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Poorer Prognosis With Ethylenediaminetetraacetic Acid-dependent Pseudothrombocytopenia

A Single-center Case–control Study

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Abstract: In ethylenediaminetetraacetic acid (EDTA)-dependent pseudothrombocytopenia (PTCP), automated platelet counts are lower than actual counts because of EDTA-induced aggregation. Factors contributing to the incidence of EDTA-PTCP are unknown, and no study has assessed the prognosis of EDTA-PTCP patients.

This retrospective study assessed characteristics in EDTA-PTCP patients and matched controls to determine differences in prognosis.

A retrospective case–control study was designed. From the University of Tokyo Hospital database, we identified patients diagnosed with EDTA-PTCP between 2009 and 2012, and performed 1:2 case:-control matching for age and sex. A control group of sex- and age-matched patients was selected at random from the same database. We investigated differences in the frequency of complications, medication history, and blood transfusion history between the groups at the time of blood collection. Prognosis was evaluated using multivariate Cox regression analysis adjusting for age, sex, autoimmune disease, liver disease, and malignant tumor.

We identified 104 EDTA-PTCP patients and 208 matched controls. The median age was 69.0 years (interquartile range: 54–76), with men comprising 51%. EDTA-PTCP patients had a higher frequency of malignant tumor and a lower frequency of hypertension and diabetes than controls. After adjustment for background factors, prognosis of EDTA-PTCP patients was significantly poorer than controls (hazard ratio, 11.8; 95% confidence intervals, 2.62–53.54). In conclusion, EDTA-PTCP patients had higher mortality, and EDTA-PTCP may need to be recognized as an indicator of worse prognosis.

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Abbreviations: EDTA = ethylenediaminetetraacetic acid, EDTA-PTCA = ethylenediaminetetraacetic acid-dependent pseudothrombocytopenia.

INTRODUCTION

Ethylenediaminetetraacetic acid (EDTA)-dependent pseudothrombocytopenia (PTCP) is a rare phenomenon whereby platelets aggregate in reaction to the anti-coagulant EDTA-2K contained in blood collection tubes. Consequently, platelet counts measured with an automated blood cell counter appear lower than they actually are.[1] Physicians are seldom aware that a normal platelet count can be obtained simply by changing the collection method in a laboratory,[2] such as using kanamycin or sodium citrate, to avoid platelet agglutination.[3,4]

EDTA-PTCP has an incidence of about 0.1% in the general population and up to 0.21% in hospitalized patients.[4–7] In addition, EDTA-PTCP is clinically recognized as a mere in vitro phenomenon, with no impact on the prognosis. However, a previous report suggested that EDTA-PTCP occurs more frequently in severely ill patients with autoimmune, neoplastic, atherosclerosis-related, and liver diseases.[8] Meanwhile, other studies reported no association with age, sex, burns, trauma, sepsis, human immunodeficiency virus, rubella, cytomegalovirus, autoimmune disorders, malignancy, cardiac surgery, or medication.[2,9] Therefore, whether EDTA-PTCP increases the risk of developing pathologies remains controversial. Furthermore, no study has examined the prognosis associated with EDTA-PTCP.

Thus, we conducted a case–control study with a group of EDTA-PTCP patients to compare the proportions of comorbidities, oral medication histories, and blood transfusion histories based on the presence of EDTA-PTCP and to examine whether EDTA-PTCP patients have a poorer prognosis than those without EDTA-PTCP.

METHODS

Study Design and Setting

This retrospective case–control study was conducted at The University of Tokyo Hospital between April 2009 and January

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2012 with follow-upuntil October 2013. This study was approved by the Ethics Committee of the University of Tokyo Hospital.

Participants

From the medical database of our hospital, which includes around 60 000 patients who underwent a blood test between April 2009 and January 2012, we identified 104 patients diagnosed with EDTA-PTCP by a blood test. Factors contributing to the incidence of EDTA-PTCP are unknown. Therefore, we only performed 1:2 case:control matching for age and sex. A control group of 208 sex-and age-matched (within 5 years) patients was selected at random from the same database. The exclusion parameters were age under 18, multiple comorbidities, or other hematologic diseases related to blood clotting that could be associated with similar comorbidities. Our facility routinely examines a smear under a microscope if a patient's platelet count is under 100 000 cells/μL or decreases over 30% compared with the previous count because visual evaluation of blood smears is regarded as the gold standard for detection of EDTA-PTCP.[10] A diagnosis of EDTA-PTCP is made via confirmation of platelet aggregation. Blood samples were subsequently collected in tubes containing 20 mg/mL of kanamycin, as excess aminoglycoside antibiotics such as kanamycin can prevent platelet agglutination in blood samples from EDTA-PTCP patients. Furthermore, platelet aggregates can even be dissociated by the addition of these antibiotics within 30 min after blood withdrawal.[3]

