Discussion
DISCUSSION

Nephrotic Syndrome is caused by primary (idiopathic) renal disease or by a variety of secondary causes. The Nephrotic Syndrome (NS) recognized as an entity for over half a century (Cameron and Hicks, 2002) is defined by massive continued urinary protein losses resulting in Hypoalbuminemia and Oedema formation. These are associated with modifications in kidney functions and with complications such as increased susceptibility to infections, thromboembolism, altered lipid and carbohydrate metabolism, and losses of binding proteins in the urine.

6.1 Proteinuria, Hypoalbuminemia and electrolyte metabolism in NS

Oedema, the usual presenting complaint, is the consequence of abnormal accumulation of interstitial fluid, and becomes obvious if in excess of 10 per cent of body weight. This is usually associated with proteinuria greater than 40 mg/m² BSA/h (greater than 3.5 g/24 h in a 70 kg adult) leading to hypoalbuminemia of less than 25 g/l. However, in slowly developing Nephrotic Syndromes, significant proteinuria and hypoalbuminemia may persist for prolonged periods without evident of oedema. Thus, there is only an approximate boundary between the NS and persisting proteinuria with borderline patients.

In adults, retention of up to 4 litre of salt and water remains undetectable, which is revealed only by weighing. With increasing oedema, ascites may appear followed by pleural effusions, which are usually bilateral, occasionally unilateral, and usually limpid, but sometimes opaque and chylous. Genital oedema may be distressing, especially in males. The oedema remains soft and pitting even when profound, but if it remains untreated for long periods it may become indurate and pit only with difficulty especially around the ankles. Ankle swelling may be asymmetrical if deep venous thrombosis supervenes. Striate may appear even if no corticosteroids are being given, and the skin may actually split and weep spontaneously. Needle stick punctures may also weep profusely. The pathogenesis of oedema formation in Nephrotics is still not entirely understood (Donckerwolcke R.A. et al., 2001).
The only symptom of profuse proteinuria itself is urinary frothing, which some patients may notice, and which may provide a valuable clue as to when major proteinuria began. Findings on urine microscopy or testing for haematuria depend upon the underlying cause of the proteinuria.

In adults, diabetes mellitus is the most common secondary cause and memranous nephropathy is the most common primary cause. Imaging studies were generally not needed, blood test should be used selectively to diagnose specific disorders rather than for a broad or unguided workup. Renal biopsy was useful in all cases to confirm an underlying disease or to identify idiopathic disease that is more likely to respond to corticosteroids. Treatment of most patients included fluid and sodium restriction, oral or intravenous diuretic and corticosteroids treatment (Kodner C., 2009). The diagnostic criterion for establishment of NS is the presence of proteinuria, hypoalbuminemia, hypercholesterolemia and finally edema. The edema was classically thought to be consequence of the decreased intracellular oncotic pressure because of the loss of protein, retention of sodium and water. When oedema formation is severe, symptomatic treatment is required, independent of any specific measures that may be available to treat the underlying condition. The first step is to ensure a reasonably low intake of sodium compatible with a relatively normal diet, but in patients with hypovolaemia who require maximal sodium retention to maintain circulatory volume, particularly children, and this policy can be dangerous. In patients with hypervolaemia, and in those requiring diuretics, a low sodium intake (50–70 mmol/24 h in adults) should be given. An additional goal of sodium restriction is potentiating of the antiproteinuric effects of ACE inhibitors. Patients with the NS often show relative resistance to diuretic (Kirschner K A et al., 1992), which has been attributed to a multifactorial decreased delivery of the drug to the active sites in the tubular brush border of the kidney (Wilcox, 2002).

Proteinuria has been demonstrated not only a representative sign of renal lesion but also a risk factor for the progression to renal failure through its injurious effects on tubulointerstitium. The responsible gene for finish type congenital Nephritic Syndrome was identified and its product was named “nephrin” which was located on slit membrane between foot processes of glomerular epithelial cells and was considered also in the induction of acquired renal lesions with proteinuria. The
monoclonal antibody against nephrin can induce proteinuria. Nephrin was regarded as the most promising and attractive molecule for the development of new therapeutic strategy (Nakatsue T. et al., 2005). The NS, mainly through alterations in concentration of plasma proteins, affects every cell and every tissue in the body. There is selective loss of low molecular weight protein in the urine. Proteins are excluded from glomerular filtrate by a mechanism only partly understood (Myers and Guasch, 1994, D'Amico and Bazzi 2003). Proteinuria alters the apolipoprotein content of lipoprotein. Proteinuria causes increased amount of the total oxylipid in the HDL-C and VLDL-C fractions. Nephrotic Syndrome alters the lipoprotein oxylipid composition, independently of an increase in total lipoprotein levels. These proteinuria induced changes may be associated with the cardiovascular risk of lipoprotein oxidation (Newman J.W. et al., 2007).

