

Nephrotic Syndrome, Steroid Therapy and Associated Cardiovascular Risk; is Annexin A5 a Biomarker?

Souparnika S¹,
Benedicta D'souza^{2,3*} and
Vivian D'souza³

- 1 Department of Biochemistry, Srinivas Institute of Medical Sciences, Mukka, Mangalore, India
- 2 Department of Biochemistry, Kasturba Medical College, Manipal University, Mangalore, India
- 3 Department of Biochemistry, Kanachur Medical College, Derelakkatte, Mangalore, India

*Corresponding author:
Benedicta D'souza

✉ biochemistryforhealth@gmail.com

Department of Biochemistry, Kasturba Medical College, Manipal University, Mangalore, India.

Tel: +919448910944

Citation: Souparnika S, D'souza B, D'souza V (2020) Nephrotic Syndrome, Steroid Therapy and Associated Cardiovascular Risk; is Annexin A5 a Biomarker? Biomark J Vol.6 No.1:66

Abstract

Aims: Thromboembolism is one of the major complications affecting nearly 25% of Nephrotic Syndrome patients. Annexin A5 is a calcium dependent positively charged glycoprotein with potent anti-coagulant activity. Previous studies have reported elevated Annexin A5 levels immediately after Myocardial Infarction and its plasma level reflected the severity of myocardial damage. Cardiovascular abnormalities during Nephrotic Syndrome and the role of Annexin A5 as a cardiac risk factor have been studied here.

Methods: 72 adult Nephrotic Syndrome patients were studied for the blood levels of Annexin A5, lipid profile, Atherogenic Index and LDL/Annexin A5 ratio. The results were compared to age and sex matched healthy controls. The patients started on steroid therapy and they were followed up for a period of ten months. 27 patients attained remission during the time period and all the biochemical assays were repeated.

Results: Nephrotic Syndrome patients exhibited dyslipidemia along with elevated Annexin A5, Atherogenic Index, Total Cholesterol/HDL ratio and LDL/Annexin A5 ratio. Annexin A5 was not correlated to cardiovascular risk but LDL/Annexin A5 ratio positively correlated with Atherogenic Index. Dyslipidemia and elevated atherogenic risk persisted in patients who attained remission.

Conclusions: Cardiovascular risk persisted during remission even after effective steroid therapy. LDL/Annexin A5 can be considered as a cardiovascular risk factor in Nephrotic Syndrome patients.

Keywords: Biomarkers; Nephrotic syndrome; Steroid therapy

Received: January 20, 2020; **Accepted:** March 03, 2020; **Published:** March 10; 2020

Introduction

Nephrotic Syndrome (NS), is a well-defined combination of clinical and laboratory findings characterized by proteinuria, hypoalbuminemia, hyperlipidemia and edema [1]. According to the National Vital Statistics Report 2012, NS and associated kidney diseases are the 9th leading cause of deaths in USA [2]. Due to cumulative steroid therapy, intermittent hyperlipidemia and overt proteinuria, thromboembolism turned up as one of the major complications of NS. The incidence of thromboembolism is 25% in adults and 3% in children [3]. The major risk factors during the series of events starting from NS to cardiovascular disease includes hyperlipidemia, elevated oxidative stress, deregulated Extra Cellular Matrix (ECM) proteases, imbalanced pro-coagulant-anti-coagulant proteins and hypoalbuminemia.

The present study deals with the role of an anticoagulant protein called Annexin A5 (AnxA5) and its function as a cardiac risk factor in NS.

Annexin is a multiprotein family consisting of more than 160 proteins. AnxA5 is a glycoprotein which binds to negatively charged phospholipids, with high affinity and in a calcium-dependent manner. This characteristic of AnxA5, also inhibits the prothrombinase complex and the tenase complex in-vitro, resulting in potent anticoagulant activity [4]. AnxA5 can bind to the phosphatidyl-serine of apoptotic cells, and thereby inhibiting the procoagulant and proinflammatory activities of the dying cells. The level of circulating AnxA5 reflects the severity of cell damage and inflammation [5]. An excellent review gives further information on its detailed structure, properties and boundless functions [4].

