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RESEARCH ARTICLE

SERUM ZINC LEVEL IN CHILDREN WITH RELAPSING NEPHROTIC SYNDROME

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ABSTRACT

Background: Zinc as a second trace element of human body plays an important role in numerous function. Abnormality in the metabolism of zinc in renal problem specially nephrotic syndrome is well documented. **The Aim of Study:** To study change in serum zinc concentration in children with relapsing nephrotic syndrome. **Patients and method:** A hospital based case control study that conducted at nephrology clinic at Al-Sadder and AL-Zahra teaching Hospitals in Al Najaf Al Ashraf included patients with nephrotic syndrom for period between 1st January 2013 to end of October 2013. A total of 60 pediatric patients with relapsing nephrotic syndrome were included in this study. They were divided in two groups, (30) patients group A (patients with infrequent relapsing) and (30) patients group B (patients with frequent relapsing). Control group consist of 32 healthy children. Serum zinc was measured by atomic absorption spectrophotometery. **Results:** Patients aged 2-14 years, boys were 40 and girls were 20. The mean age of patients was 7.5 years. The Mean serum zinc level in group B (frequent relapse) (58.45 µg/dl) that was significantly lower than that of group A (infrequent relapse) (61.58/dl)and control group(89.64 µg/dl) respectively. There was no significant difference between patients of both groups and control in the mean of serum zinc level and sex of patients. **Conclusion:** Hypozincemia can occur in chronic renal problem like nephrotic syndrome. The low level of serum zinc was mainly found in those with frequent relapses.

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INTRODUCTION

Nephrotic syndrome was primarily a pediatric disorder and was 15 times more common in children than adults. The incidence was 2-3/100,000 children per year; and the majority of affected children will have steroid-sensitive minimal change disease (Ciark, 1999). The nephrotic syndrome was caused by renal diseases that increase the permeability across the glomerular filtration barrier. It was classically characterized by four clinical features, but the first two are more common and the last two may not be seen in all patients:

- Nephrotic range proteinuria (Urinary protein excretion greater than 50 mg/kg per day) or 40 mg/m^2 per hour.
- Hypoalbuminemia (Serum albumin concentration less than 2.5 g/dL (25g/L))
- Edema
- Hyperlipidemia (Ciark, 1999)

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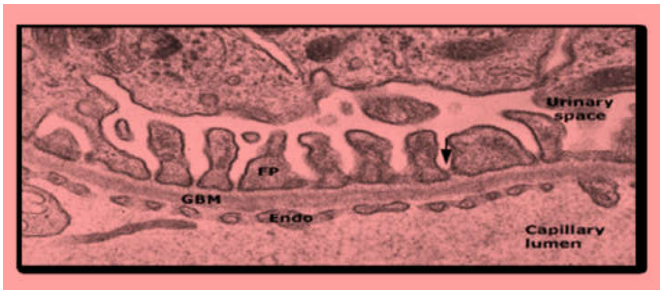
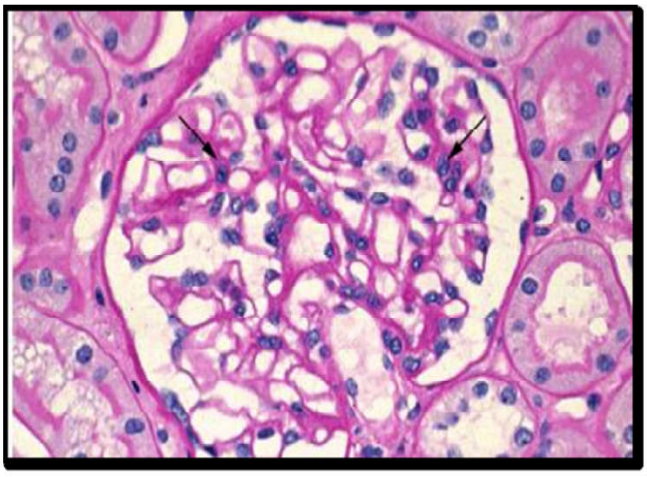
Pathogenesis: Two issues are important in the pathogenesis of nephrotic syndrome: the mechanisms of glomerular injury and proteinuria. 1- Mechanisms of glomerular injury. A variety of different, disease-specific mechanisms have been described in the nephrotic syndrome. Circulating non immune factors in minimal change disease and primary focal glomerulosclerosis. Circulating immune factors in disorders, such as membranoproliferative glomerulonephritis, post streptococcal glomerulonephritis, and lupus nephritis. Mutations in podocyte or slit diaphragm proteins (eg, CD2AP, podocin, and nephrin) in inherited forms of congenital, infantile, or glucocorticoid resistant nephrotic syndrome.