In the present study we found hypoalbuminemia were responsible for the progression of cardiovascular disease and these findings were supported by Falaschi F. et al., (2000). They observed that in patients with Nephrotic range proteinuria (greater than or equal to 3.5 gm/24 hrs) there is a significantly higher carotid intima media wall thickness than did those without (p<0.02) patients with nephrotic range proteinuria.

Some study referred that if proteinuria of sufficient amount, persists for long enough, then a series of consequences arises which results in Nephrotic Syndrome. The most notable consequence of massive proteinuria was salt and water retention leading to edema formation. In the classical "underfill", theory edema was considered to be secondary to salt retention resulting from renal hypoperfusion. According to this theory, the primary event was decrease in plasma volume due to the diminution of plasma oncotic pressure resulting from hypoalbuminemia, causing transfer of fluid from the plasma to the interstitial space "underfilling" the blood compartment and resulting to secondary renal sodium retention. This mechanism applied mainly to the Nephrotic Syndrome associated with minimal change disease. By contrast, in most adults with the Nephrotic Syndrome due to minimal change disease or other glomerular lesions such as membranous or proliferative glomerulonephritis, an initial plasma volume expansion was observed. Therefore, the primary event responsible for the sodium retention was a renal intrinsic
excretory defect, which leads to extracellular fluid expansion and edema formation. Therapy was specific for the specific glomerular disease and symptomatic for the edema (diuretics) (Palmer B.F. et al., 1997). In nephrotic patients, increased proximal as well as distal tubular sodium reabsorption are present, and this also points to extrarenal stimulation of sodium retention (Grausz et al., 1972, Vande Walle et al., 1996). All these data are arguments in favour of water and sodium retention mediated by hypovolaemia. However, such abnormalities were only found in patients with rapid progression to the nephrotic syndrome and in others with extremely low serum albumin values (Vande Walle et al., 1996).

Similarly, other studies reported about mechanism of the development of edema in the Nephrotic Syndrome and had traditionally been viewed as an under-fill mechanism. According to this view, urinary loss of protein results in hypoalbuminemia and decreased plasma oncotic pressure. As a result, plasma water translocates out of the intravascular space and results in decrease in intravascular volume. In response to the underfilled circulation, effector mechanisms were then activated that signal the kidney to secondary retain salt and water. While an underfill mechanism may be responsible for edema formation in a minority of patients, recent clinical and experimental findings would suggest that edema formation in most nephrotic patients was the result of primary salt retention. The under-fill and overflow hypothesis were usually held as mutually exclusive mechanisms for explaining sodium/water retention in nephrotic syndrome (Palmer B.F. et al., 1997). Under conditions of hypoalbuminemia the permeability of the capillary to protein seems to decrease and this will also contribute to maintenance of the oncotic gradient between capillaries and interstitium (Fadnes et al., 1975, Golden et al., 1990). Based on all these considerations, oedema formation in the nephrotic syndrome cannot be related only to reduce plasma oncotic pressure inducing hypovolaemia with secondary renal sodium retention, but a primary renal defect in sodium and water excretion must be involved. In some situations this equilibrium will not be achieved. This is the case in patients with extremely low plasma albumin concentrations, such as in children with a congenital nephrotic syndrome. In these patients, plasma colloidal osmotic pressure (COP) is so low that even a decrease of interstitial COP to values near zero will not allow achievement of a new equilibrium. Also in minimal change nephrotic syndrome during a rapidly developing relapse, the
decrease in plasma COP may be too fast to be matched by an appropriate decrease of interstitial COP, increased lymph flow and vascular refill. During this stage the disequilibrium will be associated with insufficient vascular refill and decreased circulating blood volume (Vande Walle et al., 1996 and Donckerwolcke R A et al., 2003).

It has been reported that there is a common pathology for Sodium & water retention mechanism. A central role is played by vasopressin, which is secreted in the two phases, respectively, by a volume and an osmotic stimulus; therefore, persistent sodium/water retention is maintained through the vascular and tubular effects of this peptide. In addition, vasodilation and sodium/water excretion could ensue when both stimuli for vasopressin release fade away, leading to the resolution of the syndrome ((Sala C., 2004). Resolution of salt retention in these seemed to occur in front of these persisted of massive protenuria and hypoalbuminemia in accordance with previous reports after steroids treatment. Better correlation found between log aldosterone and U(K⁺)/U(Na⁺) + U(K⁺) ratio than with other parameters measuring renal potassium handling such as transtubular potassium gradient fractional excretion of K⁺ and urine K⁺/urine Na⁺ or urine K⁺ and creatinine ratios(Sala C. et al., 2004).

