme is transported to the liver within 2 h (Kd, 700 nM) without significant urinary excretion of either isotope. Conversely, the heme–albumin complex appears to act only as a transient heme deposit before the transport of heme–Hx complexes to the liver. No experimental evidence exists about albumin-transport functions in vivo. It was also demonstrated that heme dissociates from albumin before its hepatic uptake and catabolism. Smith and Morgan demonstrated that plasma membranes isolated from rabbit liver retained the ability to interact specifically with heme–Hx and to remove heme from the complex. Finally, specific binding of heme–Hx has been demonstrated in freshly isolated hepatocytes (Kd, 50 nM) and in a murine hepatoma cell line, Hepa (Kd, 17 nM) [(79)]

Hemopexin-mediated heme uptake by the liver has been shown in vivo and in vitro to be saturable. This tissue-specific process occurs within minutes and depends on time, temperature, and energy.

Conversely, some in vitro studies suggested that Hx delayed heme uptake by hepatocytes and failed to demonstrate that Hx is the main carrier involved in heme delivery to the liver. **Taketani et al,** [(80)] observed that, in the presence of Hx, only a small amount of heme is associated with hepatocytes, unless the molar ratio of heme to Hx exceeded 1:1. The authors concluded that Hx plays a limited role in heme uptake by cultured hepatocytes and hepatoma cells, and that heme exceeding the Hx-binding capacity is taken up directly from heme–albumin. The discrepancies between the in vivo and some in vitro data can be explained by the fact that hepatocyte cultures fail to reproduce faithfully the complex architecture of the liver and the correct polarity and differentiation state of hepatic parenchymal cells. Furthermore, heme–Hx binding sites on hepatocytes may be lost during the preparation of primary cell cultures or may be downregulated in hepatoma cell lines. Thus, it is difficult to compare different experimental systems [(81).]

The heme–Hx complex is thought to be taken up by hepatic parenchymal cells by receptor-mediated endocytosis. The only known Hx receptor is LRP1, which mediates heme–Hx internalization, resulting in cellular heme uptake. Once inside the cell, the heme–Hx complex is dissociated by lysosomal activity. LRP1 is known to recycle to the plasma membrane, whereas data on Hx turnover are controversial. Some studies suggested that Hx can be recycled as an intact molecule to the extracellular milieu, whereas others proposed that following hepatic uptake of heme from heme–Hx, a certain amount of Hx returned to the circulation, and the rest is degraded in the liver. **Hvidberg et al.** [(82)] reported that most Hx is degraded in lysosomes. Accordingly, the plasma Hx level decreases in several disorders associated with heme overload in both humans and mice [(83).]

Once delivered into the hepatocyte, heme is released into the cytoplasm and used to build new hemoproteins or is catabolyzed by HO. HO is the enzyme responsible for heme catabolism, as it breaks down the porphyrin ring to yield equimolar amounts of biliverdin, free iron (Fe2+), and carbon monoxide (CO). In mammals, biliverdin is then rapidly converted into bilirubin by biliverdin reductase.

Heme Oxygenase (HO) proteins are anchored to the endoplasmic reticulum. Three isoforms of HO have been identified (HO-1, HO-2, and HO-3), encoded by three different genes. The expression, distribution, and regulation of HO isoforms differ among cell types and tissues. The regulation of HO-3 is poorly characterized and have low heme-degrading capacity. HO-1 levels have been demonstrated to be low under normal physiologic conditions but highly inducible by several stimuli, including heme and other oxidant agents. HO-2 has been described as a constitutively expressed enzyme. The activity of HO is involved in heme detoxification and may represent a source of Fe2. This problem is overcomed by ferritin (iron sequestering protein). Ferritin is generally regulated in response to iron-level changes [(84).]

Several authors reported that the heme–Hx complex was able to induce HO-1 expression in hepatoma cells and other cell types. Heme alone or bound to albumin is a stronger HO-1 inducer. HO-1 induction after heme overload or intravascular hemolysis was stronger in Hx-null mice than in wild-type controls. **Davies et al.** [85] showed that iron derived from heme transported to hepatocyte by Heme-Hx complex is rapidly delivered to ferritin.