Mechanisms of proteinuria: The proteinuria in glomerular disease was due to increased filtration of macromolecules (such as albumin) across the glomerular capillary wall. The latter consists of three components: the fenestrated endothelial cell, the glomerular basement membrane (GBM), and the epithelial cell foot processes. The pores between the foot processes are closed by a thin membrane called the slit diaphragm (picture 1). The filtration of macromolecules across the glomerular capillary wall was normally restricted by two

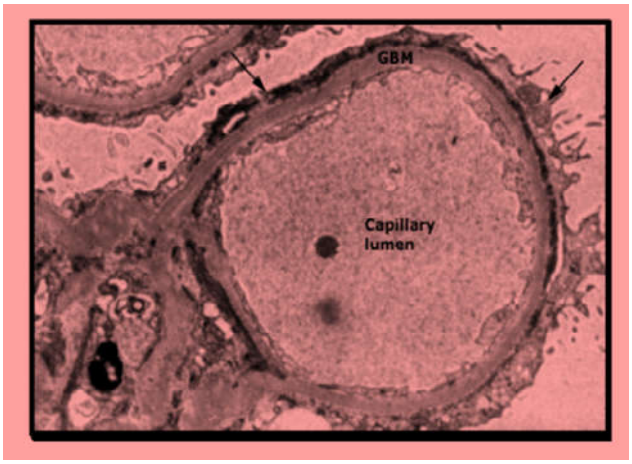
mechanisms: charge-selectivity and size-selectivity. The endothelial cells and the GBM have a net negative charge due to polyanions such as heparan sulfate proteoglycans. This creates a charge barrier to the filtration of large anions such as albumin. In comparison, circulating IgG was predominantly neutral or cationic, and its filtration was not limited by charge. In minimal change disease, the most common cause of nephrotic syndrome in children, there was a loss of anionic charge that was not accompanied by any structural damage or change to the glomerular filtration unit observed by light microscopy (picture 2). However, electron microscopy demonstrates epithelial podocyte effacement (picture 3) (Mumtaz *et al*., 2011).



Picture 1. Normal Glomular Capillary Wall High Power Electron Micrograph (Mumtaz *et al*., 2011)



Picture 2. Minimal Change Disease Light Micrograph of an Essentially Normal Glomerulus in Minimal Change Disease (Mumtaz *et al*., 2011).



Picture 3. Minimal Change Disease Electron Micrograph In Minimal Change Disease (Mumtaz *et al*., 2011)

Picture (1) shows the three layers of the normal glomerular capillary wall: the fenestrated endothelium (Endo); the glomerular basement membrane (GBM); and the epithelial cell with its foot processes (FP). The foot processes are separate by slit pores which are closed by a thin membrane, the slit diaphragm (arrow). In terms of permeability to macromolecules, the GBM and the slit diaphragms are the major sites of size-selectivity, while the endothelium and GBM are the major sites of charge-selectivity. Picture (2) There are only 1 or 2 cells per capillary tuft, the capillary lumens are open, the thickness of the glomerular capillary walls was normal, and there was neither expansion nor hypercellularity in the mesangial areas in the central or stalk regions of the tuft (arrows). Picture 3 showing a normal glomerular basement membrane (GBM), no immune deposits, and the characteristic widespread fusion of the epithelial cell foot processes (arrows).

Classification

Primary Nephrotic Syndrome: Was defined as nephrotic syndrome in the absence of systemic disease. Within this category are two subgroups. Disorders without glomerular inflammation on renal biopsy. Included in this group are idiopathic nephrotic syndrome and some cases of membranous nephropathy. Nephritic disorders associated with active urine sediment (red cells and cellular casts) and the presence of glomerular inflammation on renal biopsy. Included in this group are membranoproliferative glomerulonephritis and IgA nephropathy. Idiopathic nephrotic syndrome was the most common form of childhood nephrotic syndrome, representing more than 90 percent of cases between 1 and 10 years of age and 50 percent after 10 years of age (White *et al*., 1970). Idiopathic nephrotic syndrome was defined by the association of the clinical features of nephrotic syndrome with renal biopsy findings of diffuse foot process effacement on electron microscopy and minimal changes (called minimal change disease (MCD)), primary focal segmental glomerulosclerosis (FSGS), or mesangial proliferation on light microscopy (The primary nephrotic syndrome in children, 1981).

Glucocorticoid-responsive nephrotic syndrome: The majority of children with idiopathic nephrotic syndrome are glucocorticoid-responsive (also referred to as glucocorticoid-sensitive nephrotic syndrome). In these patients, the most likely histologic lesion was MCD, although some patients with FSGS will also respond to glucocorticoid therapy [(4)]. Patients who are glucocorticoid-responsive have a favorable long-term outcome. The steroid sensitive were divided into subgroup according to useful definition (Barrat, 1994):

- Undetermined N.S (nephrotic syndrome).: first-time diagnosed and response to steroid therapy but they still on alternative day or complete therapy but not more than 6 month.
- Frequent relapse N.S. (FRNS): two or more relapse per 6 months after remission from 1st attack. Or 4 or more relapse with any whole year.
- In frequent relapse N.S. (IFRNS): less than 2 attacks of relapse within 6 m. of 1st attack or less than 4 attacks of relapse per any year after.