Some other studies had shown that the diuretic treatments regulate sodium potassium excretion and reabsorption abnormalities. Sodium and potassium output from the body was regulated by renal excretion, which takes place predominantly in the late distal and cortical collecting tubules. The factors regulating renal sodium & potassium secretion were potassium intake, distal urinary flow, systemic acid-base equilibrium, aldosterone, antidiuretic hormone and, probably epinephrine. Renal handling of sodium & potassium was best studied by the response to the acute administration of furosemide in the present study. This loop diuretic not only increases sodium and chloride excretion but also regulate potassium and hydrogen ions recreation, reabsorption and stimulates the renin-aldosterone axis. (Sala C. et al., 2004, Donckerwalcke R.A. et al., 2003).

Differential diagnosis between these condition were easily made if attention was paid to the level of GFR, presence of sodium wasting, activity of the renin-aldosterone axis and renal response to acute administration of furosemide. Doucet A.
et al. (2007) observed that furosemide was efficient to increased urinary sodium excretion and improve serum potassium level, to reverse sodium balance and to remove edema from patients with NS.

6.2 Minerals metabolism in NS

Copper and zinc physiology is considered a matter of great interest because of the possibility of being able to activate many key enzymes in the cellular metabolism. Several investigators studied zinc & copper metabolism of patient with nephrotic syndrome. Serum level of zinc and copper mostly found below normal values. Our results confirm these observations.

Ceruloplasmin is a copper-containing serum globulin. Ninety-five per cent of the total copper content of the serum is bound to ceruloplasmin (Cartwright G.E., 1995). Nephrotic Syndrome patients may be associated with excretion of Ceruloplasmin a protein, which is normally not found in urine and urinary copper loss, is in direct proportion to the amount of Proteinuria (Saniye Sen Naci M. et al., 1991). Both ceruloplasmin and copper levels decrease in nephrotic syndrome patients (Herbert E. Spiegel, 2003). Although the copper binding protein ceruloplasmin is lost in the urine in Nephrotic Subject and its level are low in plasma and red cell, copper concentration are usually normal. Hypocupremia is associated to Nephrotic syndrome secondary to renal loss of copper proteins. Ceruloplasmin in bound copper likely plays a significant role in predisposing patient with nephrotic syndrome to copper deficiency (Reichel M.M. et al., 1992).

The most important Zinc binding protein in serum is albumin. Zinc level were reduce in parallel with serum total protein, serum albumin, but serum copper levels on the other hand reduce not as much as zinc (Lindeman R.D. et al., 1990). Most of the serum Zn is normally bound to circulating proteins. Low serum Zn concentrations might result from depletion of Zn-binding proteins. Serum proteins and Zn concentrations have been reported to be depressed in patients with acute and chronic diseases. The element Zn is the metal component or activator of many important enzymes. The tissue concentrations and activities of Zn metalloenzymes direct the rate of protein and nucleic acid syntheses, thereby influencing tissue growth and reparative processes. Nephrotic Syndrome documented zinc deficiency
was probably a consequence of reduced absorption of Zinc in conjugation with excessive urinary loss (Stek J. et al., 1990). Zinc circulate mainly bound to albumin and also to transferring and thus the reduced zinc concentration in plasma, hair & white cells in Nephrotic subjects is not surprising (Herbert E. Spiegel, 2003) over 90% of serum Zinc is bound to protein in normal subject. Though the correlation between serum zinc albumin concentrations is unimpressive. The low serum zinc concentration in the patient with nephrotic syndrome does not appear due to loss of zinc bound urinary protein (Lindeman R.D. et al., 1990). A linear correlation was found between Proteinuria and urinary zinc and copper in relapse of nephrotic syndrome. Several studies have demonstrated increased urinary losses of zinc which may occur despite high oral zinc intake, in patients without proteinuria suggesting that alteration in renal tubular excretion or reabsorption of zinc may contribute to increased urinary Zinc losses in patients with nephrotic syndrome (Stek J. et al., 1990, Perrone L et al., 1990, Mahajan S.K. et al., 1985).

Ghayour M.M. et al. (2008) measured serum Cu & Zn/Cu ratio. They were found significant difference in ratio in the dyslipidemic patients groups compared to control. This finding was in support that of the imbalance in Cu & Zn metabolism in dyslipidemic patients with NS. The imbalance in Zn/Cu metabolism may either contribute to the CHD risk or be consequence of an acute phase response of NS. Bovino G. et al. (2007) found that the serum Cu and Zn levels were below the normal range in patients with nephrotic proteinuria. Serum Zn was directly correlated with proteinuria and urinary Zn, but negatively correlated with testosterone levels in both sexes. This supported to the positive correlation of Zn and albumin in the present study. However Hughes S. et al. (2006) observed that Zn supplementation decreased Cu/Zn-SOD activity, primarily due to the antagonistic relationship high Zn intakes and Cu absorption. Besides the demonstrated adverse effect of Zn supplementation on plasma HDL-C Concentration in apparently health men, there was insufficient evidence to determine the role of Zn supplementation in influencing other risk factors for CHD such as antioxidant status and thrombogenesis.
6.3 Lipoprotein metabolism in NS

