**Reviewer’s Comments**

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**Acquired neonatal severe factor II deficiency- two cases report**

**Abstract**

We report two cases of transient acquired and isolated factor II deficiency associated with severe bleeding. Two infants were involved in severe coagulopathy. The blood clot time (CT) in case 2 was excessively prolonged over 16 hours. One-stage prothrombin time (PT) was remarkable prolonged. Haemostatic markers analysis showed an isolated deficiency of factor II at 2.5% and 4.5% respectively. No inhibitory activity against factor II could be detected. We successfully treated the deficiency with vitamin K1 during 15 days. It was interesting that in the case 2 female baby the cause of vitamin K deficiency might be breast feed problem (nutrition deficiency) and/or poor absorption from bowel. Physiopathological laboratory results and therapeutic aspects of two patients were presented.

**Keywords:** Acquired neonatal factor II deficiency; Plasma factor II activity (II:C) assay; Vitamin K1

**Introduction**

Prothrombin is a precursor to thrombin, an enzyme that convert fibrinogen into fibrin to strengthen a clot (Hemker HC,et al,1963; Davie EW and Ratnoff OD,1964;Macfarlane RG,1964, 1966; Biggs R,1972;Mial JB,1977). The gene involved in the synthesis of prothrombin is located on chromosome 11,which consists of 14 exons(Girolami A,1975;Lancellotti S,2013). Factor II deficiency(also called hypoprothrombinemia or prothrombin deficiency) was first identified in 1947 by Dr. Armand Quick(Girolami A,et al,1998). Congenital prothrombin deficiency is extremely rare,with an estimated incidence of 1:2,000,000 in the general population (DeBastos O,et al,1964;Shusterman S,Manno CS,2007;Key NS,Boles JC,2011; Imane S,et al,2012;Lancellotti S,et al,2013). There were only 100 cases with congenital prothrombin deficiency are known worldwide. Acquired factor II deficiency is caused by several factors: severe liver disease, long-term use of antibiotics, Ingestion of vitamin K antagonists such as warfarin,and impaired absorption of vitamin K from the intestines. Newborns may be born with a vitamin K deficiency. The plasma factor II deficiency is associated with a variable bleeding phenotype. Here in this paper, A case 2 with prothrombin deficiency presented the deficiency of vitamin K1 due to poor absorption from the bowel and bile tract.

**Case report**

 Case 1. In May 1985 a 13.5 year old girl had a generalized easy bruising weakness, and a petechial rash for ten days duration. A joint hemorrhage produces a strict restriction of normal activity. She presented the bleeding gums for three days. There was no family history of bleeding tendency, and chronic hepatitis albeit her hepato-chlangiostomy and "T" drain in her right abdomen was performed during early 1982. At the period of the study, she did not use oral warfarin anticoagulants.

 On examination she developed multiple sites of ecchymosis on the lower part of her legs. Repeat hemorrhages into the left knee, ankle and right elbow caused the limitation of motion activity, and both pain and swelling erosion involving joins surface. A "T" drain was still remained in right lower abdominal cavity without cholangietic jaundice. Blood foundings was hemoglobin(Hb) 83 g/l. The leukocyte count was slightly elevated with 12.5 x109/l. The platelet count was 220 x109/l. Prothrombin time was excessively prolonged,being over 1260 seconds (control time 12.6 second,see table 1 and 2). She received a week course of vitamin K1 4 mg tid oral administration. The hemorrhagic lesions disappeared. Abnormal coagulant tests recovered to normal(data not shown).

 **Case 2**. The ten months female baby was admitted to hospital because of pallor, weakness and sporadic subcutaneous ecchymosis from the begin the back purpura to lower part of legs within two days in July 1985. Two days later, hemorrhagic lesions were continously involved in head, chest, abdomen even extremities, which were also edematous. Among them, hemorrhagic lesions reached to 5 x 5 cm after the onset of purpura. No lymphoadenopathy and splenomegaly were found. There were no family history of bleeding tendency. A history of steatorrhea was noticed. She had a normal serum A/G ratio. She did not use oral warfarin anticoagulants.

 On examination, blood founding showed a marked anemia with hemoglobin 22 g/l. The leukocyte count was 4.4x109/l. The platelet count was 376x109/l. Plasma fibrinogen(factor I) 350 mg%. Prothrombin time was excessively prolonged, being over 1260 seconds(control:11.4～14.7s, see table 1,2). Thromboplastin generation test(TGT) and plasma factor II activity defined the diagnosis of severe plasma factor II deficiency.

 Treatment consists of a small volume(50 ml) of blood transfusion and of 10mg of vitamin K1 administered intravenously, and 2 mg tid of vitamin k1 was given orally. One week later, her hemoglobin recovered to 62g/l, following a prompt improved symptoms of anemia. The ecchymotic lesions did not progress, and disappeared. No further hemorrhagic manifestations was observed. During the follow up, she was still well until now.

