Retrospective Analysis of Coagulation Abnormalities in Patients with Different Types of M-proteinemia

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Abstract: M protein (MP) is an abnormal monoclonal immunoglobulin produced by the abnormal proliferation of plasma cells or B lymphocytes which is very homogeneous in amino acid composition and sequence. Clinically, it can be seen in a variety of haematological diseases. This paper compares the coagulation indexes of patients with different types of M-proteinemia and patients with different levels of M proteins and observe the effects of different types and levels of M-proteinemia on the coagulation results. Different types of M-proteinemia were classified in 103 patients and the coagulation indexes of the patients were analyzed. *Aim:* To analyze the correlation between M proteins and coagulation function by analyzing the effects of different M-proteinemia patients' serum globulin types and contents on their indicators reflecting different coagulation functions. *Methods:* 103 patients with an initial diagnosis of M protein abnormality were selected from the Affiliated Hospital of Hebei University, and the results of their coagulation, liver function, and serum protein electrophoresis were collected to compare the coagulation function between patients with different types of M-proteinemia and between patients with the same type of M-proteinemia with different levels of M proteins and to analyze the correlation between them and the content of M proteins. *Results:* The differences in prothrombin time (PT) and fibrinogen (FIB) between the heavy-chain group (including IgG, IgA and IgM groups) and the light-chain group were statistically significant ($P < 0.05$), and PT in the heavy-chain group were higher than those in the light-chain group. The difference of PT, TT and FIB between the M proteins > 30g/L group and M protein ≤ 30g/L group was statistically significant ($P < 0.05$), and the high M protein group PT and TT were higher than the other group while FIB was lower than the other group as there was no statistically significant difference of APTT comparing between the two groups ($P > 0.05$). In the M protein > 30 group, the mean values of PT and TT exceeded the upper limit of the reference interval, which had some clinical significance. *Conclusion:* There are some differences in the effect of different M protein types on PT and FIB results in patients with M-proteinemia, and the amount of serum M protein in patients has an effect on coagulation results.

Keywords: M-proteinemia; Coagulation indices; Serum globulin; Retrospective analysis

1. Background

The main role of the immune system is to protect the body from foreign pathogenic bacteria and kill tumour cells, which is commonly known as "resistance." It consists of a variety of immune cells and the proteins they secrete. Among them, the immunoglobulins secreted by B-lymphocytes and plasma cells, namely antibodies, can help the body fight against pathogens, such as antibodies against the hepatitis B virus. The body's immunoglobulins are diverse, targeting a wide range of pathogens and forming a strong defence system. However, when B lymphocytes or plasma cells are abnormally stimulated, or when mutation occurs, abnormal immunoglobulins with identical structure, i.e. monoclonal immunoglobulins, or M proteins for short, are produced, and this condition is called M-proteinemia. Non-haematopoietic system diseases include prolonged chronic liver disease, nephrotic syndrome, viral infections, colon cancer and other malignant tumours, rheumatoid arthritis and other autoimmune diseases that stimulate the body's immune cells. Lymphohaematopoietic system tumours are mainly multiple myeloma, macroglobulinemia, monoclonal gammaglobulinemia of undetermined significance (MGUS), lymphoma and so on. The onset of the disease is insidious, and in the early stage, there may only be M-proteinemia without clinical manifestations, and the symptoms of abnormal immune cells and M-protein harming the human body will appear in gradual progress. The most common plasma cell malignant tumour is multiple myeloma (MM). Due to the malignant transformation of plasma cells, a high concentration of monoclonal immunoglobulin is secreted, which leads to an abnormally high concentration of M-protein in the blood and a series of symptoms such as bone damage, kidney damage, and haematological damage. In the case of Wahl's macroglobulinemia, the concentration of monoclonal IgM antibodies in the body is abnormally high, and the patient develops hyperviscosity, as well as a series of symptoms such as thrombosis, embolism, and splenomegaly [1]. The prevalence of M-proteinemia is as high as 6% in people over 50 years of age and increases progressively with age. Both morbidity and mortality have been on the rise in recent years [2].

2. Methods

103 patients with abnormal M protein levels at the initial diagnosis of Hebei University Hospital from January 2021 to January 2022 were selected. Among them, 65 cases were male and 38 cases were female; their ages ranged from 38–88 years old, with an average age of 67 years old; they were outpatients and inpatients of various departments; the first symptoms of patients were different, but M protein was abnormal in all of them, and all of them had been tested by blood routine examination, coagulation function examination, liver function examination, immuno-serum protein level and typing test.

According to the different serum M protein components, they were divided into 54 cases of IgG, 18 cases of IgA group, 11 cases of IgM group, and 20 cases of light-chain group; according to the different serum M protein contents of the patients, they were divided into 16 cases of M protein > 30g/L group and 87 cases of M protein ≤ 30g/L group.

2.1. Research methodology
2.1.1. Instruments and reagents

(1) Experimental instruments: Coagulation function instrument (Werfen ACLTOP750 automatic coagulation analyzer, Werfen Instrumentation Laboratory MA, USA), serum electrophoresis instrument (Sebia Capillarys2 Flex Piercing automatic capillary electrophoresis), immunofixation electrophoresis instrument (Sebia HYDRASYS2).