Dyslipidemia is a contributory factor in the progression of initial glomerular injury in NS (Delvin EE. et al., 2003). In the present study, significantly increased mean serum level of TC, LDL-C, TG and significantly decreased level of HDL-C in NS was observed, although dyslipidemia is a common complication of NS. The increased level of serum TC could be attributed to impaired metabolism of mevalonate by the nephrotic kidney. This allows a greater cholesterol availability that coupled with an enhanced 3-Hydroxy Methyl Glutaryl-CoA (HMG-CoA) reductase activity leads to increased hepatic cholesterol synthesis and unbalanced lipid homeostasis (Delvin E.E. et al., 2003). HDL-C is an effective antioxidant with the capacity to inhibit oxidative modification of LDL-C. HDL-C also possesses anti-inflammatory properties. These antioxidant and anti-inflammatory properties of HDL-C may be as important as its cholesterol efflux function in terms of protecting against development of atherosclerosis (Barter P.J. et al., 2004).

Hyperlipidemia affects renal function, increases proteinuria and induce glomerulosclerosis, thus determining a higher risk of progression to CVD. 3-Hydroxy-3 methylglutaryl-coenzyme-A (HMG-CoA) reductase was the rate-limiting enzyme in cholesterol synthesis from mevalonate and its inhibitors are statins, can therefore interfere with the above mentioned consequence of hyperlipidemia. As for as kidney disease were concerned, statin therapy has been shown to prevent creatinine clearance decline and decelerate renal function loss, particularly in case of proteinuria, and its favorable effect may depend only partially on the attenuation of hyperlipidemia. (Buemi M et al., 2005).

Hypercholesterolemia was one of the major manifestations of nephrotic syndrome. Nephrotic hypercholesterolemia was associated with and in part, due to dysregulation of hepatic HMG-CoA reductase, acyl-CoA: cholesterol acyltransferase (ACAT) and cholesterol 7 alpha hydrolase, as well as lecithin: cholesterol acytransferase (LCAT), low-density lipoprotein (LDL) receptor and high-density lipoprotein (HDL) receptor deficiencies. Heavy proteinuria, hypoalbuminemia, hypercholesterolemia, elevated total cholesterol: HDL cholesterol ratio and normal creatinine clearance. This was associated with severe reductions in hepatic LDL-D receptor, hepatic HDL-C receptor and plasma LCAT.
concentration, marked upregulation of hepatic ACAT, and uncharged cholesterol 7-alpha-hydroxylase (rate-limiting step in cholesterol catabolism). Statin administration for 2 weeks ameliorated hepatic LDL-C receptor, HDL-C receptor deficiencies and significantly lowered plasma cholesterol, LDL cholesterol, total cholesterol to HDL cholesterol ratio and Proteinuria. HMG-CoA reductase inhibition improved hepatic LDL-C and HDL-C receptor deficiencies, and ameliorated the associated hyperlipidemia in the nephrotic syndrome (Vaziri N.D. et al., 2004).

In many studies statin therapy was mandatory in order to prevent atherosclerosis in patients with NS. Statin drugs were used in nephrotic syndrome patients with hypercholesterolemia and combined hyperlipidemia to test whether the drug decreases production of LDL-C and reduces levels of VLDL-C and IDL-C. Treatment of combined hyperlipidemia may require statin based drug targeted to normalize levels of VLDL-C, LDL-C and IDL-C (Miltiadous G. et al., 2006, Todo R.D. et al., 2000). In NS, early and intensive therapy is antilipidemic drugs combined with diuretics, furosemide (Matsunaga A. et al., 2009).

Dyslipidemia is widely accepted risk factor for CVD. In our study we observed significantly increased TC, TGs, LDL-C levels in NS patients. Similar results have been reported by many other studies. Few studies have shown lipid abnormalities, proteinuria and cardiovascular risks in primary nephrotic syndrome. Hyperlipidemia, a common complication, was very prevalent in patients with primary nephrotic syndrome. Hyperlipidemia was not only significantly increased the cardiovascular risk in adulthood, but also accelerated the progression of renal disease. Urinary protein excretion and the serum concentrations of Lp(a), TC, TG, HDL-C, LDL-C and apo B were higher in the primary nephrotic syndrome than in the control group (p<0.001), no correlation existed between serum lipid profiles and serum Albumin (p>0.05). Secondary dyslipidemia in patients with primary nephrotic syndrome was in parallel with the degree of urinary protein excretion (Hu P. et al., 2009).