AnxA5 levels were elevated immediately after Myocardial Infarction (MI) and it was observed to correlate with the extent of apoptosis [6]. Immunocytochemical studies identified AnxA5 and A6 in both myocytes and non-myocytes in a variety of species. Most of these reported increased concentrations of AnxA5 along the sarcolemma and Z line in cardiomyocytes [6]. During end-stage heart failure the levels of Annexin A6 fall in cardiomyocytes whereas those of annexins A2 and A5 rise [7]. The high levels of plasma AnxA5 in patients with acute MI, cardiac arrest and severe trauma reflects the severity of damage of the myocardium and/or other visceral organs, and measurement of plasma AnxA5 concentration may help to assess the prognosis of patients brought to the emergency room [8].

Kamel et al. studied the urinary AnxA5 concentration in NS and it was found to be elevated in responders of cyclophosphamide [9]. Twenty four-hour urinary AnxA5 excretion may be a prognostic marker in children with NS [10]. Matsuda et al. suggested that a high urinary AnxA5 concentration may be an indicator of acute renal injury [11]. No studies have investigated the role of AnxA5 as a cardiac risk factor in NS patients. Therefore, the present study measures the serum AnxA5 levels in NS cases before and after steroid therapy and compare with that of the healthy controls. Correlation of AnxA5 with lipid profile and atherogenic Index was also performed to assess the cardiovascular risk.

Materials and Methods

The present study comprised of two phases. The first phase was a Case-Control study in which 72 newly diagnosed primary NS patients in the age group of 18-65 years and 72 age and sex matched healthy subjects were included. Control subjects were free from any clinical illness. Informed consent was obtained from all the subjects and the study was approved by the Institutional Ethics Committee, Kasturba Medical College, Mangalore. Fasting blood samples were collected, serum separated and stored at -80°C until analysis.

Total Cholesterol (TC), High Density Lipoprotein (HDL) and triglycerides (TG) were estimated in serum by semi-autoanalyser kit method. Serum AnxA5 was estimated by Enzyme Linked Immunosorbent Assay (ELISA). Low Density Lipoprotein (LDL) and Very Low Density Lipoprotein (VLDL) were calculated by Friedwalds formula. Atherogenic Index (AI) was measured by the formula $\log(TG/HDL)$ (12). TC/HDL and LDL/AnxA5 ratios were also calculated.

All the NS patients were treated with the recommended regime of steroids depending on the type of primary NS, following the KDIGO guidelines [12,13].

In the second phase of the study, the patients were followed up during their regular check up in the hospital, for a period of ten months. Out of 72 NS patients only 27 attained remission in this time period and they were included in the remission group.

Remission: Normalization of proteinuria (to $<4 \text{ mg/m}_2/\text{hr}$ or urine albumin dipstick of zero to trace for three consecutive days) and serum albumin (at least 3.5 g/dl), along with resolution of edema [14]. All the biochemical analyses were repeated in these study subjects.

Statistical Analysis

Sample size was calculated based on the statistical formula for paired t test. IBM SPSS, version 20.0 was used for the analysis. Quantitative data was expressed as mean \pm standard deviation (SD) for normally distributed parameters and as median \pm Inter Quartile Range (IQR) for non-normal distributions. Case-control comparisons were performed by paired students t-test and Wilcoxon signed rank test for normal distribution and skewed distributions respectively. Pearsons and Spearmans tests were used for correlation analyses. In Phase II of the study, One way ANOVA and Kruskal Wallis test were used to compare the results of cases, controls and remission groups. $p < 0.05$ was considered statistically significant.