Glucocorticoid-resistant nephrotic syndrome: Pproximately 20 percent of all children with idiopathic nephrotic syndrome will not respond to glucocorticoid.

Table 1. Sample Sizes and Serum Zinc Concentration For Children With Control Group And Groups A And B, and Sample Size With Male: Female Ratio for Each Group.

Variables	Group Symbol	Mean Age, years	Sample Size		Total	M:F	Mean, mcg/dl
			Male	Female			
Control	control	7	17	15	32	1.133:1	89.64
Infrequent Relapsing	A	7.2	20	10	30	1.5:1	61.58
Frequent Relapsing	B	8.2666	20	10	30	1.5:1	58.45

Table 2. Serum Zinc Concentration for Males and Females in Control Group , and Groups A and B

Groups	Sex	Mean, mcg/dl	Standard Deviation	Standard Error	P-Value
Control	Male	92.159	17.25	4.18	*0.209
	Female	86.787	19.46	5.025	
A	Male	62.095	4.07	0.91	**0.214
	Female	60.55	5.26	1.66	
B	Male	58.45	6.60	1.47	***0.498
	Female	58.46	5.97	1.888	

Table 3. Statistical Analyses to Correlate the Different Groups

Variables	Sample Size	Mean	Standard Deviation	P-Value
Control	32	89.64	18.223	<0.001
A	30	61.58	4.469	
Control	32	89.64	18.223	<0.001
B	30	58.45	6.286	
A	30	61.58	4.469	0.028426
B	30	58.45	6.286	

The response rate was better in younger children, who are much more likely to have MCD. Patients with glucocorticoid resistant nephrotic syndrome have a worse prognosis than those who are glucocorticoid-responsive (Houser, 1984; Houser *et al*., 1986).

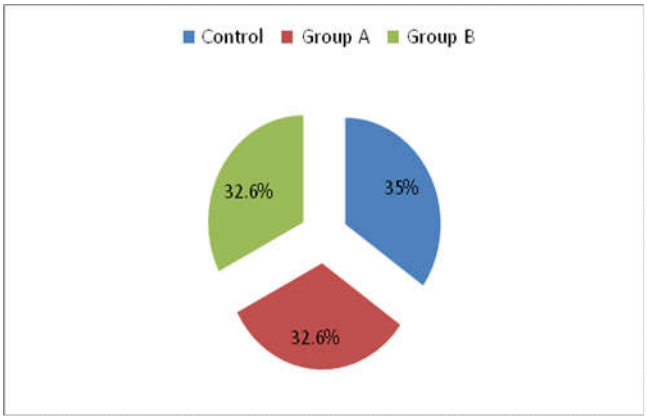


Figure 1. The Percentages of samples sizes for each group

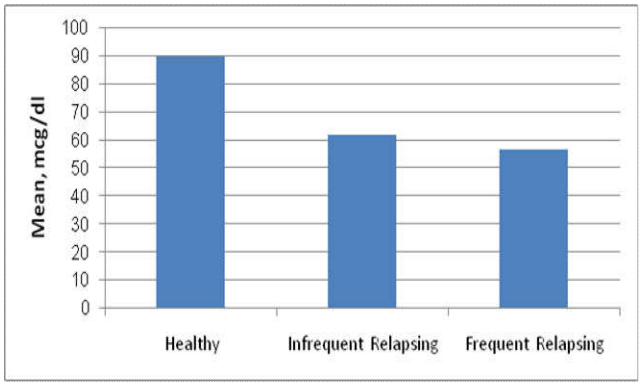


Figure 2. Comparism of Mean Serum Zinc Concentration For Control Group and Groups A With B

Secondary Nephrotic Syndrome:-Was defined as nephrotic syndrome associated with systemic diseases or was secondary to another process that causes glomerular injury.

- Post infectious glomerulonephritis and infective endocarditis.
- Systemic lupus erythematous.
- Vasculitides, such as Henoch-Schonlein purpura.

Risk factors for relapse in childhood nephrotic syndrome:- these factors are (Barrat, 1994**):** 1-Cold and infections. Cold and infections are the most common cause of relapse of nephrotic syndrome. If left untreated, illness conditions will become even worse. Children and adolescents are still in the growth period and many of their tissues and organs are still immature. Compared with adults, children patients have weak immune system and they are more susceptible to be attacked by various viruses, bacteria which often cause the proteinuria, hematuria, swelling to reoccur. 2-Poor compliance. Different from adult patients, small children usually do not understand why they need to take so many bitter drugs and the importance to have the right dosage on schedule. Often the reduction of dosage or stopping taking the prescribed medicines often cause relapse of illness. Therefore it is very important that parents should supervise the children to have medicines at the right time and with the right dosage.Proper treatment is very important to prevent relapse of nephrotic syndrome in children. Hormones and immunosuppressants are effective at relieving symptoms but cannot solve the root problems, that is why patients often become dependent on these drugs and illness often relapse when the dosage is reduced or the drug is stoped. Often these drugs have some side effects and harms to the kidneys if taken for long time, that is why nephrotic syndrome will continue to become worse after each relapse. Infection is an important cause of morbidity and mortality in nephrotic children. Patients with(steroid sensitive nephrotic syndrome) SSNS have increased susceptibility to bacterial nfections and