Some studies had reported raised serum TG levels in NS. TGs levels were raised in NS because of the presence of a combined defect: A decrease in endothelial-bound LPL that occurred as a consequence of reduced serum albumin
concentration and a defect in VLDL-C binding to endothelium bound LPL. This later defect occurs only in the presence of proteinuria and was conferred by HDL-C (Shearer G.C. et al., 2001).

Patients with NS had impaired endothelial function probably related to dyslipidemia; endothelial function was assessed at the baseline, after 12 weeks of treatment with statins. Serum lipids were significantly lowered following (p<0.001). There was a trend to an increase in serum albumin and flow mediated dilation improved significantly following treatment. Studies found that statins therapy significantly improved dyslipidemia and branchial artery endothelial function in patients with NS (Beumi M. et al., 2005 and Cheung C.Y. et al., 2009). These findings also suggested a role for dyslipidemia in endothelial dysfunction and the risk for cardiovascular disease in NS. There was significant improvement in the clinical parameters and resolution of symptoms after the introduction of statins. Our study also confirmed previous findings and the role of apolipoproteins and their interaction with lipoprotein abnormalities in the pathogenesis of lipoprotein glomerulopathy in NS. In our study we reported that prednisone (steroid anti-inflammatory and immunosuppressive), simvastatin (for dyslipidemia) furosemid (diuretics) were used for the treatment of NS, this treatment regime was supported by many other studies.

6.4 Lp(a) metabolism in NS

In the present study all patients had elevated Lp(a) level. Saibaba KSS et al. (2004), reported that LP(a) levels correlated positively with severity of atherosclerosis (p<0.001). The Lp(a) levels in healthy population reported in Indian studies had taken Lp(a) level 25 mg/dl as cut of value and analyzed its association with cardiac heart disease using logistic regression and observed significant relationship. This study confirmed positive correlation of LP(a) with CHD at greater than 25 mg/dl. Proteinuria, lipid profile, high levels of LDL-C & Lp(a) treated by prednisone therapy in NS, result proteinuria disappeared after one month & average normalization of lipid profile and lipoproteinuria level (Merouni A et al., 2003).

Recently, Lp(a) abnormalities in NS has been demonstrated. The atherogenic serum Lp(a) was significantly elevated in patients with nephrotic syndrome. The
primary cause may be markedly elevated number of low-molecular-weight apo(a) phenotypes which were usually associated with high Lp(a) levels. In addition, secondary causes for the pathogenetic mechanisms of the nephrotic syndrome, itself resulted in increased level of Lp(a) in the various apo(a) isoform groups. The tremendously increased Lp(a) levels in nephrotic syndrome were caused by primary genetic as well as disease-related mechanisms. However some studies reported that in NS patients, lipid profile disturbances persist during nephrotic syndrome remission, elevation of genetic polymorphisms of proteins involved in lipoprotein metabolism in children and adolescents with nephrotic syndrome (Ksiazek J. et al., 2009).

Some studies have been reported the mechanism of the thrombotic role of Lp(a) in NS. First quantitative evidence that binding of Lp(a) to lysine residues of fibrin and cell surfaces was directly related to circulating levels of both plasminogen and Lp(a) and that these glycoproteins may interact as competitive ligands for these biological surfaces into vivo. This mechanism may be of relevance to the atherothrombotic role of Lp(a), particularly in nephrotic patients (Saulat T. et al., 2000).

An increased fapo(a) level in renal patients possibly could be one of the reasons contributing to the high incidence of vascular diseases in these patients, because fapo(a) not covalently linked with Lp(a) is even more easily able to inhibit the fibrinolytic system than the complete Lp(a) (Herrman W. et al., 2000).

It has been observed that hyperlipidemia and lipoprotein abnormalities were often encountered in patients with nephrotic syndrome. Even though the significance of lipid deposition in renal tissue and the role of lipoproteins in the pathogenesis of renal disease in man were unclear, experimental and clinical data indicate a possible damaging effect of a disturbed lipid metabolism on the kidney. There were strong indications that lipoproteins play a critical role in mediating the development of glomerulopathies. These all above findings were further endorsed by the observation of our study and others that significant correlation with this.

Kuge Y. et al. (2005) were observed severe proteinuria hyperlipidemia hypoalbuminemia, and very high level of Lp(a) in the serum of nephrotic
syndrome patients. Severe atherosclerosis was also found in nephrotic syndrome, which was abdominal aortic syndrome (AAS). Abdominal aortic aneurysm (AAA) and coronary artery disease (CAD) were detected and treated by statins and prednisone. Markedly elevated LP(a) plasma levels in patients had played an important role in the progression of atherosclerosis. Kronenberg F. et al. (2004) found high Lp(a) concentration that significantly contribute to the measured or calculated LDL-C level. It was expected that the LDL-C lowering effect of statins diminished in patients who had pronounced elevation of Lp(a) level accompanied by only moderate elevation of LDL-C. Patients showed a tremendous elevation of Lp(a) concentration when compared to controls (p<0.001). The tremendously increased Lp(a) levels in nephrotic syndrome was explained primarily by genetic defect and secondary to disease related (pathogenetic) mechanism. There was no gender difference in Lp(a) levels of healthy as well as NS patients(Pedreno J, et al., 2000). Though Saibaba KSS et al. (2004) reported influence of gender on serum Lp(a) level, significantly high level was in female than in male. This may be due to the lowering effect of testosterone in males and presence of menopause status.