Data Sources/Measurements

At the time of blood collection, the following data were collected from patients' medical records: patients' age; sex; comorbidities including hypertension, diabetes, autoimmune disease, malignant tumor, liver disease, and heart disease; medications including aspirin, other antiplatelet, warfarin, steroids, anti-cancer drugs, Chinese herbal medicines, and anti-convulsants; and blood transfusion history. Patients' deaths by the end of October 2013 were confirmed by medical record or telephone survey (author NO). If a patient had died, the date of death was also collected.

The primary outcome of the study was mortality. In addition, we also examined factors contributing to the incidence of EDTA-PTCP by comparing characteristics between patients with and without EDTA-PTCP.

Statistical Methods

We examined differences in the frequency of complications, medication use, and history of blood transfusion between the EDTA-PTCP and control groups. Categorical data were compared with the χ^2 test. Univariate and multivariate Cox regression analyses were then conducted to investigate the association between EDTA-PTCP and mortality. Factors used in the final model were age, sex, malignancy, autoimmune disease, and liver disease. Previous studies have suggested that these diseases were associated with EDTA-PTCP pathogenesis.[2,8] The level of significance was defined as a P value <0.05. All statistical analyses were performed using STATA Special Edition version 13.1 (StataCorp, College Station, TX).

RESULTS

Participants

Table 1 illustrates patient demographics of 104 EDTA-PTCP patients and 208 matched controls. The median age of the patients was 69 years (interquartile range [IQR]: 54–76 years), with the majority aged >60 years. Men accounted for 51%. The median length of patient follow-up was 308 days (IQR 768–1562 days).

TABLE 1. Patient Characteristics, Complications, and Drugs Compared Between Patients With and Without EDTA-PTCP

	EDTA-PTCP (n = 104)	Control (n = 208)	*P*
Median (IQR) age, y	69.0 (54–76)	68.5 (54–76)	0.918
Age distribution, y			
<18	1	2	
18–29	3	6	
30–39	6	12	
40–49	11	22	
50–59	14	28	
60–69	19	38	
70–79	38	76	
>80	12	24	
Sex (male, %)	53(51)	106(51)	1
Complications			
Hypertension, %	37 (36)	99 (48)	0.044*
Diabetes mellitus, %	18 (17)	82 (39)	<0.001*
Heart disease, %	11 (11)	39 (19)	0.064
Malignancy, %	46 (44)	45 (22)	<0.001*
Autoimmune disease, %	26 (25)	37 (18)	0.135
Hepatic disease, %	29 (28)	41 (20)	0.103
Previous blood transfusion, %	22 (21)	38 (18)	0.542
Drugs			
Aspirin, %	19 (18)	58 (28)	0.113
Other antiplatelet drug, %	10 (10)	34 (16)	0.141
Warfarin, %	19 (18)	47 (23)	0.493
Steroid, %	9 (8.7)	29 (14)	0.169
Anticancer drug, %	5 (4.8)	9 (4.3)	0.835
Chinese herbal medicine, %	3 (2.9)	6 (2.9)	0.390
Anticonvulsant, %	2 (1.9)	13 (6.3)	0.091

EDTA-PTCP = ethylenediaminetetraacetic acid-dependent pseudothrombocytopenia; IQR = interquartile range.

Descriptive Data

Tables 1 and 2 show comparisons of background conditions between the cases and controls. The cases were less likely to have hypertension (36% vs 48%, $P = 0.044$) and diabetes mellitus (17% vs 39%, $P = 0.001$) than the controls. On the other hand, the cases were more likely have malignancy (44% vs 22%, $P < 0.001$).

There were no significant differences between the 2 groups in frequency of heart disease, autoimmune disease, liver disease, history of blood transfusion, medication use, and type of cancer.

Main Results

Table 3 shows the results of univariate and multivariate Cox regression analyses. The loss-to-follow-up value was 1/208

TABLE 2. Types of Cancer

	EDTA-PTCP (n = 38)	Control (n = 31)	*P*
			0.204
Liver cancer	11	6	
Uterus cancer	1	3	
Lung cancer	4	1	
Colon cancer	3	1	
Pharyngeal cancer	2	0	
Pancreatic cancer	2	4	
Blood cancer	6	3	
Gastric cancer	3	3	
Renal cancer	1	0	
Breast cancer	4	1	
Skin cancer	1	1	
Eye cancer	0	1	
Bladder cancer	0	3	
Ovary cancer	0	1	
Esophageal cancer	0	1	
Prostatic cancer	0	2	

EDTA-PTCP = ethylenediaminetetraacetic acid-dependent pseudothrombocytopenia.

