Low triiodothyronine syndrome is associated with platelet function in patients with nephrotic syndrome

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INTRODUCTION

Nephrotic syndrome (NS) is characterized by massive proteinuria, hypoalbuminemia, edema, and hyperlipidemia. The loss of anticoagulant substances from urine, abnormal platelet function, hyperlipidemia and blood concentration, and the use of hormones and diuretics in clinical treatment cause NS patients to have hypercoagulability and the complication of thromboembolism. Platelet dysfunction is one of the main causes of thrombosis, which can manifest as abnormal activation of platelets and hyperfunction of platelet aggregation. Low thyroid hormone concentrations, especially low serum T3 levels are a common finding in NS patients. The primary pathophysiological mechanism underlying low circulating T3 is the reduced enzyme activity of 5’-monodeiodinase responsible for converting T4 into T3 in peripheral tissues.

SUMMARY

OBJECTIVE: The objective of this study was to investigate the effects of low triiodothyronine syndrome (LT3S) on platelet function and clotting factors in patients with nephrotic syndrome (NS).

METHODS: Patients with primary nephrotic syndrome were divided into two groups, normal thyroid function (group A) and LT3S (group B), based on whether they had LT3S or not. Healthy subjects were selected as the control group (group C). Blood coagulation function was detected in each group. The platelet activation function (CD62P, CD63) was determined by flow cytometry. The platelet aggregation rate was detected by an optical method using adenosine diphosphate and arachidonic acid as inducers.

RESULTS: The proportion of primary nephrotic syndrome with LT3S was 23.2% (69/298). Compared with group C, group A had higher CD62P and PAgTADP, and group B had higher CD62P, CD63, PAgTAA, and PAgTADP; the difference was statistically significant (all P < 0.05). There was no significant difference in renal pathology between group A and group B (X2 = 4.957, P = 0.421). Compared with group A, the 24-hour urine protein, CD63, PAgTAA, and PAgTADP were higher in group B, and APTT and Alb were lower. The difference was statistically significant (P < 0.05). Logistic regression analysis showed that LT3S was associated with CD63 (OR: 3.516; 95% CI: 1.742–8.186; P = 0.004) and PAgTAA (OR: 0.442; 95% CI: 1.001–1.251; P = 0.037).

CONCLUSION: NS patients are prone to LT3S. Patients with LT3S may have abnormal platelet activation and increase of platelet aggregation.

KEY WORDS: Nephrotic syndrome; low triiodothyronine syndrome; platelet activation; platelet aggregation
euthyroid sick syndrome, which is widely believed to be an adaptive mechanism for energy conservation. At present, there are few studies on the relationship between LT3S and platelet function in NS patients. The purpose of this study is to evaluate thyroid function, nutritional status, platelet activation function, and platelet aggregation function. The objective is to provide a reference for future clinical work.

METHODS

Patients and inclusion criteria

From January 2016 to May 2018, patients with primary nephrotic syndrome treated in the Department of Nephrology of the Affiliated Hospital to Nantong University were enrolled. Diagnostic criteria for NS: 1) urine protein greater than 3.5 g/day; 2) plasma albumin lower than 30 g/L; 3) edema; 4) elevated blood lipids. Items 1 and 2 are required for diagnosis. Exclusion criteria: 1) heart, liver, lung, and other major organ diseases; 2) primary thyroid disease; 3) secondary nephrotic syndrome; 4) mental illness or inability to cooperate. Thyroid function was detected after admission. Patients with primary nephrotic syndrome were grouped according to whether they had LT3S or not. Normal thyroid function group (Group A): Normal free triiodothyronine (FT3), normal free thyroid hormone (FT4), and normal hypersensitivity human thyroid-stimulating hormone (TSH). LT3S group (Group B): FT3 < 3.8 pmol/L and normal TSH. In addition, 60 healthy subjects were selected as the control group. All the subjects in the control group did not have any history of kidney disease or severe diseases.

Outcome measures

General data were collected, such as sex and age, as well as thyroid and blood coagulation function, blood cytology and blood biochemical indexes, including FT3, FT4, TSH, prothrombin time (PT), activated partial thrombin time (APTT), fibrinogen (Fib), thrombin time (TT), platelet count (PLT), hemoglobin (Hb), serum albumin (Alb), 24h urine protein, serum creatinine (SCR) and urea (BUN).

Platelet function tests

Platelet Activation: 1 ml of blood was collected using a vacuum blood collection tube containing 3.18% sodium citrate 0.3 ml. Platelet-rich plasma was extracted by centrifugation, fixed with 1% paraformaldehyde, and labeled with fluorescein isothiocyanate (FITC). CD62P and CD63 were labeled as CD62P-FITC and CD63-FITC, and IgG-FITC was used as a negative control (reagents were purchased from Beekman, USA). Flow cytometry (Beckman Coulter Epics XL) counted 5,000-10,000 platelets and determined the percentage of fluorescently labeled platelets.