various infections may result in relapses or steroid resistance or may trigger the onset of disease. Relapses in SSNS often follow infections of upper airway or gastrointestinal tract and cellulitis. It is estimated that 52–70% of relapses among children in developing countries chiefly follow the upper respiratory tract infection (Houser, 1984; Houser *et al*., 1986). Common infections associated with either onset of disease or in the course of disease are acute upper and lower respiratory infections (ARI) including pneumonia with or without empyema, skin infections including impetigo and cellulitis, acute watery or invasive diarrhea, urinary tract infections (UTI) and primary peritonitis (Barrat *et al*., 1994). Studies have shown that use of prophylactic antibiotics, immunoglobulins replacement therapy, vaccination against streptococci pneumoniae, thymosin as immuno-modulating agent, and zinc supplements may have a role in prevention of these infections (Lonnerdal, 2000). However, in a recent Cochrane Database of Systemic Review, it has been concluded that there is no strong evidence for any of above interventions for prevention of infection in nephrotic syndrome. Though pneumococcal peritonitis and cellulitis are decreased with use of pneumococcal vaccine and antibiotics but these infections are still responsible for 1.4-10% of mortality and repeated relapses in more than 80% of cases, requiring high dose steroids and hospitalization (Houser, 1984). A high frequency of infections in children with nephrotic syndrome (38–83%) has been reported from developing countries like India, Pakistan & Bangladesh in different studies (White, 1970). Studies from developing countries have also suggested that increasing the maintenance dose of steroid from alternate day in a child with remission to daily during the episode of mild infections can prevent relapse (Tumer *et al*., 1989; Mumtaz *et al*., 2011). Thus a strong suspicion regarding infections in a nephrotic child is important not only for treatment but also to prevent infection associated relapse.

Clinical manifestations: Primary nephrotic syndrome was more common in younger children, particularly those less than six years of age (White, 1970; Barrat, 1994). There was an increased incidence of nephrotic syndrome in family members when compared to the general population (Hambidge, 1986; Subar, 1989). In affected siblings, nephrotic syndrome usually presents at the same age with the same histopathology and outcome (Subar, 1989).

Edema:- Childhood nephrotic syndrome generally presents with edema and often occurs after an inciting event, such as an upper respiratory infection or an insect bite.[11] Edema increases gradually and becomes detectable when fluid retention exceeds 3 to 5 percent of body weight. Typically, periorbital edema was noted first and was often misdiagnosed as a manifestation of allergy. The edema was gravity dependent, and thus, over the day, periorbital edema decreases while edema of the lower extremities increases. In the reclining position, edema localizes to the back and sacral area. Other dependent areas that can become edematous include the scrotum, penis, or labia. The affected edematous areas are white, soft, and pitting (White, 1970; Barrat *et al*., 1994)**.** Some patients develop anasarca (ie, generalized and massive edema) with marked peripheral edema, abdominal distension resulting from ascites, marked scrotal or vulvar edema, and severe periorbital edema resulting in swollen shut eyelids (White, 1970; Barrat, 1994). Despite the marked increase in extracellular fluid volume, some children with nephrotic syndrome, primarily those with MCD, present with or develop signs of a decrease in effective circulating volume, such as tachycardia, peripheral vasoconstriction, oliguria, decreased glomerular filtration rate, elevation of plasma renin aldosterone, and norepinephrine (Lonnerdal, 2000). In such children, a further insult such as diuretic therapy, sepsis, or diarrhea can lead to hypotension and rarely shock (Shankar *et al*., 1998).

Umbilical or Inguinal Hernias

Abdominal Pain due to Rapid Fluid Accumulation or Peritonitis

Dyspnea: Which was most often due to respiratory compromise from pleural effusions or marked ascites. Infrequently, respiratory symptoms may be due to pneumonia or, rarely, pulmonary embolus due to the hypercoagulable state associated with the nephrotic syndrome (Shankar *et al*., 1998).

Nonspecific Complaints:-Including headache, irritability, malaise, and fatigue are common at presentation (Barrat *et al*., 1994).

Complications: The major complications directly related to the underlying nephrotic syndrome in children with idiopathic nephrotic syndrome (Shankar *et al*., 1998; Prasad, 1988; Bao *et al*., 2003; Bovio *et al*., 2007).

- Infection
- Anasarca
- Thrombosis
- Renal insufficiency
- Hypovolemia
- Poor growth.

