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Original Article

Paediatrics Section

Platelet Functions and Coagulation Changes in Indian Children with Nephrotic Syndrome

ALIZA MITTAL[1], KAILASH CHANDRA AGGARWAL[2], SUMITA SALUJA[3], ARCHANA AGGARWAL[4], BINIT SUREKA[5]

ABSTRACT

Introduction: Only little is known on the effect of the platelet function in the paediatric nephrotic syndrome. The earlier studies which had been done on hypercoagulability have mainly featured the secondary forms of the nephrotic syndrome and the data on the minimal change type of disease is limited.

We therefore, made an effort to study the platelet functions and the coagulation profile in children with the nephrotic syndrome,to find the relationship between the steroid response and the coagulation profile, and to look for the correlation between thromboembolism and the hypercoagulable states.

Methodology: Twenty nine children with the steroid responsive nephrotic syndromewere studied to see the platelet aggregation and the coagulation parameters and their response to the steroid therapy. Doppler studies were done for the renal vein and the inferior vena cava (IVC) thrombus.

Results: It was seen that an increased aggregability of the platelets occurred with Adenosine diphosphate (ADP) and collagen (out of the four agonists, ADP, Collagen, Ristocetin and Arachidonic acid) which were used as agonists for the assay.

We also observed that the Partial thromboplastin time (PTT) had become prolonged and a significant decline in the high values of the procoagulant proteins (Protein C and Protein S) was seen after the steroid therapy and when the children went into remission. These findings were suggestive of a reversibility of the changes in the steroid responsive nephrotic syndrome with the steroid therapy.

One child was found to have thrombosis of the inferior vena cava (IVC) on Doppler studies, which resolved with treatment subsequently.

Conclusions: An increased platelet aggregability contributes to the hypercoagulable states, that may increase the incidence of thrombosis in such patients. Although the incidence of such complications is very low, in a given child with the hypercoagulable states, Doppler may be used to look for any evidence of a latent thrombus and, an early intervention could be instituted. A change in the coagulation parameters points to the reversibility of the changes which are produced in a diseased state.

Key words: Nephrotic syndrome, Platelet aggregation, Deep Vein Thrombosis, Hypercoagulable State, Coagulation profile

INTRODUCTION

The nephrotic syndrome has been considered a hypercoagulable state, which may be complicated by thrombotic episodes of the venous or arterial circulation [1-4].

This study was conducted with the aim of studying the platelet functions and the coagulation profile in paediatric patients with the steroid responsive nephrotic syndrome, the relationship between the steroid response and the coagulation profile, and the association between hypercoagulability and the Doppler studies of the renal vein and the inferior vena cava, for any evidence of thrombosis, so that a rapid therapeutic intervention which was made could become feasible.

SUBJECT AND METHODS

This study was conducted in the Department of Paediatrics of a tertiary care hospital in New Delhi, over a period of one year (Feb. 2010 to Feb. 2011). The patients were included in the study after taking written informed consent from them and the study was initiated after getting clearance from the ethical committee of the institute. This study was conducted on 29 patients with the steroid responsive nephrotic syndrome, who attended the Paediatric Nephrology Clinic and were admitted to the wards in the hospital. Blood samples were taken for platelet aggregation studies (to study the platelet function) and for studying the coagulation parameters at the time of a first episode or a relapse of the steroid responsive nephrotic syndrome, before starting the therapy. Platelet aggregation was performed by using a CHRONO LOG optical platelet aggregometer. The four agonists which were used for measuring the platelet aggregation were Adenosine diphosphate (ADP), collagen, arachidonic acid (AA) and ristocetin. It was performed against two concentrations of ADP (5 µl and 2.5 µl) and two concentrations of collagen (2.5µl and 1 µl).The coagulation parameters which were tested were the Prothrombin Time (PT), the Partial Thromboplastin Time(PTT), the Thrombin Time (TT), Protein C, Protein S and Antithrombin III. All the patients were re-evaluated for the coagulation functions when they were in remission after the completion of six weeks of the steroid therapy. A Colour Doppler ultrasound was performed at the time of the induction of the steroid treatment and after the completion of the treatment, for any evidence of thrombosis in the renal veins and in the Inferior vena cava(IVC). Five healthy children were investigated to obtain a baseline range for our laboratory, as these parameters have to be individualised for each laboratory and for the calibration of the aggregometer. The patients with the steroid resistant nephrotic syndrome, those who were suffering from other infections and were on medications for the same, those with secondary causes of the nephrotic syndrome and those with liver disease were excluded from the study.