(2) Experimental reagents: Fibrinogen-CXL, RecombiPlasTin 2G, SynthASil, Thrombin time, D-Dimer

test (Werfen, Instrumentation Laboratory Company reagents); Immunofixation electrophoresis and serum electrophoresis (Sebia reagents).

2.1.2. Statistical methods

Data were analyzed using IBM SPSS Statistics 21.0 statistical software to process the data, and the paired-sample *t*-test was used for comparison between groups, with a test level of α = 0.05, and a difference of *P* < 0.05 was considered statistically significant.

2.1.3. Evaluation criteria

Clinically, the PT reference interval was 9.4–12.5 s; the APTT reference interval was 28.0–42.0 s; the FIB reference interval was 2.00–4.00 g/L; and the TT reference interval was 10.3–16.6 s.

3. Results

3.1. Comparison of coagulation indices between the heavy-chain group and the light-chain group

Comparison between the heavy-chain group and light-chain group, the PT and FIB values were statistically different by independent samples *t*-test (*P* < 0.05). In the heavy-chain group, PT was higher than the other group and FIB was lower than the light-chain group, while there was no statistically significant difference in APTT and TT compared between the two groups (*P* > 0.05). In the IgA group and IgM group, the mean values of PT exceeded the upper limit of the reference interval, which had some clinical significance. Refer to **Table 1.**

A comparison of the groups from the perspective of PT prolongation rate revealed that the PT prolongation rate was 35.2% in the IgG group, 33.3% in the IgA group, 45.5% in the IgM group and 10% in the light-chain group.

Table 1. Comparison of coagulation indices in the groups (Mean ± Standard Deviation, SD)

Groups	PT (s)	APTT (s)	FIB (g/L)	TT (s)
Light-chain group (*n* = 20)	11.77 ± 1.00 [2,3,4,5]	31.88 ± 2.83 [2,4,5]	3.98 ± 1.20	14.66 ± 2.36
Heavy-chain group (*n* = 83)	12.29 ± 1.82 [1]	33.19 ± 5.27[1]	3.32 ± 1.08	15.39 ± 3.01
IgA group (*n* = 18)	12.56 ± 2.00 [1]	34.81 ± 3.78	3.47 ± 1.12	14.54 ± 3.69
IgG group (*n* = 54)	12.10 ± 1.51 [1]	31.96 ± 5.24 [1]	3.34 ± 1.12	15.85 ± 2.63
IgM group (*n* = 11)	12.76 ± 2.76 [1]	36.52 ± 5.84 [1]	2.97 ± 0.69	14.57 ± 3.33
P-value	0.087	0.132	0.034	0.242

Significance level = 0.05

3.2. Comparison of coagulation indices by grouping according to serum M protein levels

All patients were divided into two groups M protein ≤ 30g/L and M protein > 30g/L and the values of PT, APTT, FIB and TT were compared between the two groups. The difference of PT, TT and FIB between the two groups was statistically significant (*P* < 0.05), and the high M protein group PT and TT were higher than the other group and FIB was lower than the other group, while there was no statistically significant difference of APTT comparing between the two groups (*P* > 0.05). In the M protein > 30 group, the mean values of PT and TT exceeded the upper limit of the reference interval, which had some clinical significance. Refer **Table 2**.

Table 2 Comparison of coagulation indices between M protein ≤ 30 g/L and M protein > 30 g/L groups (Mean ± SD)

Groups	PT (s)	APTT (s)	TT (s)	FIB (g/L)
M protein ≤ 30 g/L (n = 87)	11.76 ± 1.26	32.63 ± 4.17	14.64 ± 2.13	3.63 ± 1.113
M protein > 30 g/L (n = 16)	14.56 ± 2.09	34.08 ± 6.48	17.14 ± 4.83	2.85 ± 0.93
P-value	0.000	0.375	0.045	0.004

4. Conclusions

M protein is an abnormal monoclonal immunoglobulin produced by monoclonal aberrant proliferation of B lymphocytes or plasma cells that is very homogeneous in amino acid composition and sequence [6]. M protein is clinically seen in multiple myeloma, hypergammaglobulinemia, malignant lymphoma, heavy-chain disease, light-chain disease, etc.

Abnormalities of coagulation and fibrinolytic system in patients with malignant haematological diseases [7]. In recent years, there have been remarkable results in the study of multiple myeloma (MM) [4]. For example, MM is a malignant proliferative tumour of B-lymphocytes characterized by the secretion of large amounts of monoclonal immunoglobulin, which is the most common and typical disease in M-proteinemia [8]. In the report of Zhang et al. (2018), reported that the prolongation of PT in MM patients was due to the combination of elevated serum globulin and coagulation factors in patients [5]. However, there are not many studies on the analysis of coagulation function in patients with different types of M-proteinemia.