Abnormalities of Zn metabolism are well documented in patients with chronic renal disease, especially those with nephrotic disease and uremia. The causes of Zn deficiency in kidney disease are not clear. Decreased dietary Zn intake and intestinal absorption, increased endogenous Zn secretion, and increased urinary Zn excretion (as in the nephrotic syndrome and in renal transplant recipients) all may contribute to altered Zn metabolism. Zn depletion may account for decreased taste, sexual and gonadal dysfunction, hyperprolactinemia, glucose intolerance, hyperlipidemia, growth retardation in children, neuropathy, anemia, abnormalities of neutrophil and lymphocyte function, and delayed wound healing. The benefit of pharmacologic doses of Zn, in the treatment of such manifestations, requires further evaluation under controlled conditions. Before use of Zn routinely for therapeutic purposes in uremic subjects, the cause(s) of abnormal Zn metabolism should be identified.

Diagnosis: The urinalysis reveals 3+ or 4+ proteinuria, and microscopic hematuria was present in 20% of children. A spot urine protein: creatinine ratio exceeds 2.0, and urinary protein excretion exceeds 40 $mg/m^2/hr$. The serum creatinine value was usually normal, but it may be abnormally elevated if there was diminished renal perfusion from contraction of the intravascular volume. The serum albumin level was <2.5 g/dL, and serum cholesterol and triglyceride levels are elevated. Serum complement levels are normal. A renal biopsy was not routinely performed if the patient fits the standard clinical picture of MCNS (Reimold, 1980; Tumer *et al*., 1989).

Treatment: If a child has the typical features of nephrotic syndrome, treatment consists of efforts to reduce edema and therapy with prednisone. Because more than 80% of children 1 to 7 years old with typical MCNS respond to corticosteroids, steroid therapy may be undertaken without a renal biopsy. Specific therapy for MCNS was prednisone, 2 mg/kg/day (60 mg/m2/24 hours) in a single daily dose. Approximately 92% of children who respond to steroids do so within 4 weeks. The optimal duration of steroid therapy for responders was 12 weeks. If a child does not respond to daily prednisone therapy, a renal biopsy was indicated because steroid resistance greatly increases the chance that the underlying pathology was something other than MCNS. Frequent relapses or steroid resistance in MCNS may necessitate immune-suppressive therapy (Mumtaz *et al*., 2011). More than 80% of patients with FSGS do not respond to corticosteroid therapy. Aggressive medical therapy of familial congenital nephrotic syndrome, with early nephrectomy, dialysis, and subsequent transplantation, was the only effective approach to this syndrome. Edema due to nephrotic syndrome was treated with restriction of salt intake. Severe edema may require the use of diuretic therapy. Aggressive efforts with diuretics may lead to profound hypovolemia, however. Occasionally, even adding diuretic therapy to salt and water restriction was ineffective in alleviating severe edema. In these situations, cautious parenteral administration of 25% albumin (0.5 g/kg intravenously over1 to 2 hours) with an IV loop diuretic (furosemide) given during or immediately after albumin usually results in diuresis. The albumin was excreted rapidly, in light of the proteinuria. Effusions may require drainage. [19] Acute hypertension was treated with -blockers or calcium channel blockers. Persistent hypertension was often responsive to angiotensin-converting enzyme inhibitors (Mumtaz *et al*., 2011).

Zinc: Zinc has been recognized as a trace element since 1509, but was not identified as an essential mineral until the 1900s. In 1961, a link was established between zinc deficiency, endemic hypogonadism, and dwarfism in rural Iran (Perrone *et al*., 1990). Zinc was an essential trace element. Zinc intake was closely related to protein intake; where diet rich in proteins stimulate zinc absorption as a result, it was an important component of nutritionally related morbidity worldwide (Perrone *et al*., 1990). The usual oral intake of zinc was approximately 4 to 14 mg/day; the recommended dietary allowance (RDA) was 8 mg/day for children ages 9 to 11years; [21]. Primary dietary sources of zinc include animal products such as meat, seafood, and milk. Ready-to-eat cereal contains the greatest amount of zinc consumed from plant products (Rahi *et al*., 2009). Approximately 10 to 40 percent of dietary zinc was absorbed in the small bowel; absorption was inhibited by the presence of phytates and fiber in the diet that bind to zinc, as well as dietary iron, cadmium, calcium, copper and phosphorus in high amounts (Sarker *et al*., 2012).

Approximately 0.5 to 1.0 mg/day was secreted in the biliary tract and excreted in the stool. Zinc circulates at a concentration of 70 to 120Mg/dL with 60 percent loosely bound to albumin and 30 percent tightly bound to macroglobulin. Urinary excretion typically ranges from 0.5 to 0.8 mg/day. The primary stores of zinc include the liver and kidney. Most of the body zinc stores are intracellular where zinc was bound to metalloproteins (Rahi *et al*., 2009). Relapses in steroid-sensitive nephrotic syndrome (SSNS) often follow infections of the respiratory or gastrointestinal tract. Based on data that zinc supplements reduce the risk of infections, we examined the efficacy of such supplements in reducing relapse rates in the patient (Tumer *et al*., 1989).