Hemopexin in Counteracting Heme Toxicity

The formation of heme–Hx or heme–albumin complexes allow heme to be transported in the body in a nontoxic form. After heme binding, Hx induces heme detoxification in the liver through receptor-mediated endocytosis of the complex, then heme degradation or reutilization. No receptors for the heme–albumin complex have been identified. Albumin-bound heme is thought to be transferred to Hx because of its higher affinity or, when Hx is saturated, to pass the lipophilic plasma membrane. Alternatively, heme could be up taken by cells through a heme importer [86].

Several studies demonstrated Hx as a potent antioxidant of heme. The binding of Heme to Hx has been demonstrated to reduce the production of heme-mediated free radical from organic peroxides. Furthermore, in vitro studies by **Grinberg et al.** [87] demonstrated that Hx strongly decreased the peroxidative and catalytic activity of heme through forming inactive heme–protein complexes. Moreover, heme activities were found to be inhibited by 80–90% with Hx but only by 50–60% with either human or bovine albumin. Hx binds and inhibit heme-catalyzed lipid peroxidation in artificial liposomes, rat liver microsomes, and plasma low-density lipoprotein. In vitro, it was demonstrated that Hx can prevent heme-induced oxidative damage and cell death in Hepa cells [88].

Much experimental evidence also supports the antioxidant function of Hx in vivo. Hx-null mice have been demonstrated to be particularly sensitive to heme overload and more prone to heme-induced oxidative damage and inflammation during hemolytic processes. Furthermore, in vivo studies showed that the most-damaged tissues with heme overload conditions are the vasculature, the liver, and the kidney [83]

After heme overload, Hx-null mice have shown an increased induction of the adhesion molecules ICAM1 and VCAM1 in the endothelium and an increased vascular permeability compared with wild-type mice. Hx activity is required to prevent heme-induced vasopermeabilization and endothelial activation. Heme-overloaded Hx-nullmice showed a higher expression of HO-1 in the endothelium than did wild-type animals. The induction of HO-1 before heme overload preserved endothelial integrity. In this way, Hx and HO-1 work in the same pathway to counteract the toxic effect of heme [83].

The other tissues are more sensitive to heme-mediated damage when Hx is lacking. After phenylhydrazine-induced hemolysis, Hx-null mice recovered more slowly and had severe renal damage compared with control mice. These animals had prolonged higher iron loading, hemoglobinuria, and lipid peroxidation in the kidney than did wild-type mice. Additionally, after heme injection, there is evidence of increased oxidative damage in the Hx-null kidney [83]

Lacking Hx leads to heme overload, then marked liver congestion accompanied by red blood cell stasis and sinusoidal dilation around the centrolobular vein. Lacking Hx is also, associated with abnormal iron deposits, increased lipid peroxidation, and massive leukocyte infiltrates the compound mutant mice showed lacking both Hx and haptoglobin, hence, more evidence of liver damage after intravascular hemolysis [89].

Hemopexin and Minimal change nephrotic syndrome

Protease Activity of Hemopexin and Albuminuria

A vasoactive plasma factor was isolated from plasma of normal subjects and patients with MCNS. This plasma factor has a molecular weight ranging from 70 to 100 kD, denoted as 100KF, [90]

This vasoactive plasma factor was known to contain serine protease activity and to be related to the pathogenesis of MCNS. The characteristic glomerular alterations can be induced by 100KF. These glomerular alterations include a loss of glomerular extracellular matrix molecules, a loss of anionic sites at the ultrastructural level exclusively along the lamina rara interna (LRI) of the glomerular basement membrane. This pattern is like that described in biopsies from subjects with MCNS in relapse [91].