Results

Phase I

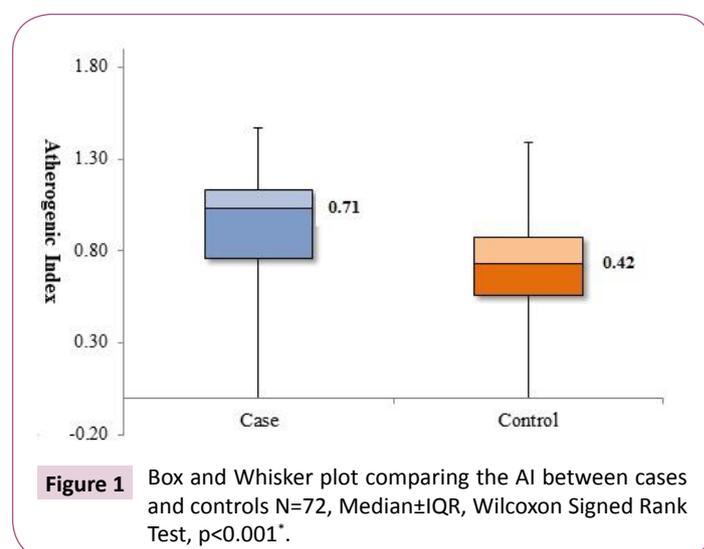
There is profound dyslipidemia in NS patients when compared to their age and sex matched healthy controls (Table 1). Patients exhibited higher levels of TC, TG, LDL, VLDL and TC/HDL ratio. Atherogenic Index (AI) was also significantly high in NS cases (Figure 1).

According to the International Lipid Information Bureau (2000), a TC/HDL ratio >5 (for men) and >4.5 (for women) is considered as the risk level for the primary prevention of cardiovascular diseases [15]. An Atherogenic Index [Log (TG/HDL)] over 0.5

Table 1 Comparison of Lipid profile between NS Cases and Healthy Controls.

Parameters	Cases (n=72)	Controls (n=72)	p value
T. Cholesterol (mg/dL) [#]	304.4 \pm 149.9	162.15 \pm 55.47	<0.001*
Triglycerides (mg/dL) [#]	201.0 \pm 143.75	123.51 \pm 88.67	<0.001*
HDL (mg/dL) [§]	47.31 \pm 16.90	48.16 \pm 12.13	0.703
LDL (mg/dL) [#]	193.9 \pm 129.08	91.5 \pm 56.63	<0.001*
VLDL (mg/dL) [#]	40.2 \pm 28.75	24.7 \pm 17.74	<0.001*
TC/HDL [#]	5.83 \pm 4.59	3.56 \pm 1.89	<0.001*

[§]Mean \pm SD paired t test [#]Median \pm IQR Wilcoxon Signed Rank test, * $p < 0.05$ statistically significant



has been proposed as the cut-off point indicating atherogenic risk [16]. The NS cases have a TC/HDL value of 5.83 and an AI value of 0.71, with increased LDL, TG, and VLDL. This infers that the cardiovascular risk in adult NS cases is high when compared to their age and sex matched healthy controls. 71% of the NS patients were falling in the high risk group.

AnxA5 levels were elevated in cases when compared to healthy controls (Figure 2). LDL/AnxA5 ratio was also high in cases (Figure 3). There was a significant positive correlation of LDL/AnxA5 ratio and AI in NS patients with an r value of 0.351 (Figure 4). But AnxA5 was not found correlating with AI or any of the lipid parameters.

Out of 72 NS patients, renal biopsy was performed for 45 subjects. Out of these, 12 were diagnosed with IgA Nephropathy (IgAN), 11 with Minimal Change Disease (MCD), 10 with Focal Segmental Glomerulosclerosis (FSGS), 9 with Membranous Nephropathy (MN) and 3 with Membranoproliferative Glomerulonephritis (MPGN). (Table 2) shows the results of subtype analysis in NS patients. The results of TC/HDL ratio, AI and LDL/AnxA5 ratio are mentioned in the table with respect to different subgroups of NS. All three parameters were found to be lowest in MCD. Thus, it

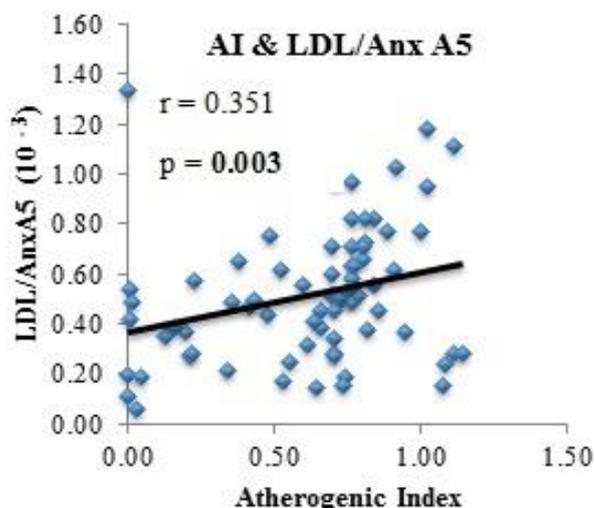


Figure 4 Correlation of AI and LDL/AnxA5 ratio in Nephrotic Syndrome. LDL/AnxA5 ratio was positively correlating with AI in Nephrotic Syndrome patients. N=72, Spearman's test, r=0.351, p=0.003*.