Plasma Zinc:- Circulating zinc concentration closely correlate with the major carrier protein, albumin. Thus lowered plasma zinc concentrations observed in hypolbuminemic conditions such as hepatic cirrhosis and malnutrition, may reflect depressed plasma binding of zinc (Shankar *et al*., 1998). Many forms of steroids will depress circulation concentrations of zinc, for example, exogenous cortical steroids, exogenous gonadal steroids in the form of oral contraceptives and endogenous gonadal steroids during pregnancy. Depressed plasma zinc has been associated with infection and inflammation (Shankar *et al*., 1998). Abnormalities of Zn metabolism are well documented in patients with chronic renal disease, especially those with nephrotic disease and uremia. The causes of Zn deficiency in kidney disease are not clear. Decreased dietary Zn intake and intestinal absorption, increased endogenous Zn secretion, and increased urinary Zn excretion (as in the nephrotic syndrome and in renal transplant recipients) all may contribute to altered Zn metabolism. Zn depletion may account for decreased taste, sexual and gonadal dysfunction, hyperprolactinemia, glucose intolerance, hyperlipidemia, growth retardation in children, neuropathy, anemia, abnormalities of neutrophil and lymphocyte function, and delayed wound healing.

The benefit of pharmacologic doses of Zn, in the treatment of such manifestations, requires further evaluation under controlled conditions. Before use of Zn routinely for therapeutic purposes in uremic subjects, the cause(s) of abnormal Zn metabolism should be identified (Mumtaz *et al*., 2011). Minerals like zinc, iron and copper help the body better process and utilize food. “They are needed to make energy for cells and promote growth and repair body tissues," according to the National Kidney Foundation. “Chronic kidney disease changes your body's need for certain vitamins and minerals (Rahi, Kasim, 2009). Zinc plays an important role in many bodily functions including metabolism, the immune system, smell and taste and wound healing (Rahi, Kasim, 2009). Individuals with decreased kidney function typically experience deficient levels of bodily zinc. “Abnormalities of Zn (Zinc) metabolism are well documented in patients with chronic renal disease,” said Dr. S. K. Mahajan in a 1989 report for the Journal of the American College of Nutrition (Prasad, 1988). The exact cause of zinc deficiency in kidney disease remains unclear, but it is more prevalent in those suffering from nephrotic kidney disease, a disorder where glomeruli, the minute blood vessels that help filter the blood, are damaged (Prasad, 1988).

Actions: Zinc was the intrinsic metal component or activating cofactor for more than 70 important enzyme systems, including carbonic anhydrase, the alkaline phosphatases, dehydrogenases, and carboxypeptidases. It was involved in the regulation of nucleoproteins and the activity of various inflammatory cells and plays a role in growth, tissue repair and wound healing, carbohydrate tolerance, and synthesis of testicular hormones (Prasad, 1988). Zinc was involved in the immune response and the response to infection. Zinc deficiency was associated with impaired phagocytic function, lymphocyte depletion, decreased immunoglobulin production, a reduction in the T4+/T8+ ratio, and decreased interleukin (IL)-2 production (Prasad, 1988; Hambidge, 2007).

Zinc Deficiency

Etiology: Dietary zinc depletion: caused by both inadequate zinc intake and the binding of ingested zinc to fiber and phytates in unleavened bread and clay. Both breast milk and the complementary foods typically offered in developing countries provide inadequate amounts of zinc (Kury *et al*., 2002). Breast feeding: Zinc deficiency occurs rarely in exclusively breast-fed infants (usually premature) whose mothers have a low level of zinc in their breast milk. Zinc malabsorption: Acrodermatitis enteropathica was a recessively inherited partial defect in intestinal zinc absorption (Wang, 2001; Naber *et al*., 1998). Affected infants develop an erythematous and vesiculobullous dermatitis alopecia, ophthalmic disorders, diarrhea, severe growth retardation, delayed sexual maturation, neuropsychiatric manifestations, and frequent infections. The syndrome was associated with severe zinc depletion and responds to oral supplementation. Crohn disease: Low plasma zinc concentrations occur in a significant number of patients with active Crohn disease.(30) Sickle cell disease: Low zinc levels can occur in children and adolescents with sickle cell disease, particularly in association with poor or delayed growth (Leonard *et al*., 1998; Phebus *et al*., 1988). Caused by a renal tubular defect and perhaps chronic hemolysis or impaired absorption, not inadequate dietary intake (Leonard *et al*., 1998; Phebus *et al*., 1988). Liver disease: Children and adults with severe, chronic liver disease have an increased incidence of low plasma zinc levels (Perrone *et al*., 1990). The mechanism may be multifactorial, including hypoalbuminemia, reduced intake, and increased urinary excretion. Renal disease: Zinc deficiency caused by increased urinary excretion can complicate nephrotic syndrome in children through loss of protein-zinc complexes in proteinuria or to decreased tubular reabsorption of zinc (Shankar, 1998; Mahajan, 1989). Adolescent athletes: related to inadequate intake associated with overall dietary restrictions caused by a desire to maintain weight as well as possibly increased losses in sweat (Kay, 1975).