In addition to these histochemical alterations, there is significant enhancement of albumin permeability. In contrast, the heat-inactivated 100KF caused neither enhanced glomerular permeability nor loss of glomerular

extracellular matrix molecules. The active moiety of 100KF has an enzymatic nature, suggesting that impairment of glomerular extracellular matrix molecules may be due to the serine protease activity of 100KF]. Accordingly, the serine protease inhibitor phenylmethylsulfonyl fluoride can inhibit 100KF then, prevents loss of glomerular polyanion in vitro. In 1999, this factor was reported as plasma Hemopexin (Hx) or an isoform of this plasma constituent [(92)]

Both recombinant human Hx and 100KF show identical in vitro and ex vivo properties. The recombinant human Hx on binding with heme differs to the native form as it loses the catalytic triad that is characteristic of many serine proteases. Recombinant Hx, and active isoforms of human plasma Hx derived from normal human pooled serum is detected at 85 kD with rabbit polyclonal antihemopexin IgG by Western blotting. Incubation of rat and human cryostat kidney sections with either Hx or recombinant Hx followed by washing. Then, staining of the sections for glomerular extracellular matrix molecules, i.e., sialoglycoproteins and ectoapyrase, resulted in a significant loss of these molecules in comparison to control sections. This effect could be inhibited by serine protease inhibitors, while heat inactivation of the Hx preparation abolishes its protease activity [(93)]

The unilateral perfusion of recombinant Hx into the rat kidney induces enhanced glomerular permeability for plasma proteins, like that demonstrated with native Hx . An evaluation of capillary loop segments at the ultrastructural level following Hx infusion showed effacement of epithelial foot processes, in contrast to heat-inactivated Hx (HI-Hx) in which foot process effacement occurred to a significantly lesser degree. Also, a reduction of polyethyleneimine, as a cationic marker, and positive punctate staining occurring in the LRI of the glomerular basement membrane were seen exclusively in the Hx-infused versus the HI-Hx-treated animals. It is noted that these findings after Hx infusion are reversible as is observed in MCNS patients. The effects of active Hx are observed on human podocyte and glomerular endothelial cells (GEnC) monolayers, and an additional mechanism for proteinuria. After Hx treatment, both podocytes and GEnC demonstrated a reduction in the expression of glycocalyx. The glycocalyx is composed of glycoproteins including proteoglycans and which coats the luminal surface of the glomerular capillaries. The disruption of glycocalyx coating GEnC was associated with an increase in the flux of albumin, without any changes in the morphology of GEnC monolayers. Glycocalyx has albumin-restrictive properties. These properties contribute to the barrier to flux of albumin across the cell layer [(94).]

Foot process effacement has been demonstrated after infusion of Hx. Changes observed in the filamentous actin cytoskeleton of human podocytes are reported. Wild-type (WT) podocytes showed dramatic reorganization of actin with loss of stress fibers. They also showed development of membrane ruffles and cytoplasmic aggregates. The effects of Hx on actin were reversible within 4 h. This occurs in keeping with the time course of proteinuria seen after Hx infusion in rats. This reversibility of action could be explained by receptor-binding dynamics or by the physiologic stability of Hx. On the other hand, nephrin-deficient (ND) podocytes did not show actin reorganization after Hx treatment. This indicate that the Hx effect on actin was dependent on the expression of nephrin. This finding was further supported by nephrin siRNA knockdown experiments in WT podocytes. There was a significant reduction in actin reorganization after Hx treatment. The reconstitution of nephrin in ND podocytes was associated with changes in the actin cytoskeleton after Hx. This was comparable to the changes in WT podocytes. These observations firmly give a link between Hx treatment, nephrin expression, and actin reorganization in podocytes. Therefore, it is hypothesized that nephrin plays a key role in the downstream relay of intracellular signaling leading to actin reorganization in podocytes in MCNS. The mechanism of Hx's effects on the actin cytoskeleton was explored by studying key cytoskeletal signaling pathways [(95).]

WT and ND podocytes showed differences in activation of key cytoskeletal signaling pathways after Hpx treatment. RhoA is a small GTPase. When RhoA is activated, it acts as a molecular switch that can lead to actin reorganization. In WT podocytes, there was minimal basal activation of RhoA. There was increased activation of RhoA after Hx perfusion. In addition to this, there was observed actin reorganization. Contrarily, ND cells had no change in RhoA activation after Hx. In addition to this, ND podocytes had no associated actin reorganization. These data suggest that nephrin is required to regulate RhoA activation in response to

Hx. Protein kinase B (PKB) is a central enzyme in many intracellular signaling pathways, and it has been related to actin reorganization and nephrin signaling [96].