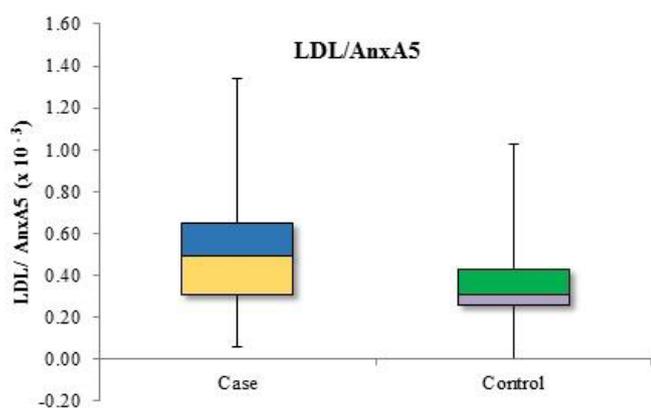


Figure 2 Bar Graph comparing Annexin A5 between cases and control N=72, Mean±SD, Paired t test, p<0.001*.

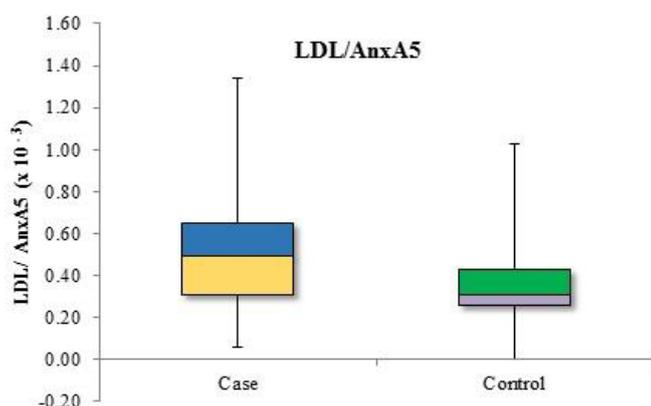


Figure 3 Box and Whisker plot comparing LDL/AnxA5 between cases and control N=72, Median ± IQR, Wilcoxon Signed Rank Test, p<0.001*.

Table 2 Subtype analysis in NS.

Parameter	MCD	FSGS	MN	IgAN	MPGN
N	11	10	9	12	3
AI	0.4	0.7	0.71	0.72	0.76
TC/HDL	4.98	5.32	5.84	6.19	7.51
LDL/AnxA5	0.49	0.51	0.49	0.5	0.68

can be inferred that cardiovascular risk is lowest in MCD when compared to the rest of the subgroups.

Phase II

In phase II of the study, results of biochemical parameters of 27 remission subjects (after steroid therapy) were compared to their results before the initiation of steroid therapy and also with their corresponding age and sex matched healthy controls. Thus there are three groups involved in the second phase. Group I: Age and sex matched healthy controls (n=27); Group II: Patients during acute NS (n=27); Group III: Same patients during remission after steroid therapy (n=27).

Except TC, none of the other lipid parameters showed a significant variation during remission when compared to their acute disease stage. Also, except HDL, all the parameters were significantly higher than their age and sex matched healthy controls (Table 3). AI continued in the high risk range during remission (Figures 5 and 6). Shows the percentage of NS patients in the high risk group before and after steroid therapy. Thus it can be inferred that even after steroid therapy, NS patients are still having elevated cardiovascular risk.

AnxA5 and LDL/AnxA5 ratio slightly decreased but it was not significant. LDL/AnxA5 ratio was still significantly higher than their age and sex matched healthy counterparts (Table 4).