STATISTICAL ANALYSIS

The results were compared with the baseline values and they were analyzed by using the SPSS, version 10 software.

RESULTS

The cohort consisted of 23 male and 6 female patients. The mean platelet count in our study was 3,07,000 ± 49099.5 /cumm, which was within the normal range.

The Results of the Platelet Aggregation Studies

Platelet aggregation studies were done on all the patients at the times of their inclusion in the study. The platelet aggregation was found to be increased against ADP, Collagen and Arachidonic acid, which were used as agonists. The mean aggregation in response to ADP was 79.9% and that to collagen was 79.2%.When this mean was compared withour baseline values, it was seen that the difference was significant. The p value for the mean aggregation against ADP was 0.043, and that against collagen was 0.037($p<0.05$).For different concentrations of the reagents which were used, it was found that there was a significantly increased aggregability with both ADP and Collagen. ($p=0.002$ and 0.028 for ADP at 5µl and 2.5 µl respectively) and ($p=0.003$ and 0.000 for collagen at 2.5µl and 1 µl respectively.)

However, the platelet aggregation to arachidonic acid was 72.6 ± 24.32 against our baseline values, where it was 64.0 ± 10.24. This difference was not significant $p=0.200$. The aggregation with ristocetin was not increased.

The Results of the Coagulation Studies

In our study, it was seen that the mean prothrombin times before (PT1) and after the steroid therapy (PT2) were 15.17 s and 15.93 s respectively, which were within normal limits and the difference was not significant. The partial thromboplastin times were 36.21 s and 39.31s respectively before (PTT1) and after (PTT2) the steroid therapy. The difference was significant, with a p value of <0.05.The values for the Thrombin times were 18.07 s and 18.28s before (TT1) and after the treatment (TT2) respectively ($p>0.05$) [Table/Fig-1].

Apart from this, on measuring the values of the pro and the anti-coagulants, it was seen that the mean value for Protein C was 137.95% against a normal range of 72–160%, which lay in the upper part of the normal range. The mean value for Protein S was found to be 108.44% against a normal range of 50-120 %, which was high to normal. Antithrombin III was found to be 76.69 mg% against a normal range of 72–125 mg%, which lay in the lower part of the normal range, which was suggestive of a decreased Antithrombin III level.

Protein C and Protein S were again estimated after the steroid therapy (Prt C2 and Prt S2).The mean value was noted to be 92.5% for Protein C. The mean protein S value was 82.75%.

Although all these values were within the normal range, they were in the lower normal limit and were suggestive of a decline in the levels of the procoagulant proteins [Table/Fig-2].

It was found that there was a significant decline in the level of protein C ($p=0.041$) after the treatment. The difference was not significant for protein S ($p>0.05$).

The Results of the Doppler Study

Ultrasound of the abdomen was performed by using renal Doppler and it was found that mean size of the right kidney was 7.15 × 3.07 cm. The mean size of the left kidney was 7.02 × 3.19 cm.

There was an evidence of ascites in six patients, among which two had both ascites and pleural effusions and one had an IVC thrombus in the middle portion of the IVC.Two other patients had only pleural effusions.On calculating the flow velocity, the mean Peak Systolic Velocity (PSV) in IVC was found to be 2.6 cm/sec and the End Diastolic Velocity (EDV) was found to be 16.27 cm/sec.

DISCUSSION

The incidence of clinical vascular thromboembolic complications in children with the nephrotic syndrome has been reported to be low (1.5-66%), while it is relatively high in adults, ranging from 9%-70%. Renal vein thrombosis is now generally considered to be a complication, rather than a cause of the nephrotic syndrome [2]. Although deep vein thrombosis (DVT), renal vein thrombosis, and pulmonary embolism are most frequently encountered, axillary, subclavian, femoral, coronary, and mesenteric arterial thrombosis have also been reported [5-7].

The occurrence of venous thrombosis may be influenced by the factors such as an increase in the platelet aggregability and loss of the low molecular weight regulatory proteins in urine and it may be made worse by an increase in the viscosity which results from the use of diuretics [2]. An increased hepatic protein synthesis is not sufficient to compensate for the renal loss of the proteins which have molecular weight below 70000 daltons. However, it leads to a marked increase in the plasma proteins which have molecular weights of more than 100000 daltons, which are not filtered in selective proteinuria [8]. An anti-thrombin III deficiency [7] and various anomalies of the platelet function, as well as the release of different products (Adenosine di phosphate (ADP), thrombin, collagen and arachidonic acid (AA))by the platelets have been linked to an increased thromboembolic phenomenon [9–12]. A particularly high incidence of renal vein thrombosis has been reported in the patients with membranous and membrano proliferative glomerulonephritis and a causal relationship with these specific renal disease processes has been suggested [1].