Platelet aggregation: 2.7 ml of blood was taken using a vacuum blood collection tube containing 3.18% sodium citrate 0.3 ml. Platelet-rich plasma and platelet-poor plasma were extracted by centrifugation. The platelet-poor plasma was used as a negative control and placed in a platelet aggregation instrument (Beijing Plymouth LBY-NJ2). The platelet aggregation rate (PAgT) was determined by preheating at 37 °C for 3 min with 0.5 mol/L arachidonic acid (AA) and 10 μmol/L adenosine diphosphate (ADP) as inducers. Both AA and ADP were purchased from Shanghai Dusheng Biological Company.

Statistical analyses

The main statistical indicators were used to test for normality. The measurement data were expressed as mean ± standard deviation ($X \pm S$ SD), the count data were analyzed by $X^2$ test. The t-test was used for comparison between the groups, and logistic regression was used to analyze influencing factors. Statistics were made using the SPSS 20 software package. P < 0.05 was statistically significant.

RESULTS

General Information

A total of 298 NS patients were enrolled in this study, with 229 cases of normal thyroid function (group A), of which 94 cases (41%) were women, with an average age of 46.8 ± 14.3 years. There were 69 cases of LT3S (group B). The proportion of NS with low LT3S was 23.2% (69/298), including 28 cases (40.1%) in women, with an average age of 44.3 ± 14.2 years. There were 60 patients in the control group, including 26 women (43.3%), with an average age of 45.5 ± 14.8 years. There was no significant difference in gender and age distribution between the three groups. Compared with group C, 24-hour urine protein, PLT, SCR, BUN were higher in group A and group B, and Alb and APTT were lower; the difference was statistically significant (P < 0.05). The urine protein level of patients in group B was higher than that in group A. APTT and Alb were lower than in group A, and the difference was statistically significant (P < 0.05), as showed in Table 1.
Renal Pathology Composition

In group A, 66 cases (28.82%) were minimal change disease (MCD), 18 (7.86%) were focal segmental glomerulosclerosis (FSGS), 57 (24.80%) non-IgA mesangial glomerulonephritis (MsPGN), 22 (9.61%) IgA nephropathy (IgAN), 59 (25.76%) membranous nephropathy (MN), and 4 cases (2.63%) were membranous proliferative glomerulonephritis (MPGN). Of the patients in group B, 18 (26.01%) were MCD, 6 (8.70%) FSGS, 13 (18.85%) MsPGN, 7 (10.15%) IgAN, 19 (27.54%) MN, and 6 (8.69%) MPGN. There was no significant difference in the distribution of renal pathology types between the two groups (X^2=4.957; P=0.421), as shown in Fig. 1.

Comparison of platelet function

Compared with group C, CD62P and PAgTADP were higher in group A, while CD62P, CD63, PAgTAA, and PAgTADP were higher in group B; the difference was statistically significant (all P < 0.05); Compared with patients in group A, patients in group B had higher levels of CD63, PAgTAA, and PAgTADP, and the difference was statistically significant (all P < 0.05), as shown in Fig. 2.

Regression analysis

CD62P, CD63, PAgTAA, 24-hour urine protein, APTT, and Alb were used as independent variables, and LT3S was used as the dependent variable for logistic regression analysis. The results showed an association with CD36 (OR:3.516; 95%CI:1.742~8.186; P = 0.004) and PAgTAA (OR:0.442; 95%CI:1.001~1.251; P = 0.037) after correction for age, gender and other related factors (Table 2).

DISCUSSION

LT3S is an abnormal level of serum thyroid hormone caused by non-thyroid diseases, with a decline in FT3 and normal or reduced TSH, often associated with systemic disease. Under normal physiological conditions, T3 exists in both free and bound states, with the state of binding to thyroid-binding globulin...
Patients with NS may have LT3S due to the loss of various hormone-binding proteins including albumin, thyroid-binding protein, and thyroxine transporter from urine. This study showed that the incidence of LT3S in NS patients was 23.2% (69/298). There was no significant difference in the distribution of renal pathology types in the two groups. Our further studies showed that the 24-hour urine protein in the patients with LT3S was higher, and the serum albumin was lower. The results of our study are similar to those of previous reports.

The results indicate that LT3S is associated with the severity of the disease in NS patients, suggesting that LT3S may be a self-protective mechanism under the disease state. By reducing the concentration of thyroid hormone, protein catabolism is reduced, resulting in a decrease in the basal metabolic rate and reduced energy consumption.