**Table 1. Coagulation studies in two patients with factor II deficiency**

Test Case 1 Case 2 Case 2 following VitK1 Control Values

CT(minutes) 72.5 >900,<1260 6 4～12

clot plug overnight

BT(minutes) 2 1～3

Complete clot 3 24 6～24

retraction(hours)

PRT(seconds) 1290 >1260 134 105～135

Cross-PRT for

anticoagulants No circulating anticoagulants

PT(seconds)

(Quick methods) >1260 >1260 12.6 11.4～14.7

KPTT(seconds) 930 >1260 31.5 31～38.8

TT(seconds) 15.3 16.4 12.6 13～19

TGT(Biggs method) No abnormal results

Factor XIII assay Normal Normal

Factor II activity 0.045 0.025 0.596～1.05

(II:C) (u/ml)

VIIIR:Ag(%) 194.5 71.52～173.11

Abbreviation: CT: coagulation time; BT: bleeding time; PRT: plasma recalcification time; PT: prothrombin time(Biggs R,Denson KWE,1967;Mial JB and Lafond DJ,1969a;Quick AJ,1971); KPTT: Kaolinpartial thromboplastin time; TT: thrombin time; TGT: thromboplastin generation test; VIIIR:Ag: Factor VIII-related antigen

**Table 2. Differential PRT,PT and KPTT studies in factor II deficiency**

Test materials PRT PT KPTT

case 1 case 1 case 2 case 1 case 2

patient's plasma 1290s >1260s >1260s 930s >1260s

patient's plasma + normal plasma 150s 13s 19s 44.7s 50.8s

patient's plasma + normal serum 760s >1260s >1260s 262.5s >1260s

patient's plasma+BaSO4-absorbed

plasma >900s >1260s >1260s >900s >1260s

control values 135s 11s 15s 38.8～41.6s

Note: s: seconds

**Discussion and Conclusion**

At present two young infants have been reported with the clinical situation of severe hemorrhagic disease without a history of bleeding tendency in her family. On the basis of *in vitro* experiments it has been suggested that a deficiency of prothrombin might be diagnosed. The laboratory data presented available that prothrombin time was excessively prolonged, which suggest that a defective in mostly involvement of thrombinogenesis (II, V,VII and X deficiency) (phase II of the process of blood coagulation). Failure to correct prothrombin time with the reagents of normal serum (deficient in I,II,V,VII,XIII,but contain "activated" VII,XI,X,XI and XII) and BaSO4-absorbed plasma(deficient in II,VII,XI and X, but contain I,V,VIII,XI and XII) indicated a deficiency of factor II. The further determination of the factor II activity (II:C) in two patients was 4.3% and 2.5% of normal level respectively. In those cases of factor II deficiency, TGT, as evidence of normal generation of thromboplastin and differentiation of plasma factor VIII,IX and XI deficiencies, should be normal time. The normal results of plasma fibrinogen(factor I) and thrombin time, a function of the integrity of the phase III(fibrin formation) of coagulation, reflected no heparin substances and circulating anticoagulants. It is unknown that the combined deficiency of factor II and mild factor IX in case 2 due to the partial correction of KPTT time with normal serum. Unfortunately, protein C and protein S, two vitamin K-dependent proteins, were not measured during the period of treatment (Zhu YJ,Li JX,1989;Zhu G,Broekmans AW,Bertina RM, 2020).

Prothrombin deficiency is usually characterized by mild to moderately bleeding disorder, and prolonged PT and PTT and normal TT (Shusterman S,Manno CS,2007;Key NS,Boles JC,2011;Roman E,et al,2018). Symptoms include easy bruising, frequent nose bleeds, umbilical cord bleeding and hemorrhage after surgery or trauma. The diagnosis is made based on a low factor II activity and/or antigen measurements. Usually, activity levels less than 10% of normal are found in homozygotes, and between 40 and 60% in heterozygotes (Girolami A,et al,1998). In all those vitamin K-dependent factors, including plasma protein C and protein S, are low. Treatment for prothrombin deficiency includes plasma at a dose of 15-25 ml/kg followed by 3 ml/kg every 12-24 hours to achieve levels of approximately 30% (Roman E,et al,2018). PCCs (prothrombin complex concentrates) can be used to the indication of patients with life-threating bleeds. A minium targets prothrombin level of 20-30 IU/dl has been suggested for hemostasis (Shusterman S,Manno CS,2007;Key NS,Boles JC,2011)

From clinical situation, the pathogenesis of prothrombin deficiency in case 1 was contributed to the vitamin K deficiency due to the obstructive jaudice, following the cholelithotomy due to cholelithiasis. Defective synthesis of vitamin K in case 2,which can result from any long-standing gastrointestinal disorder, particulary in steatorrhea complicated by poor absorption and/or the the absence of bile salts in a bowel, may take into account for the explanation of factor II deficiency. Vitamin K deficiency in case 2 may develop during the first few months of life as a result of vitamin K-deficient diet(breast feeding without the supplements of vitamin K or nutritional deficiency).

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**Conflict of interest**

**Author’s Contribution**

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