6.5 Antioxidant imbalance in NS

Nephrotic Syndrome is a consequence of an imbalance between oxidants and antioxidants. In the present study significantly increased TC, TG and LDL-C level and decreased levels of HDL-C TAC, Zinc and Copper were noticed in the patients with Nephrotic Syndrome. Caraba A et al. (2007) studies endothelial dysfunction they assessed and correlated it with dyslipidemia and markers of inflammation and endothelial dysfunction in patients with nephrotic syndrome. The most important factors involved in atherosclerosis, were the endothelial dysfunction in the NS with LDL-C, TC, and their treatment.

Recent study had shown that the reactive oxygen species (ROS) play a role in inducing the proteinuria in nephrotic syndrome (NS). Patients in the active phase of NS had significantly lower PON (Paraoxonase), and TAR (total antioxidant response) levels and higher Oxidative stress index (OSI) and total peroxide values than those in full remission. Significant correlations were found between PON, TAR, and total peroxide. Serum total protein and significantly positive correlation
with PON and negative correlation with total peroxide in acute-period NS patients. (Ece A. et al., 2005).

Some studies found that nephrotic syndrome (NS) was stressful condition for patients where oxidative damage would also influence the response of these patients undergoing therapy.

It has been reported that erythrocyte superoxide dismutase (SOD) concentrations were significantly decreased in NS group when compared with controls. Thus, antioxidant concentrations change considerably, indicating a compensatory mechanism to cope up with increased pro-oxidant status in such cases (Kamireddy R. et al., 2002) Antioxidant status and reliable factors involved in antioxidant protection with nephrotic syndrome (NS). There was increased lipid peroxidation and insufficient antioxidant defense in nephrotic syndrome (Mader W. et al., 2001).

Some studies had been found that increased oxidative stress correlated with glomerular injury. Decreased expression of glomerular antioxidant enzymes (AOEs), including Cu/Zn superoxide dismutase (SOD) in a wide variety of glomerular diseases. Similarly other data reported that the mean vitamin E, vitamin C was significantly lowered during the proteinuria phase of the disease. There was significant elevation in the serum level of malondialdehyde (MDA) during this phase. In addition, all these parameters tended to improve during remission (Mathew J.L. et al., 2002).

E.I. Melegy et al. (2008) recently reported significantly higher serum levels of malondialdehyde, oxidized LDL, TC, LDL-C, TG, apolipoprotein A-I and apolipoprotein B. The serum levels of albumin, glutathione peroxidase activity, Vitamin C Vitamin E and HDL-C were significantly lowered, a significant relationship between the oxidant/antioxidant status and dyslipidemia were documented in patients with steroid sensitive nephrotic syndrome, especially among relapsers. These findings were in support of our finding where no normalization of the biochemical indices were observed despite the use of glucocorticoids. Therefore the combined use of steroid, antioxidant therapy and lipid lowering therapy can be recommended in such patients.
6.6 tHcy metabolism in NS

Homocysteine (Hcy), as a cardiovascular risk factor, was studied over 30 years ago, through the observation of extensive atherosclerotic lesions during autopsies of patients affected by certain genetic variants of homocystinuria. Thereon, Hcy has been investigated as a factor in the genesis of atherosclerosis (Perna A.F., 2001). In the present study controlled NS 4.8% patients have greater than 15 μmol/L tHcy, in managed NS 5.7% have tHcy greater than 15 μmol/L, in uncontrolled NS 30.5% have greater than 30 μmol/L tHcy, 5.7% have greater than 15 μmol/L tHcy. Some other study was in agreement of this concept Majumdar VS et al. (2001), showed that tHcy mediated impairment of endothelial dependent vasodilation were reserved by co-incubation of tHcy with nicotinamide (an inhibitor of peroxinitrate and nitrotyrosine) suggesting a role of tHcy in redox mediating endothelial dysfunction and nitrotyrosine formation which was supported to oxidative stress by tHcy. tHcy was negatively correlated with serum total protein and Albumin. These findings were in agreement with the findings of Gurusharan D. et al., (2001). In their study they observed that tHcy was significantly correlated with serum creatinine (=0.58; p<0.01) and calculated GFR (r=0.45; p<0.05). There was no significant correlation with urinary protein or serum albumin, our results is in supports of this. Increased tHcy level was due to renal failure for effective amino acids clearance. After treatment significant improvement in serum albumin and marked reduction in proteinuria consists with remission of nephrosis. However Margret A. et al. (2001) showed significantly lower tHcy level in NS patients than nonephrotic patients. tHcy correlated significantly with serum concentration of creatinine (r=0.53; p<0.050) and albumin (r=0.43; p<0.05) GFRs r=0.42; p<0.05) and urinary albumin excretion (r=0.47; p<0.05). Plasma tHcy concentration did not change significantly during treatment. There is no significant reduction in serum tHcy in managed NS.