The nephrotic syndrome is per se regarded as a hypercoagulable state [5]. The mechanisms and the factors which are involved in the development of the thromboembolic complications in the nephrotic syndrome can be attributed to the interplay of three components: endothelial cell injury, platelet hyperaggregability

N=29	PT1 (s)	PT2 (s)	PTT1 (s)	PTT2 (s)	TT1 (s)	TT2 (s)
Minimum	13	13	32	32	18	18
Maximum	20	19	40	48	19	20
Mean	15.17	15.93	36.21	39.31	18.07	18.28
Std. Deviation	1.713	1.850	2.194	4.560	.258	.649

[Table/Fig-1]: Test of significance applied to coagulation parametersbefore and after therapy
PT-Prothrombin Time, PTT-Partial Thromboplastin Time, TT-Thrombin Time
1-Values before steroid therapy, 2-Values after steroid therapy

		Paired Differences				
					95% Confidence Interval of the Difference	
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper
Pair 1	Prt C1-Prt C2	59.688	67.648	23.917	3.132	116.243
Pair 2	PrtS 1 - PrtS 2	19.262	38.477	13.604	-12.905	51.430

[Table/Fig-2]: Comparative statistics of protein C and Protein S before and after steroid treatment

and hypercoagulability. The patients with the nephrotic syndrome have increased releases of arachidonic acid (AA), an increased production of the AA metabolites which include thromboxane A2 (TXA2), and a shortened platelet survival, which have been shown to be hyperaggregable to ADP, collagen and AA. This increased aggregability has been attributed to hypoalbuminaemia, because albumin inhibits the AA–induced platelet aggregation and the conversion of the AA which is released from the platelet phospholipids to endoperoxide intermediates and TXA2. The additional mechanisms which are involved may include hypocalcaemia and hypercholesteraemia (which are commonly seen in the nephrotic syndrome) [13].

In our study it was seen that an increased aggregation was present with ADP and collagen, which were used as agonists for the platelet aggregation [14–15]. The mean aggregation in response to ADP (79.9%) and collagen (79.2%), when it was compared with the baseline values,was significant. The studies which were performed by Remuzzi et al., [16], Machleidt et al., [17] and Kuhlman et al., [18] also found an increase in the aggregability of the platelets against these two agents which were used as agonists .

We performed the test against decreasing concentrations of the agonists. It was found that a significant change was seen in the platelet aggregation with ADP as an agonist but not with collagen. The platelet aggregation to arachidonic acid was increased.

However Kayima et al., [19] have reported a significant decrease in the platelet adhesiveness (p value less than 0.001), as well as a prolonged platelet aggregation time (p value less than 0.001). It was explained as a difference in the way platelets metabolised arachidonic acid to the potent aggregating agents, in the African patients as compared to that which was seen in the patients who were studied elsewhere. So, the hypercoagulable states in these may be explained on the basis of the alterations in other haemostatic parameters rather than in the platelet function.

Abnormalities in the coagulation cascade have also been reported [20–21]. These include increased levels of the coagulation factors V and VIII, vWF, factor VII and fibrinogen. An increased plasma fibrinogen concentrationis believed to reflect the protein's increased hepatic synthesis, a contracted intravascular distribution and a normal degradation rate [22]. In our patients, the partial thromboplastin times were 36.21s and 39.31s respectively before and after the steroid therapy. These changes in the partial thromboplastin times may be explained by the fact that hypercoagulable states existed in the nephrotic syndrome. This was primarily due to an increased hepatic synthesis of the coagulation factors. The reversal of this phenomenon after the treatment, would lead to normalisation of the clotting factor synthesis.Thus, a relative deficiency of the clotting factors would occur, which would manifest as a prolonged PTT. This may not be clinically significant, as there is no evidence of bleeding diathesis in any patient. According to Robert et al., fibrinolysis has been reported to be normal or impaired in the nephrotic syndrome, due to increased fibrinogen and α2-antiplasmin levels, decreased plasminogen levels, and decreased levels of the profibrinolytic contact factors, XII and prekallikrein. The decreased level of factor XII could be caused by its reduced synthesis, increased catabolism, unusual losses, and/or an increased extra–vascular distribution. While this sometimes manifests as a prolonged PTT during a routine coagulation screening, these patients are usually haemostatically competent [9].

The mean value for Protein C was 137.95 % and that for Protein S was 108.44%, both of which are high normal. Antithrombin(AT) III was found to lie in the lower part of the normal range, which was suggestive of a decreased antithrombin III level.