Clinical manifestations: Numerous signs and symptoms have been associated with zinc depletion. Mild zinc deficiency was associated with depressed immunity, impaired taste and smell, onset of night blindness, and decreased spermatogenesis. Severe zinc deficiency was characterized by severely depressed immune function, frequent infections, bullous pustular dermatitis, diarrhea, and alopecia, growth retardation (Kilic *et al*., 1998). In some situations, zinc depletion was documented by measurement of zinc concentration in plasma, lymphocytes, or neutrophils. Because zinc was a cofactor for alkaline phosphatase activity, alkaline phosphatase serves as a serologic marker for zinc depletion (Wood, 2000).

Diagnosis: Zinc status can be assessed by measurement of zinc in plasma, erythrocytes, neutrophils, lymphocytes, and hair. Low plasma zinc usually was defined as a value less than 70 mcg/dL .Because much of plasma zinc was bound to albumin correcting values for the level of serum albumin in conditions associated with hypoalbuminemia was important. Some investigators argue that plasma zinc measurements are relatively insensitive and that mild zinc deficiency occurs with normal plasma levels (Prasad, 1984). Zinc levels in neutrophils or lymphocytes may be more sensitive (Meftah, 1991). The criteria for zinc deficiency are decreased zinc level in either lymphocytes (<50 mcg/10(10) cells) or granulocytes (<42 mcg/10(10) cells) (Black, 1998).

Depressed serum alkaline phosphatase levels for age provide supportive evidence for zinc deficiency.

Zinc Supplementation: Zinc deficiency, associated with impaired immunity and propensity to infection, was thought to be common in children in developing countries in which children experience high rates of serious infections. Zinc supplementation has been evaluated both as a therapeutic agent and as a potential prophylactic agent in children in these populations (Aggarwal *et al*., 2007). Prevention of diarrhea and pneumonia, numerous studies in developing countries has shown that routine oral zinc supplementation reduces the incidence of diarrheal disease and pneumonia (Aggarwal *et al*., 2007; Schlesinger *et al*., 1992).

- Treatment of acute diarrhea
- Treatment of persistent diarrhea
- Prevention or treatment of malaria
- Treatment of the common cold
- Enhancement of growth: Some studies have described improved linear growth in infants who receive zinc supplementation, especially in those who were stunted or had low plasma zinc concentrations at baseline (Barceloux, 1999).

Zinc Toxicity: Little toxicity occurs with zinc supplementation. Ingestion of up to ten times the recommended daily intake produces no symptoms. The acute ingestion of 1 to 2 g zinc sulfate produces nausea and vomiting associated with irritation and corrosion of the gastrointestinal tract. Large doses of zinc compounds also can produce acute renal failure caused by tubular necrosis or interstitial nephritis (Ekkehard, 1980) .

The aim of study: To study change in serum zinc concentration in patients with relapsing nephrotic syndrome.

Patients and method: This was a hospital based case control study on samples at nephrology clinic at Al-Sadder and AL-Zahra teaching Hospitals for period between 1st January 2013 to end of October 2013. A total of 60 pediatric patients age 2-14 years with relapsing nephrotic syndrome were included in this study, male were 40 and female were 20. They were separated into two groups:

- Group (A) infrequent relapsing, 30 children, consist of 20 males and 10 females (relapse once time during 6 months since diagnosis of disease).
- Group (B) frequent relapsing, 30 children, consist of 20 males and 10 females (relapse two or more during 6 months since diagnosis of disease).

These patients were compared with 32 children without nephrotic syndrome of corresponding age called "control group", consist of 17 males and 15 females. For all conditions, control, infrequent relapsing, and frequent relapsing, blood samples were collected to measure the serum zinc concentration level by using Spectrophotometer. This occur by spectrophotometry where zinc reacts with chromogen present in reagent forming coloured compound which colour intensity proportional to the zinc concentration present in sample.

Laboratory work: Used serum by collection 3 ml of blood by venipuncture then centrifugation, the specimens then frozen at -20^{C} for long term storage. Reagents used as following:

- **Reagent A:** Borate buffer, saliciladoxims and preservatives.
- **Reagent B:** Nitro-PAPS.S
- **Standard:** zinc ion 200mcg/dl (stabilizers and preservatives).

The working reagent was Prepared by adding 2ml of vial B to a vial reagent A. The test procedure was made according to "Diagnostica e Tecniche di Laboratorio", Itally as following:

- We delivered 50 ML specimens into a test tube, then be mixed with 1ml of working reagents`(prepared previously) to form samples used in spectrophotometry.
- Then we prepared the Blank by delivering 50ML of distalled water and be added to 1ml of Working Reagent to reset the spectrophotometry.
- Preparing the standard by mixing 50mml of Standard Reagent into test tube and 1 ml of Working Reagent.
- To measure the zinc level, first, recording the readings of spectrophotometry for the prepared standard, second, recording reading of spectrophotometry for the prepared sample, then using Equation (1): -

$$\text{Zinc, mcg/dl} = (A_{sample} / A_{standard}) \times 200$$

For each sample, after 5 minutes as minimum and 30 minutes as maximum for mixing duration, the reading by spectrophotometry was obtained (A_{sample}) at wave length of 520-570 nm.