37.5% of MCD patients, 9% of FSGS patients, 25% of MN patients

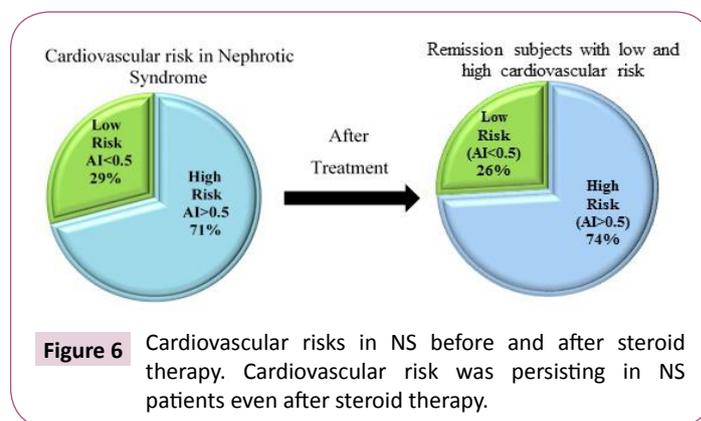
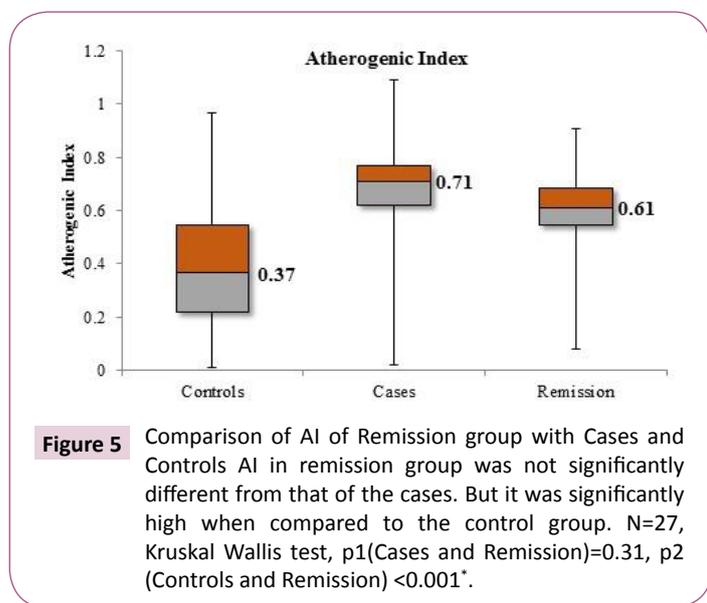
Table 3 Comparison of Lipid Profile between NS cases, NS remission and Healthy Controls.

Parameter	Controls (Group 1)	Cases (Group 2)	Remission (Group 3)	p1 (1&3)	p2 (2&3)
N	27	27	27		
TC (mg/dL) [‡]	166.95 ± 49.40	283.73 ± 109.07	230.12 ± 69.76	0.013*	0.042*
LDL (mg/dL) [§]	91.48 ± 51.62	195.67 ± 93.96	150.89 ± 61.79	<0.001*	0.35
HDL (mg/dL) [‡]	49.62 ± 11.72	44.61 ± 14.50	44.54 ± 11.77	0.31	1
VLDL (mg/dL) [§]	25.85 ± 11.14	43.44 ± 20.99	34.69 ± 11.90	<0.001*	0.49
TG (mg/dL) [§]	129.24 ± 55.72	217.20 ± 104.96	173.46 ± 59.51	<0.001*	0.49
TC/HDL [‡]	3.67 ± 1.66	6.57 ± 2.15	5.31 ± 1.45	<0.001*	0.24

[‡]Mean ± SD One Way Anova test, [§]Median ± IQR Kruskal-Wallis test, *p<0.05 statistically significant.

Table 4 Comparison of AnxA5 and LDL/AnxA5 of Remission group with cases and controls.

Parameters	Controls (n=27)	Cases (n=27)	Remission (n=27)	p1	p2
AnxA5	338.93	393.42	363.63	0.28	0.33
LDL/AnxA5	0.3	0.52	0.43	0.97	0.001



and 7.4% of IgAN patients attained remission during the study period. Remission rate was higher in MCD when compared to other subgroups.