Otto Mehls [23] studied children with the nephrotic syndrome and found that Protein C was significantly elevated in the children with the nephrotic syndrome, but it was normal in adults.

Reduced Antithrombin (AT) levels were also seen [14] and they were attributed to urinary loss and/or consumption during an accelerated intravascular coagulation. The reduction in the plasma concentrations of the clotting factors is generally attributed to a urinary protein loss.

In our study, the protein C and protein S values after the treatment were noted and it was found that there was a significant decline in the level of protein C ($p<0.05$) after the treatment.

Our study features the reversibility of the changes which occur in the nephrotic syndrome with the steroid therapy. In a study which was published in 1996 by al-Mugeiren et al., [24] during a relapse, there was a marked increase in the plasma levels of fibrinogen, protein C, and protein S and reduced plasma ATIII levels. During remission, the protein C level had either remained elevated or it had increased further, but in some patients it had decreased. Similar findings for protein C were reported by Wygledowska et al., [25]. They found in their study, that the activity of protein C had increased at the onset of the nephrotic syndrome, that it had decreased progressively and significantly during the improvement stage and that it had again increased markedly during remission. The above investigators concluded that in the relapse of childhood nephrosis, despite the existence of a significant prothrombotic tendency, as was featured by the hyperfibrinogenaemia and the markedly reduced ATIII levels, the simultaneous elevation of the natural anticoagulant, the protein C level and the enhanced fibrinolysis that persist until the remission phase, seem to be major preventive mechanisms which guard the nephrotic children against thromboembolic phenomena [25].

Unfortunately, there are few reliable predictors of the individual risk. Still, low serum albumin levels (<25g/l), high rates of protein excretion (>10g/24hours), high fibrinogen levels, low AT III levels (< 75%), and hypovolaemia are associated with an excessive risk of the thromboembolic complications [13].

The thromboembolic complications are major hazards in the patients with the nephrotic syndrome. Venous thrombosis, which includes renal vein thrombosis (which is frequently seen in the patients with membranous glomerulonephritis), pulmonary embolism and deep vein thrombosis are common [13]. The incidence of such complications in the minimal change nephrotic syndrome is low. In our study, it was found that only one patient had a thrombus in the intrahepatic portion of the IVC. This resolved consequently after the treatment which was given for the nephrotic syndrome and adequate hydration was also maintained. Since only one case was found to be positive, no correlations could be made with the coagulation parameters. The rest of the parameters which were studied were the kidney size, and the IVC flow velocities, which were normal in all the cases.

It was thus concluded that there was an increased platelet aggregability in the patients with the steroid responsive nephrotic syndrome, which contributed to the hypercoagulable state that existed in these children. However, this hypercoagulable state is reversible with the institution of the appropriate therapy.

These patients should therefore be looked into, for a clinical evidence of deep vein thrombus. If a higher aggregation response is seen to the platelet aggregation and if the coagulation studies indicate a hypercoagulable state, Doppler can be used to look for any evidence of a thrombus and an early intervention can be instituted.

LIMITATIONS OF THE STUDY

The small sample size and the time bound nature of the study. We had only limited resources, as platelet aggregometry is an expensive investigation.