In this study, the correlation between the abnormal changes in coagulation function of 103 patients with M-proteinemia and the correlation between the patients' M-protein types and serum M-protein levels are analyzed retrospectively. In the comparison of the coagulation function of 20 patients in the light-chain type, 54 in the IgG, 18 in the IgA group, and 11 in the IgM group, it was found that patients in the heavy-chain type were more likely to have abnormal PT results than patients in the light-chain type. In the comparison of the coagulation function of 16 patients in the M protein > 30 g/L group and 87 patients in the M protein ≤ 30 g/L group, it was shown that patients in the M protein > 30 g/L group were more likely to have abnormalities in coagulation function, and such results indicate that the amount of M protein affects coagulation results.

The reason for the altered coagulation function in the heavy-chain type may be that: (1) IgG immunoglobulin can lead to abnormal coagulation, which covers platelets and hinders the normal platelet aggregation process [5]; (2) IgG and IgA can easily combine with themselves to form polymers or with other plasma proteins in the plasma or with coagulation factors in the plasma to make the blood turns viscous so that the patient's tendency to bleed increases. In patients with light-chain type [6], the risk of infection is greatly increased in light-chain patients compared to heavy-chain patients because of the reduced level of intact immunoglobulins in their serum.

The mechanism of coagulation abnormality caused by M protein is complicated. These reasons together led to the prolongation of PT and TT in patients in the hyper M-proteinemia group, which was consistent with the collected results.

(1) M protein inhibits the binding site of negatively charged thrombin, and the binding of IgG to thrombin accelerates the antithrombin-thrombin reaction, thus prolonging the prothrombin time. Moreover, IgG inhibits thrombin-activating factor VIII and may prolong APTT by interfering with the formation of factor IXa-VIIIa complex, which is consistent with the prolongation of APTT in the high M protein group.

(2) M protein may interfere with the normal coagulation process by encapsulating platelets to seal off their receptors, affecting fibrin polymerization.

(3) Heparin-like substances are increased in patients with M-proteinemia, and the anticoagulant effect is strengthened *in vivo*.

(4) Plasma cell bone marrow infiltration, so that the haematopoietic function is inhibited, causing thrombocytopenia.

(5) Vascular wall damage caused by hyperimmunoglobulinemia and amyloidosis.

Patients with M-proteinemia produce large amounts of M-protein due to abnormal proliferation of immune cells [3]. Not only can they induce bleeding tendency by inhibiting the activity of antithrombin or coagulation factors, affecting platelet adhesion by covering the platelet surface, and selectively inhibiting the polymerization of fibrinogen, but globulin is closely related to the body's immune function and plasma viscosity [9]. At the same time, globulins are closely related to immune function and plasma viscosity [4]. The increase of M-protein will reduce the repulsive force between charges on the surface of erythrocytes so that the M protein content is positively correlated with the blood viscosity. The increase of abnormal M protein can increase the blood viscosity, which will lead to the occurrence of poor blood flow and thromboembolism, and eventually the clinical manifestations of hyperviscosity, such as haemorrhage and blurring of vision, will appear.

In this study, IgG type accounted for the largest number of cases, followed by light-chain type, IgA type, and IgM type, and according to a database on monoclonal immunoglobulin cases [10]. According to a database of monoclonal immunoglobulin cases, IgG, IgM, and IgA types ranked the top three in the number of cases, respectively, and cases of each type were collected more comprehensively in this study, especially the addition of the understudied IgM type. However, as the number of IgM cases was still small, there may be other mechanisms of IgM's influence on the coagulation function of patients with M-proteinemia that were not found in the present study, and there was no statistically significant difference between the results of the κ-type and λ-type groupings. There were fewer domestic and international studies on the comparison of the light-chain subtypes, so it is necessary to further study and explore the differences in coagulation function of the different light-chain types and the related mechanisms.

Plasma prothrombin time (PT) is the most sensitive and commonly used screening test in the exogenous coagulation system [11]. The activated partial thromboplastin time (APTT) is the most sensitive and commonly used screening test in the endogenous coagulation system, and has been used as a routine coagulation programme for the prediction of hypercoagulability and coagulation-fibrinolytic function, but it is poor in sensitivity and specificity. In Zhou et al. (2018)'s study, it was mentioned that TAT, as a complex generated by the 1:1 binding of thrombin and antithrombin, has the advantages of long half-life, irreversibility, inactivity, easy to be detected, etc., thus it is recommended to be used as a sensitive marker for coagulation abnormality [12]. However, this marker is still not popularized in daily testing and is not as commonly used as coagulation indexes such as PT, APTT, etc.

Proteinemia is common in a variety of primary and secondary diseases [13]. The presence of one or more abnormal coagulation indexes in patients with M-proteinemia is related to the type and level of abnormal immunoglobulins in their bodies. The effect of M protein level on the coagulation function of patients with M-proteinemia is more obvious, which can provide a simple and quick basis for the clinical consideration of M proteinemia earlier through the abnormality of coagulation indexes of the patients, and guide the clinic to make the next step in the examination. However, there is still a need for further research on the difference in serum globulin type on the coagulation function of patients with different types of M-proteinemia. However, the differences in the effects of serum globulin types on the coagulation function of patients with M-proteinemia need to be studied in depth.

Disclosure statement

The authors declare no conflict of interest.

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