Discussion

In NS, there is an uncontrolled increase in the concentration of lipoproteins in mesangial cells and glomerular capillaries, which results in their proliferation [17]. In the mesangium, lipoproteins are oxidized (oxLDL) and in this form, they stimulate the production of antibodies against themselves resulting in podocyte apoptosis, loss of glomeruli function and chronic kidney disease [18]. Lipid metabolism is thoroughly impaired in NS, which is evident from the outcome of this study (Table 1).

AI exhibits a positive correlation with HDL esterification rate and a negative correlation with LDL size. Ultimately this ratio reflects the percentage of small HDL, small-dense LDL and thus the complex interactions of overall lipoprotein metabolism making it a useful predictor of plasma atherogenicity [19]. da Luz et al. (2008) reported that, the elevation of AI is the most effective predictor of coronary heart disease irrespective of other lipid

serum markers. Since there is an increase in TG and decrease in HDL, AI is markedly increased in NS. An AI over 0.5 has been proposed as the cut-off point indicating cardiovascular risk [19]. In our study, the NS cases have an AI value of 0.71, in addition to the increased LDL, TG, VLDL and TC/HDL. This infers that the cardiovascular risk in adult NS cases is high when compared to their age and sex matched healthy controls.

In our study, serum AnxA5 levels were elevated in NS cases when compared to healthy controls (Figure 1). Dyslipidemia leading to increased apoptosis could be the reason for the increased secretion of AnxA5. The tubular reabsorption of AnxA5 owing to its small protein size of 36 kD with a positive charge also could be a reason for elevated AnxA5 level in NS. Owing to its inability for fibrinolysis, the antithrombotic property of AnxA5 is attributed to its ability to form a shield on the surface of endothelium. According to Cederholm et al. [20], in SLE, autoantibodies on endothelial surface prevent the formation of the shield thereby increasing the AnxA5 levels in the circulation. At the same time, endothelium is more prone to bind with the coagulation factors thereby initiating the thrombus formation. Thus, elevated AnxA5 in the circulation represents a novel mechanism of atherothrombosis. It can be hypothesized that, in NS, altered ECM texture of the endothelium due to reduced activity of Matrix Metalloproteinase-9 (MMP-9) in NS [21] may prevent the shield formation of AnxA5, thereby leading to elevated plasma concentration and increased thrombogenesis. However, this requires further in-depth research.

But AnxA5 did not correlate with any of the cardiovascular risk factors. Also, it was not associated with the severity of NS. Werner et al. reported circulating CD31+/annexin V+ apoptotic microparticles numbers as an independent predictor for an impaired endothelial-dependent vasorelaxation in patients with manifestations of CAD [22]. No studies have reported a direct correlation of AnxA5 with cardiovascular risk factors.

According to van Tits [23], plasma AnxA5 levels inversely correlates with the severity of atherosclerosis. He proposed that OxLDL/AnxA5 is a better measure for assessing the severity of atherosclerosis. In this study, LDL/AnxA5 ratio was found to be significantly high in cases when compared to healthy controls. Moreover, it positively correlated with AI in adult NS patients ($r=0.351$, $p=0.003$).

On subtype analysis of NS, it was found that TC/HDL ratio, AI and LDL/AnxA5 ratio were lowest in MCD. The cardiovascular risk was higher in all the other subtypes of NS. Owing to the minimal mesangial prominence, which is reversible by appropriate treatment in 75% of MCD subjects, cardiovascular risk is comparatively lower in them when compared to other subtypes like FSGS, MN, and IgAN where there is poorer prognosis and increased resistance to the treatment.

Except for a significant decrease in TC, dyslipidaemia persisted during remission. LDL, VLDL, and TG levels were still in the high-risk range during remission. TC/HDL ratio was also higher than the advisable range of 4.5. Our previous results show that the oxidative enzyme Myeloperoxidase (MPO) levels continued to be in the higher range during the remission stage. Since albumin levels were normalized, dyslipidaemia observed during remission cannot be the after-effect of proteinuria. It can be hypothesized that persisting oxidative stress may be responsible for the deregulation of lipoprotein metabolism [24,25].