Recent experimental evidences suggested that an increased concentration of tHcy resulted in vascular changes through several mechanisms. Hyperhomocysteinemia arised from disrupted tHcy metabolism. Severe hyperhomocysteinemia was due to rare genetic defects resulting in deficiencies in cystathionine-β-synthase, methylene tetrahydrofolate reductase (MTHFR) and an
activator of cystathionine-β-synthase or an enzyme involved in methylcobalamine synthesis and tHcy methylation. High levels of tHcy induce sustained injury of arterial endothelial cells. Proliferation of arterial smooth muscle cells and enhance expression/activity of key participants in vascular inflammation, atherogenesis, and vulnerability of the established atherosclerosis plaque. These effects were mediated through its oxidation and the concomitant production of reactive oxygen species (ROS). Changes in metabolism of tHcy may have an influence on endothelial dysfunction and change the redox balance (Tkaczyk M. et al., 2009).

It has been demonstrated that dietary supplementation with folic acid and Vitamin B₁₂ and Vitamin B₆ were efficient means to decrease plasma tHcy. Endothelial dysfunction was underlying cause leading to proatherogenic effect associated with hyperhomocysteinemia. Folic acid and Vitamin B₁₂ deficiencies should be corrected by supplementation in hyperhomocysteinemia. Increased folate intake by dietary changes or fortification can also lower plasma tHcy levels in vitamin depleted subjects (Sydow K. et al., 2001).

Recent findings also suggested that remethylation of homocysteine to methionine in the kidneys was of great importance for homocysteine clearance. Markedly increased concentrations of methylmalonic acid, a metabolic marker of vitamin B₁₂ deficiency, have been found in approximately 70% of renal patients. The lowering of methylmalonic acid and tHcy concentrations in renal patients after Vitamin B₁₂ administration may indicate the presence of intracellular pre-treatment deficiency. They administered folic acid (5 mg) plus vitamin B₆ (10 mg) and B₁₂ (0.7 mg) three times per week intravenously to hyperhomocysteinemic nephrotic patients. tHcy was decreased after 4 weeks by 51%. Serum concentrations of methylmalonic acid and cystathionine were reduced by 28% and 26% respectively. (Ciaccio M. et al., 2010 and Herrman W. et al., 2005).

The mechanism through which elevated circulating level of tHcy cause vascular injury and promote thrombosis remain elusive. Coppola A. et al. (2000) found that in homocysteinuric patients which are homozygotes for mutations of the gene coding for the cystathionine β synthase enzyme, abnormalities of coagulation variables reflect a hypercoagulable state. During the autooxidation of tHcy in
plasma, reactive oxygen species were generated. The later initiated lipid peroxidation in cell membranes (potentially responsible for endothelial dysfunction) in circulating lipoprotein, oxidized LDL-C, trigger platelet activation as well as some of the homeostatic abnormalities. In such patients, thus the oxidative stress induced by tHcy is a key process in the pathogenesis of thrombosis in NS.

An elevated plasma level of homocysteine (tHcy) was associated with increased risk of thrombotic, atherosclerotic vascular disease and endothelial dysfunction (Austin RC et al., 2004). However in renal diseases, folic acid treatment (1-5 mg/day) ameliorates the plasma homocysteine level in most of the cases but hyperhomocysteinaemia persists in the majority of patients. Primary fasting hyperhomocysteinaemia treated with folic acid (0.5-5 mg/day). Hyperhomocysteinaemia treated with a combination of pyridoxine and folic acid. In the absence of close-effect studies, a combination of pyridoxine (50 mg) and folic acid (5 mg) was advised (Van Stehouwer C.D., 2001).

The results of present study matched to the increasingly accepted hypothesis that tHcy is not only a cardiac risk factor but also an important factor for oxidative stress induced endothelial dysfunction and nitrotyrosine formation. tHcy was negatively correlated with serum albumin. These findings were in agreement with the findings of Gurusharan D. et al. (2001), who found that tHcy was significantly correlated with serum creatinine (=0.58; p<0.01) and calculated GFR (r=0.45; p<0.05) but not with urinary protein or serum albumin. This increased tHcy level was due to renal failure for effective amino acids clearance. After treatment significant improvement in serum albumin and marked reduction in proteinuria consistent with remission of nephrosis. However Margret A. et al. (2001) found significantly lower tHcy level in NS patients than nonephrotic patients. tHcy was correlated significantly with serum concentration of creatinine (r=0.53; p<0.050) and albumin (r=0.43; p<0.05) GFRs r=0.42; p<0.05) and urinary albumin excretion (r=0.47; p<0.05). In our study we also reported that Plasma tHcy concentration did not change significantly during treatment.