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REFERENCES

[1] Citak A, Emre S, Sairin A, Bilge I, Nayir A. Haemostatic problems and thromboembolic complications in nephrotic children. *Pediatr Nephrol.* 2000;14:138-42.
[2] Anand NK, Chand G, Talib VH, Chellani H,Pande J. Haemostatic profile in nephrotic syndrome. *Indian Pediatr.* 1996;33:1802-03.
[3] Abella EM. Hemostatic problems associated with renal disease. *Int J Pediatr Hematol Oncol.* 1994;1:43-51.
[4] Rabelink JT, Zwaginga JJ, Koomans HA, Sixma JJ. Thrombosis & hemostasis in renal disease. *Kidney Int.* 1994;46:287-96.
[5] Kanfer A. Coagulation factors in nephrotic syndrome. *Am J Nephrol* 1990;10 (suppl1):63-8.
[6] Bernard DB. Extrarenal complications of the nephrotic syndrome. *Kidney Int.* 1988;33:1184-1202.
[7] Drijfhout HH, Knot EAR, Cate JW. Antithrombin III metabolism in two patients with a nephrotic syndrome caused by minimal change nephritis and primary amyloidosis. *Haemostasis.* 1987;17:286-92
[8] Hoyer PF, Gonda S, Barthels M, Krohn HP, Brodehl J. Thromboembolic complications in children with nephrotic syndrome. Risk and incidence. *Acta Paediatr Scand.* 1986;75:804-10
[9] Marcus AJ, Coleman RW, Hirsh J, Ivarder VJ, Salzman EW. Hemostasis and thrombosis: Basic Principles and Clinical Practice. 472. JB Lippincott Company, Philadelphia 1982.
[10] E. Walter, D. Deppermann, K. Andrassy, J. Koderisch.Platelet hyperaggregability as a consequence of the nephrotic syndrome. *Thromb Res*. 1981;23: 473-79
[11] Bennett A, Cameron JS. Platelet hyperaggregability in the nephrotic syndrome which is not dependent on arachidonic acid metabolism or on plasma albumin concentration. *Clin Nephrol*. 1987;27:182-8
[12] Kuhlmann U, Steurer J, Rhyner K, von Felten A, Briner J, Siegenthaler W. Platelet aggregation and beta-thromboglobulin levels in nephrotic patients with and without thrombosis. *Clin Nephrol*. 1981;15:229-35.
[13] Robert A, Olmer M, Sampol J, Gugliotta JE, Casanova P. Clinical correlation between hypercoagulability and thrombo-embolic phenomena. *Kidney Int.* 1987;31:830-5.
[14] Taira K, Okajima C, Yamashita T, Kawahara S. Ristocetin induced platelet aggregation in children with nephrotic syndrome. *Nippon Jinzo Gakkai Shi*. 1990;32:659-66.
[15] Takagawa K, Nakajima M, Taira K, Sakagami Y .Shear stress-induced platelet aggregation in children with minimal change nephrotic syndrome. *Nippon Jinzo Gakkai Shi*. 2002;44:380-8
[16] Remuzzi G, Mecca G, Marchesi D, Livio M, Gaetano G, Donati MB et al.Platelet hyperaggregability and the nephrotic syndrome. *Thromb Res.* 1979;15:345-354.
[17] Machleidt C, Mettang T, Stärz E, Weber J, Risler T, Kuhlmann U. Multifactorial genesis of enhanced platelet aggregability in patients with nephrotic syndrome. *Kidney Int.* 1989;36:1119–24.
[18] Kuhlmann U, Steurer J, Bollinger A, Pouliadis G, Briner J, Siegenthaler W. Incidence and clinical significance of thromboses and thrombo-embolic complications in nephrotic syndrome patients. *Schweiz Med Wochenschr*. 1981 ;111:1034-40.
[19] Kayima JK, Otieno LS, McLigeyo SO, Kyobe J. Platelet function in nephrotic syndrome patients at the Kenyatta National Hospital. *East Afr Med J*. 1990 ;67:109-12
[20] F. Panicucci, A. Sagripanti, M. Vispi, E. Pinori, L. Lecchini, G. Barsotti, et al. Comprehensive Study of Haemostasis in Nephrotic Syndrome. *Nephron*. 1983;33:9-13.
[21] Norishi Ueda, Shinji Kawaguchi, Yukihiko Niinomi, Toru Nonoda, Junko Matsumoto, Masazumi Ohnishi et al. Effect of corticosteroids on coagulation factors in children with nephrotic syndrome. *Pediatr Nephrol*. 1987;1:286-89.
[22] Rasedee A, Feldman BF. Nephrotic syndrome: a platelet hyperaggregability state. *Vet Res Commun.* 1985;9:199-211.
[23] Mehls O, Andrassy K, Koderisch J, Herzog U, Ritz E. Hemostasis and thromboembolism in children with nephrotic syndrome: differences from adults. *J Pediatr.* 1987;110:862-67
[24] Al-Mugeiren MM, Gader AM, Al-Rasheed SA, Bahakim HM, Al-Momen AK, Al-Salloum A. Coagulopathy of childhood nephrotic syndrome--a reappraisal of the role of natural anticoagulants and fibrinolysis. *Haemostasis.* 1996;26:304-10.
[25] Wygledowska G, Grygalewicz J, Matuszewska E. Natural coagulation inhibitors; antithrombin III, protein C, protein S in children with hypercoagulation due to nephrotic syndrome. *Med Wieku Rozwoj.* 2001;5:377-88.

PARTICULARS OF CONTRIBUTORS:

1. Senior Resident, Department of Paediatrics, Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi, India.
2. Head of Department, Department of Paediatrics, Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi, India.
3. Associate Professor, Department of Hematology, Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi, India.
4. Consultant, Department of Radiodiagnosis, Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi, India.
5. Senior Resident, Department of Radiodiagnosis, Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Aliza Mittal,
Department of Pediatrics, Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi, India.
Phone: 9968629988, E-mail: alizamittal@gmail.com

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