Statistical analysis: Using MS Excel 2010, statistical analyses were made to the results of zinc level for different groups included t-test (mean and P-value), standard deviation, and standard error.

RESULTS

A total of 60 pediatric patients age 2-14 years with relapsing nephrotic syndrome were included in this study, male were 40 and female were 20. They were separated into two groups: group A is infrequent relapsing (30 children) where the M:F ratio was 1.5:1, and group B is frequent relapsing (30 children) where the M:F ratio was 1.5:1. These patients were compared with 32 children without nephrotic syndrome of corresponding age called "control group", where the M:F ratio was 1.133:1, as in Table (1). Figure (1) shows the percentages samples sizes for each group. The mean serum zinc concentration in healthy children (control group) was 89.64mcg/dl, while in children with infrequent relapsing nephrotic syndrome (group A) was 61.58mcg/dl and children with frequent relapsing nephrotic syndrome (group B) was 58.45mcg/dl. Table 2 shows the results of statistical analyses to correlate the zinc level with respect to the sex for each group. The mean serum zinc concentration in control group males and females were 92.159 mcg/dl and 86.787mcg/dl respectively and P-Value was 0.209, while in group A, the mean was 62.095 mcg/dl for males and 60.55 mcg/dl for females, the P-Value was 0.214, whereas in group B, the mean was 58.45 mcg/dl for males and 58.46 mcg/dl for females, P-Value was 0.498. These findings indicate that there is insignificant difference corresponding to sex in all groups of patients studied. For the control group, deviation the standard was 17.25 with standard error of 4.18 for males while females show a standard deviation of 19.46 at standard error of 5.025. Group A, the standared deviation was 4.07 and standard error of 0.91 for males, but for females, the standared deviation was 5.26 and standard error of 1.66. In group B, for males, the standared deviation was 6.60 and standared error of 1.47, then again, for females, the standared deviation was 5.97 and standared error of 1.888. Table 3 shows the statistical analyses to correlate the zinc serum level in the different groups. There was significant difference between control group and group A with P-value < 0.001, also, there was significant difference between control group and group B with P-value < 0.001. Similarly the difference between group A and group B was significant with P-value of 0.028426.

DISCUSSION

In this study the mean serum zinc level in group B (frequent relapse) (58.45 µg/dl) that was significantly lower than that of group A (infrequent relapse) (61.58/dl) with P-value of 0.028426, likewise both groups (A &B) are significantly lower than control group (89.64 µg/dl) with P-value <0.001. In pediatric nephrology studies done by Sasiarun and *et al*. (1998) where relaps occure in steroid sensitive nephrotic syndrome often follow infection of upper respiratory tract infection and gastrointestinal tract infection, based on data that zinc supplimatation notation reduce relapsing in these patients so subjecte receiving zinc (10mg/day). Reducing relapsing i.e decrease in relapsing 20% so zinc play important role in remition and relapsing of nephrotic syndrome so this study goes with my study. Another study saw zinc supplimantation reducing of relapse in patients with nephrotic syndrome done by Pankaj Hari and et.al in Pakistan at department of pediatric nephrology in January at 26 in 2012 for children with range(2-15) years old dividing in to zinc group(Z.G) receive zinc and placebo group(P.G) without zinc supplementation result mean infection and relapse rate were 1.92+_1.47 and 1.14+_0.37 respectively compare with P.G mean infection and relapse rate 2+_1.53 and1.3+_0.48 respectively so show relapsing in ZGchildren7.28%lower than compared with PG10.34% so zinc helpful in reducing relapse in nephrotic syndrome as in my study (Hambidge *et al*., 2007). Also other study done by Ashima Gulati and *et al*. (2012) on 100 patients to see the effect of zinc on relapse nephrotic syndrome and results showed that daily supplimantation of zinc resulted in 59%reduction frequency of relapsing (Sarker *et al*., 2012) so support my study in the effect of zinc in reduction of relapsing in patients with nephrotic syndrome. This study certifies insignificant difference corresponding to sex in all groups of patients studied. This result was also stated by Rahi *et al.* 2009, with M:F ratio of 1.8:1. This result moreover was stated by Sarker, MN *et al*., 2012, with M:F ratio of 2:1,so these studies go with my study where P-value between male and female insignificant where in control group was 0.209 while in group A was0.214 and in group B was 0.498 so as mention previously all studies were insignificant.

Conclusion

Hypozincemia can occur in chronic renal problem like nephrotic syndrome. The low level of serum zinc mainly found in those with frequent relapses there is no effect of sex on mean serum level in all group studied

Recommendations

For future work, we suggest the followings:

- Measurement of serum zinc concentration in all patients with relapsing nephrotic syndrome
- The need for giving zinc supplement for patients with relapsing nephrotic syndrome especially in patients with frequent relapsing syndrome.
- Further studies are needed to show the effect of zinc supplement on the decreasing the relapses.

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