In the present study it was found that the serum Cu & Zn concentration were decreased in NS and nephropathic patients. Our study correlated the cardiac risk and
imbalance antioxidant status with changes in serum Cu and Zn levels. Many other studies supported this evidence.

In the present study it was found that the serum HCY was negatively correlated to the Cu & Zn. Hughes et al. (2008) showed that elevated level of HCY were involved in dilated cardiomyopathy, thCy chelates copper and impairs Cu dependent enzymes, Cu deficiency has been linked to hyperhomocysteinemia & cardiovascular disease. These studies had suggested that Cu supplement action help to improve cardiac function in a pressure overload dilated cardiomyopathy. These findings were in agreement of our study where decreased level of Cu due to increased level of thCy in nephrotic syndrome patients and Cu deficiency was related to risk of cardiac diseases. Kenkeni M. et al. (2008) reported low activity of GSH-Px, SOD and Zn concentration were associated with Hyperhomocysteinemi.

Ghayour M.M. et al. (2008) measured that serum Cu & Zn/Cu ratio were significantly different in the dyslipidemic patients compared to healthy subjects. These findings were supported disturbances in Cu and Zn metabolism in dyslipidemic patients with NS. The imbalance in Zn/Cu metabolism may either contribute to the CHD risk or be consequence of an acute phase response of NS. Bovino G. et al. (2007) found that the serum Cu and Zn levels were below the normal range in patients with nephrotic proteinuria. Serum Zn was directly correlated with proteinuria and urinary Zn, but negatively correlated with testosterone levels in both sexes.

Endothelial dysfunction and homeostatic disturbances were common features of nephrotic syndrome (NS) due to hyperhomocysteinemia, hyperlipoproteinemia, hypoproteinemia. Some studies reported that in the nephrotic patients there was an evidence of endothelial cell injury. Similarly other studies reported that the endothelial functions in NS were inversely correlated with serum LDL-C. Dyslipoproteinaemia probably contribute to the increased risk of CVD as seen in NS. Hypoalbuminemia increases the delivery of lipoproteins to endothelial cells thereby impairing the synthesis of lipoproteins (Dogra et a., 2002).

The results of present study further supported by accepting the hypothesis that the endothelial functions impairment constitute a link between nephrotic
syndrome, atherosclerosis and other complications. These disturbances were
dependent on the degree of proteinuria and serum albumin concentration. The
patients with severe clinical course of nephrotic syndrome were at high risk of
accelerated atherogenesis.

Nephrotic syndrome is characterized by proteinuria > 3.5g/24h, oedema.
Hypoalbuminaemia and hyperlipidaemia. Several glomerular diseases, either
primary or secondary, may lead to nephrotic syndrome. Renal biopsy was often
mandatory. Urinary protein loss leads to several complications: water and sodium
retention, hyperlipidaemia, increased risk of thromboembolism, infection, anemia
and alteration of mineral metabolism. Each of these complications must be identified
(De Seinguex et al. (2009). Adult patients with primary nephrotic syndrome
associated with IgA nephropathy, used immunosuppressive therapy resulted in a
high percentage of achieved remissions (Rasic S. et al., 2008).

Some studies had been reported that glucocorticoid activates glomerular
antioxidant enzymes and protects glomeruli from oxidant injuries (Tetsuya et al.,
1991). These studies indicated that the mechanism for therapeutic effect of
glucocorticoids on ROS-mediated renal injuries include an enhancement of
endogenous glomerular AOE activities, which attenuates lipid peroxidation of
glomerular tissue (Tetsuya et al., 1991).

Some studies had shown that the minimal change nephrotic syndrome
(MSNS) was a common form of nephrotic syndrome in children and young adults.
Clinical presentations of older patients (> 50 years) with MCNS were similar to
younger patients’ apart form the age related decline of renal function and higher
prevalence of hypertension. Both groups had similar steroid responsiveness, but
older patients tend to had fewer secondary nephropathies (Kai Chung et al., 2003).

Some study referred to membranous nephropathy in the elderly. MN had the
same risks for progression as in male and females individuals thus, therapy for
hypertension, hyperlipidemia, edema and proteinuria should be instituted.
In the present study we reported that prednisone (steroid, anti-inflammatory and immunosuppressive), simvastatin (For dyslipidemia) furosemid (diuretics) were used for the treatment of NS.
REFERENCES


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