(0.48%) in control and 4/104 (3.70%) in EDTA-PTCP patients. In a univariate analysis, patients with EDTA-PTCP had a hazard ratio of 17.0 (95% CI 3.89–74.36) for mortality, compared with those without EDTA-PTCP. Male sex, malignancy, and hepatic disease were also significantly associated with mortality.

In the multivariate analysis, the hazard ratio between patients with and without EDTA-PTCP decreased to 11.8 (95% CI 2.62–53.54), but EDTA-PTCP was still significantly associated with mortality (Table 3). Other conditions were not significantly associated with mortality in the multivariate analysis, whereas there was weak evidence of an association between malignancy and death (hazard ratio 2.67; 95% CI 0.85–8.36).

DISCUSSION

This large case–control study revealed that EDTA-PTCP patients had a higher frequency of malignancy as a comorbidity and higher mortality than age- and sex-matched controls.

These findings are consistent with an earlier report indicating that malignant tumor is a common comorbidity in EDTA-PTCP patients.[8] Furthermore, the data support a previously reported mechanism for the occurrence of EDTA-PTCP. When antigens of EDTA-dependent anti-platelet antibodies undergo cryptogenic exposure, antibodies against these antigens are produced. These antigens subsequently bind to platelets and cause agglutination in the presence of EDTA in vitro.[11] The likelihood of crypto-antigens in the blood, as well as the production of EDTA-dependent anti-platelet antibodies, may increase with malignant tumors, liver disease, or autoimmune disease.[12] Of these factors, we demonstrated that malignant tumor increased the risk of EDTA-PTCP. Although the clinical relevance of EDTA-dependent anti-platelet autoantibodies in vivo remains uncertain,[13] EDTA-PTCP patients may need further evaluation.

We demonstrated that EDTA-PTCP patients have a higher mortality than do other patients, even after adjustment for age, sex, malignant tumors, liver disease, and autoimmune disease. We have previously discussed the possibility that EDTA-PTCP can be a cause of eosinophilic disease in a patient with eosinophilic pneumonia.[12] Additionally, others have shown that EDTA-dependent anti-platelet antibodies cause platelet aggregation in the presence of substances contained in blood transfusions such as citric acid.[14–16] These in vivo studies have suggested that EDTA-PTCP is a systemic condition affecting the body through platelet stimulation. Therefore, EDTA-PTCP should be recognized not only as a benign in vitro phenomenon, but also as an indicator of a disease that affects patients' outcomes.

Further studies are needed to determine the impact of the location and stage of malignant tumors on EDTA-PTCP and to assess whether EDTA-PTCP develops beyond a specific stage of cancer.

LIMITATIONS

This study has certain limitations. First, this single-center study cannot account for different blood collection practices that would be reflected in a multi-center study. In fact, the number of people with malignant tumors, autoimmune disease, or liver disease might be higher than that in the general population. In addition, this study was performed with a hospital control match. Thus, the proportion of hypertension or diabetes is higher than that in the general population. Second, by definition, this retrospective study was limited to the parameters available in medical records. Although we included important measured confounders reported in previous studies, a Cox regression analysis could not account for unmeasured confounders. In particular, we could not differentiate the affected organ,

TABLE 3. Univariate and Multivariate Cox Regression Analyses for Mortality

	Univariate			Multivariate		
Variable	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***
EDTA-PTCP	17.00	3.89–74.36	<0.001	11.84	2.62–53.54	<0.001
Age	1.04	0.99–1.08	0.088	1.03	0.98–1.08	0.203
Sex	0.31	0.10–0.96	0.042	0.39	0.12–1.24	0.11
Malignancy	6.21	2.19–17.6	0.001	2.67	0.85–8.36	0.092
Autoimmune disease	1.11	0.36–3.43	0.85	1.37	0.43–4.38	0.6
Hepatic disease	3.79	1.46–9.82	0.006	1.73	0.60–5.01	0.314

HR = hazard ratio, CI = confidence interval, EDTA-PTCP = ethylenediaminetetraacetic acid-dependent pseudothrombocytopenia.

pathological type, and stage classification of malignancy, which appeared to be residual confounding factors in the association between EDTA-PTCP and mortality.

CONCLUSION

This study demonstrated that patients with EDTA-PTCP have a significantly higher mortality rate than age- and sex-matched patients without EDTA-PTCP.

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