According to the study conducted by Merouani [26], though HDL concentration was normal during NS remission, there was a significant increase of smaller HDL-3 cholesterol when compared to larger HDL-2 cholesterol. This decreased HDL-2/HDL-3 ratio impairs the HDL maturation leading to elevated levels of an HDL subclass, which is a less effective transporter of apoprotein C-II, an essential cofactor in Lipoprotein Lipase activity required for lipoprotein clearance. NS duration and relapse frequencies are important factors which determine the severity and persistence of dyslipidaemia.

References

- 1 Pal A, Kaskel F (2016) History of Nephrotic Syndrome and Evolution of its Treatment. *Front Pediatr* 4: 56-61.
- 2 Heron, M (2016) Deaths: Leading Causes for 2013. *Natl Vital Stat Rep* 65: 1-95.
- 3 Kerlin BA, Blatt NB, Fuh B, Zhao S, Lehman A, et al. (2009) Epidemiology and risk factors for thromboembolic complications of childhood nephrotic syndrome: A Midwest Pediatric Nephrology Consortium (MWPNC) study. *J Pediatr* 155: 105-110.

Out of the 27 NS cases in remission, only 25.9% ($n=7$) had an AI value less than 0.5. Remaining subjects were still in the high-risk range. Thus, there was no significant change in the percentage of high cardiovascular risk group even during remission of NS. Persisting elevation of AI can be attributed to dyslipidaemia with increased triglyceride-rich lipoproteins and dysfunctional HDL due to the sustained high level of MPO during remission.

Though not significant, AnxA5 levels decreased slightly during remission. It can be due to the effect of steroids leading to reduced inflammation and apoptosis and thereby decrease in the release of AnxA5 from the cells.

According to Ece A (26), steroids neither suppress the oxidative stress nor correct the lipid abnormalities in NS. Instead they recommended the requirement of some other modality of treatment to correct this.

Though AnxA5 increased in NS, since there was no correlation with any cardiac risk factors, it cannot be considered as a biomarker for cardiovascular risk in NS patients. But LDL/AnxA5 ratio was significantly elevated in NS cases and positively correlated with AI. Therefore LDL/AnxA5 ratio can be considered as a better marker for cardiovascular risk than AnxA5 alone in NS patients.

Conclusion

Dyslipidaemia with elevated TC/HDL ratio and AI in NS triggers increased cardiovascular risk and the risk sustained during remission. The cardiovascular risk in NS patients with MCD was comparatively lesser when compared to other subtypes. LDL/AnxA5 ratio can be considered as a better marker for cardiovascular risk than AnxA5 alone in NS patients.

Funding

This work was supported by Council of Scientific and Industrial research, Govt. of India [08/048(0001)/2011-EMR-1].

Acknowledgement

I acknowledge the Council for Scientific and Industrial Research for providing the research fellowship.

Conflict of Interest

None declared.

- 4 Gerke V, Moss SE (2002) Annexins: from structure to function. *Physiol Rev* 82: 331-371.
- 5 Hrycek A, Cieslik P (2012) Annexin A5 and anti-annexin antibodies in patients with systemic lupus erythematosus. *Rheumatol Int* 32: 1335-1342.
- 6 Alhusseiny AH, Al-Nimer MS, Mohammad FI, Ali Jadoo SA (2016) Concomitant measurements of serum annexin A5 levels and hematological indices as markers in recent and old myocardial infarction with low ejection fraction: A preliminary study. *Int J Cardiol* 223: 514-518.