Thrombosis in NS patients is closely related to a proteinuria-related imbalance of thrombosis inhibition and thrombotropic factor and abnormal platelet function. Platelet hyperfunction can lead to the sustained expression of platelet surface activation markers, increased release of active substances, and increased platelet aggregation. Platelet activation is the starting factor of thrombosis. When platelets are activated, the pipeline system is open, and the internal alpha particles are fused with the plasma membrane to increase the expression of platelet membrane glycoprotein CD62P and CD63. Thus, CD62P and CD63 are considered specific markers of platelet activation. The platelet aggregation rate (PAGT) was determined by an optical method with adenosine diphosphate (ADP) and arachidonic acid (AA) as inducers, so PAGTA and PAGTADP could evaluate the platelet aggregation function. Recently studies have concluded that low serum albumin is an independent correlator of platelet hyperactivity in NS patients. Similar to this result, our study also found that patients with LT3S had higher 24h urinary protein and lower serum albumin. Our further studies showed that patients with LT3S had lower APTT, higher CD36, PAGTAA, and PAGTADP. Logistic regression adjusted for age, gender, and other related factors showed that LT3S was associated with APTT, CD36, and PAGTAA, suggesting that patients with LT3S have abnormal platelet activation and increased platelet aggregation. Prior studies show that LT3S is a common complication, and patients after stroke are associated with greater stroke severity and worse outcomes.

Findings of the present report suggest that pro-coagulative state associated with LT3S can be a significant underlying factor of the observed associations in strokes. LT3S may affect platelet function in NS patients by the following mechanisms: on the one hand, albumin can bind free arachidonic acid (AA) in a normal physiological state, inhibiting its conversion to thromboxane A2 (TXA2) and platelet metabolism. The low bioavailability of free arachidonic acid increases the production of thromboxane A2 under the action of platelet activator, further promoting platelet activation and aggregation. On the other hand, low T3 levels can...
lead to abnormal relaxation of vascular smooth muscle cells, inhibit the use of endothelial cells on vasodilator nitric oxide, and reduce the formation of nitric oxide to cause endothelial dysfunction, collagen exposure after vascular endothelial injury initiates endogenous and exogenous coagulation pathways, resulting in abnormal activation of platelets and hyperfunction of platelet aggregation. It is well known that patients with membranous nephropathy are prone to thrombosis complications, but our study did not find LT3S to be associated with renal pathology. This study is single centered with few enrolled patients, so it is necessary to further expand the sample size and conduct a comprehensive and in-depth multi-center study.

CONCLUSION

In conclusion, our findings suggest that NS patients are prone to complications with LT3S, and patients with LT3S have abnormal platelet activation and platelet aggregation. NS patients with LT3S should be monitored for platelet function and treated accordingly.

RESUMO

OBJETIVO: O objetivo deste estudo foi investigar os efeitos da síndrome do baixo triiodotironina (LT3S) na função plaquetária e nos fatores de coagulação em pacientes com síndrome nefrótica (SN).

MÉTODOS: Pacientes com síndrome nefrótica primária foram divididos em dois grupos, função tireoidiana normal (grupo A) e LT3S (grupo B), com base na presença ou não de LT3S. Indivíduos saudáveis foram selecionados como grupo de controle (grupo C). A função de coagulação do sangue foi analisada em cada grupo. A função de ativação plaquetária (CD62P, CD63) foi determinada por citometria de fluxo. A taxa de agregação plaquetária foi detectada por um método óptico usando adenosina difosfato e ácido araquidônico como indutores.

RESULTADOS: A proporção de síndrome nefrótica primária com LT3S foi de 23,2% (69/298). Em comparação com o grupo C, o grupo A apresentou níveis mais altos de CD62P e PAgTADP, e o grupo B apresentou maiores CD62P, CD63, PAgTAA e PAgTADP; a diferença teve significância estatística (P < 0,05). Não houve diferença significativa na patologia renal entre o grupo A e o grupo B (X² = 4,957, P = 0,021). Em comparação com o grupo A, o proteína em urina de 24 horas, CD63, PAgTAA e PAgTADP foram maiores no grupo B, já APTT e Alb foram maiores. A diferença apresentou significância estatística (P < 0,05). A análise de regressão logística mostrou uma associação entre LT3S e CD36 (OR: 3,516; 95% IC: 1,742~8,186; P = 0,004) e PAgTAA (OR: 0,442; 95% IC: 1,001~1,251; P = 0,037).

CONCLUSÃO: Pacientes com síndrome nefrótica estão propensos à síndrome do baixo triiodotironina (LT3S). Pacientes com LT3S podem ter ativação plaquetária anormal e aumento da agregação plaquetária.

PALAVRAS-CHAVE: síndrome nefrótica; Baixa triiodotironina, síndrome de ativação plaquetária; agregação plaquetária.

REFERENCES