Thus, Hx has been shown to affect every layer of the glomerular filtration barrier with its protease activity in histochemistry and in molecular cell biology findings. The mechanisms of Hx in causing MCNS still remain unclear. For example, Hx binds a cell surface receptor is unknown. The heme– Hx complex is known to bind to a specific receptor low-density lipoprotein receptor-related protein 1(LPR1) on hepatocytes and allow heme transfer. Hx is not known to be a ligand for other receptors. Because of having serine protease activity, Hx may act via the protease-activated receptors. More than 500 proteins have domains showing homology to the Hx molecule. The matrix metalloproteinases are included in these proteins. These proteins have been implicated in many disease processes. Some studies investigated the signaling axis formed by the matrix metalloproteinase–protease-activated receptor 1 (PAR1). The proteases present in nephrotic plasma obtained from patients with focal segmental glomerulosclerosis (FSGS) can activate PAR1. This leads to the podocin-dependent phosphorylation of actin-associated protein called vasodilator-stimulated phosphoprotein (VASP). This phosphorylation in human podocyte suggests a novel role for proteases and PARs in the pathogenesis of FSGS [97]

Hemopexin and Relapse of Pathophysiological Mechanisms in MCNS Patients

The mechanism of onset of relapse of MCNS caused by active Hx is unclear. Various isoforms of Hx are suggested to exist. In normal conditions, the circulating Hx is inactive. Under certain circumstances Hx becomes activated as a serine protease. MCNS in relapse demonstrates altered activity of plasma Hx. There was a decreased mean titer of plasma Hx in relapsed subjects as compared with remission [93]

Different Western blot patterns demonstrate Increased Hx protease activity exclusively in plasma from MCNS relapsed subjects. This finding was seen compared to remission plasma and plasma from other proteinuric subjects with FSGS, membranoproliferative glomerulonephritis (MPGN), or IgA nephropathy or healthy control individuals. The preincubation of podocytes in normal human plasma prevented actin reorganization after Hx treatment. This indicates that factors in normal plasma act to protect podocytes. Loss of such plasma factors in MCNS may leave podocytes to exposure to the effects of activated Hx. The plasma factors may act directly on podocytes to regulate the expression of receptors or to maintain the integrity of the slit diaphragm complex. These plasma factors may alternatively act, as direct inhibitors of active Hx in a similar manner to other circulating proteases that have circulating inhibitors. This theory is supported effect of serine protease inhibitor that reduce the effect of Hx on the actin cytoskeleton in WT cells and on glomerular extracellular matrix molecules [98]

In contrast, there is a report of activation mechanism of Hx in endothelial and mesangial cells in paracrine manner in glomeruli. In vitro, human mesangial cells stimulated with tumor necrosis factor alpha (TNF-α) release Hx in a corticosteroid-dependent manner. Extracellular nucleotides like ADP or ATP may have a role in inactivation of Hx isoforms. Treatment with soluble apyrase may restore he protease activity of inactivated Hx yielding the enzymatic active form of this molecule could be restored. Inflammatory agents like lipopolysaccharide or TNF-α can upregulate ecto-ADPase of endothelial or mesangial cells in vitro. Then the inactive isoform of Hx can be converted to active isoform by ecto-ADPase present along the surface of these cells. Prednisolone can downregulate ectoapyrase in stimulated endothelial or mesangial cells, and in consequence, potentially inhibit the conversion of Hx to its active isoform. This hypothesis may explain the clinical feature of onset or relapse of MCNS triggered by viral infection. Also, TNF-α synthesis in peripheral mononuclear cells from relapse is increased. The promoter region of TNF-α in naı¨ve T-helper cells from relapse has a significant reduction in DNA methylation. In comparison to that finding from remission in the same patients indicating predisposition of TNF-α synthesis in relapse [99]

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