- 7 Jans SW, De Jong YF, Reutelingsperger CP, Van Der Vusse GJ, Van Bilsen M (1998) Differential expression and localization of annexin V in cardiac myocytes during growth and hypertrophy. *Mol Cell Biochem* 178: 229-236.
- 8 Matsuda R, Kaneko N, Kikuchi M, Chiwaki F, Toda M, et al. (2003) Clinical significance of measurement of plasma annexin V concentration of patients in the emergency room. *Resuscitation* 57: 171-177.
- 9 Kamel YH, Bazaraa HM, Elwan AE, Fahmy NA, Shaker O (2009) Apoptotic markers in childhood nephrotic syndrome. *J Biol Sci* 9: 509-513.
- 10 Simsek B, Buyukcelik M, Soran M, Bayazit AK, Noyan A, et al. (2008) Urinary annexin V in children with nephrotic syndrome: a new prognostic marker? *Pediatr Nephrol* 23: 79-82.
- 11 Matsuda R, Kaneko N, Horikawa Y, Chiwaki F, Shinozaki M, et al. (2000) Measurement of urinary annexin V by ELISA and its significance as a new urinary-marker of kidney disease. *Clin Chim Acta* 298: 29-43.
- 12 Da Luz PL, Favarato D, Faria-Neto JR, Lemos P, Chagas ACP (2008) High ratio of triglycerides to HDL-cholesterol predicts extensive coronary disease. *Clinics (Sao Paulo)* 63: 427-432.
- 13 KDIGO (2012) Clinical Practice Guideline for Glomerulonephritis. *Kidney Int Suppl* 2: 132-274.
- 14 Gbadegesin R, Smoyer WE (2008) Nephrotic Syndrome. In: Geary DF, Schaefer F. *Comprehensive Pediatric Nephrology*, 1st ed. Philadelphia: Mosby Elsevier 205-218.
- 15 Gotto AM, Assmann G, Carmena R (2000) In: Gotto M. *The ILIB lipid handbook for clinical practice: blood lipids and coronary heart disease*, (2nd edn). New York, NY: International Lipid Information Bureau. 52:53-201.
- 16 Dobiášová M, Frohlich J (2001) The plasma parameter log (TG/HDL-C) as an atherogenic index: correlation with lipoprotein particle size and esterification rate in apoB-lipoprotein-depleted plasma (FERHDL). *Clin Biochem* 34: 583-588.
- 17 Dijkman H, Wetzels J, Gemmink J, Baede J, Levchenko E, et al. (2007) Glomerular involution in children with frequently relapsing minimal change nephritic syndrome: an unrecognized form of glomerulosclerosis? *Kidney Int* 71: 44-52.
- 18 Fernando RL, Varghese Z, Moorhead JF (1993) Oxidation of low-density lipoproteins by rat mesangial cells and the interaction of oxidized low-density lipoproteins with rat mesangial cells in vitro. *Nephrol Dial Transplant* 8: 512-518.
- 19 Dobiášová M (2004) Atherogenic index of plasma [Log (triglycerides/HDL-cholesterol)]: theoretical and practical implications. *Clin Chem* 50: 1113-1115.
- 20 Cederholm A, Svenungsson E, Jensen-Urstad K, Trollmo C, Ulfgren A, et al. (2005) Decreased Binding of Annexin V to Endothelial Cells. A Potential Mechanism in Atherothrombosis of Patients With Systemic Lupus Erythematosus. *Arterioscler Thromb Vasc Biol* 25: 198-203.
- 21 Souparnika S, D'Souza B, D'Souza V, Kumar S, Manjrekar PA, et al. (2016) Matrix Metalloproteinase-9-A connecting link between Nephrotic Syndrome and its impending cardiovascular risk. *Biomedicine* 36: 47-51.
- 22 Werner N, Wassmann S, Ahlers P, Kosiol S, Nickenig G (2006) Circulating CD31+/Annexin V+ Apoptotic Microparticles Correlate With Coronary Endothelial Function in Patients With Coronary Artery Disease. *Arterioscler Thromb Vasc Biol* 26: 112-116.
- 23 van Tits LJ, van Heerde WL, van der Vleuten GM, de Graaf J, Grobbee DE (2007) Plasma annexin A5 level relates inversely to the severity of coronary stenosis. *Biochem Biophys Res Commun* 356: 674-680.
- 24 Souparnika S, D'Souza B, D'Souza V, Kumar S, Manjrekar PA (2015) Emerging Role of Myeloperoxidase in the Prognosis of Nephrotic Syndrome Patients Before and After Steroid Therapy. *J Clin Diagn Res* 9: BC01-BC04.
- 25 Mérouani A, Lévy E, Mongeau JG, Robitaille P, Lambert M (2003) Hyperlipidemic profiles during remission in childhood idiopathic nephrotic syndrome. *Clin Biochem* 36: 571-574.
- 26 Ece A, Atamer Y, Gurkan F, Davutoğlu M, Koçyiğit Y, et al. (2005) Paraoxonase, total antioxidant response, and peroxide levels in children with steroid-sensitive nephrotic syndrome. *Pediatr Nephrol* 20: 1279-1284.