Uncoupling of Protein C and Antithrombin III Activity in Cerebral Ischemia Patients Associated with Cutis Marmorata

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Abstract-

Purpose: Cutis marmorata is a cutaneous livedoid disorder which can be differentiated from livedo reticularis in both clinical and pathological presentations. Unlike Sneddon syndrome, a detailed immunocoagulation profile has not yet been delineated for cutis marmorata in patients with cerebral ischemia.

Methods: To analyze the immunocoagulation profile in cutis marmorata patients associated with cerebral ischemia (CMCI) in a series of 135 cerebral ischemia patients.

Results: A total of 32 patients were found to have cutis marmorata. The blood protein C activity, protein S activity, antithrombin III activity, platelet count, fibrinogen and frequency of abnormal antiphospholipid antibody level were similar among 32 CMCI patients, 103 cerebral ischemia patients without cutis marmorata, and 35 healthy subjects. However, uncoupling of protein C and anti-thrombin III was observed in CMCI patients. Serum antinuclear antibody and Venereal Disease Research Laboratory were not detected in these patients.

Conclusion: Cutis marmorata is not uncommon in our ischemic stroke patient population, and is characterized by uncoupling of protein C and antithrombin III with altered thrombin hemostasis. Our findings raise the need for a careful cutaneous examination in patients with ischemic stroke. Abnormal immunocoagulating profile should alert physicians to the risk for cerebral ischemia even in the absence of other cardiovascular risk factors.

Key Words: Cutis marmorata, Protein C, Antithrombin III, Protein S, Cerebral ischemia

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INTRODUCTION

In 1907, Salomon Ehrmann[1], a Viennese dermatologist, identified a livedoid disorder different to cutaneous change in late syphilis. He described the skin as "tendril-like bluish pattern reminiscent of forked lightning which intensified in the cold as a sign of passive hyperemia, somewhat cooler than the surrounding skin". Clinically, this livedoid disorder is reddish-purple, reticulated, mottled skin lesion primarily found on the trunk

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and extremities. Since then, a number of classifications for cutaneous livedoid disorder were made based on clinical presentations.

The European medical literature has classified two types of cutaneous livedoid disorder. If discoloration persists upon warming, it is classified as generalized livedo racemosa; if disappears after warming, it is classified as livedo reticularis[2]. In American medical terminology, the former is termed livedo reticularis while the latter is termed cutis marmorata[2]. The American terminology of livedo reticularis and cutis marmorata is used throughout the remainder of this study report[2]. The diagnosis of cutis marmorata is clinically based on the presence of reddish-purple, reticulated, mottled skin change occurring or exacerbated upon exposure to cold whereas attenuated or subsiding upon exposure to heat[2-4]. Cutis marmorata is rather common in newborn infants, and generally subsides before 2 years of age. Occasionally, cutis marmorata is seen in women and children upon exposure to cold temperature or when crying[3].

Sneddon syndrome is a clinical entity resulting from a concomitance of livedo reticularis and cerebral ischemia[2]. An immunocoagulating disturbance has recently been established for Sneddon syndrome since livedo reticularis is occasionally associated with autoimmune diseases, such as systemic lupus erythrematosus[5] or antiphospholipid antibody syndrome[6-7]. Meanwhile, the relationship between cutis marmorata and cerebral ischemia (CMCI) has not yet been described. Cutis marmorata has been proposed to result from instability or immaturity of the nerve supply to the superficial capillary blood vessels in skin, resulting in dilatation or constriction of blood vessels which produces the reticulated red and white marbled pattern[3]. It is considered a benign cutaneous phenomenon and rarely reported in association with symptomatic occlusive disease. In this study, we attempt to elucidate the frequency of autoimmune and coagulation parameters in CMCI patients.

PATIENTS AND METHODS

Sampling population

We have been investigating the role of antiphospholipid antibody, autoimmunity and coagulopathy in cerebral ischemia in our patient population. In this study, patients with ischemic stroke who had been registered in our database between 2004 and 2005 were included. The diagnosis of cerebral ischemia was based on sudden onset of focal neurological deficits, corresponding infarct in cranial computed tomography or magnetic resonance imaging, and exclusion of possible nonvascular disorders. Further exclusions were: (1) previous or current history of systemic lupus erythrematosus or known collagen disease; (2) recent craniofacial trauma; (3) consumption of herbal remedies, anticoagulants, estrogen, androgen, or corticosteroids; (4) abnormal renal function (serum creatinine >1.4 mg/dl) or hepatic (SGOT > 40 IU/L or cirrhosis); (5) abnormal thyroid or adrenal function; and (6) transient ischemic attack. Systemic lupus erythrematosus was diagnosed according to the 1997 American College of Rheumatology guidelines.

Healthy volunteers free of medical or neurological disorders served as controls. They were either hospital employees, family of patients followed in the neurology clinic or individuals who presented for neurological examination.

Baseline examination

The blood anti-beta2-glycoprotein I IgG-antibody, anticardiolipin IgG-antibody, lupus anticoagulant inhibitor, protein C (PC) activity, protein S (PS) activity, anti-thrombin III (AT-III) activity, prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, complete blood count, albumin, aspartate aminotransferase, alanine aminotransferase and creatinine were examined for both patients and the healthy controls. Venous blood was collected from an antecubital vein using a sterile vacutainer system following an overnight fast lasting ten hours. Blood was mixed with 3.8% trisodium citrate (1:9 citrate:blood). For testing blood anti-beta 2-glycoprotein I IgG-antibody, anticardiolipin IgG-antibody, lupus anticoagulant inhibitor, PC activity, PS activity, AT-III activity, PT and aPTT, blood was centrifuged within one hour after collection (Universal 16R Hettich Zentrifgen for 10 minutes at 3000 g under 4˚C) to yield platelet-poor plasma. The PT and aPTT were performed within 2 hours after collection. The platelet-poor plasma was aliquoted and stored

at -20˚C for batch measurement during the next 14 days. For complete blood count, blood was examined within 4 hours after collection by Sysmex SE-9000 automated counter (Australian Diagnostics Corporation, Syndey, Australia). For fibrinogen, albumin, aminotransferase and creatinine, serum was examined within 2 hours after collection (Hitachi 7450 autoanalyzer, Tokyo, Japan)

Assays for antiphospholipid antibody, proteases and hemostatic parameters

The procedures have been described in detail in our previously published work[8,9]. In brief, the PT and aPTT assays were performed on the Sysmex CA-6000 coagulometer (Australian Diagnostics Corporation, Syndey, Australia). Thromboplastins used were APTT-LS (Fisher Diagnostics, VA, USA) for the aPTT and Innovin (Dade Behring Marburg GmbH, Marburg, Germany) for the PT. ELISA method was applied to measure the blood anti-beta2-glycoprotein I IgG-antibody and anticardiolipin IgG-antibody (Varelisa test-kit, Pharmacia & Upjohn, Germany). Lupus anticoagulant inhibitor was measured by the dilute Russell's Viper Venom test (LA Screen/LA Confirm, Gradipore Ltd., Australia). Furthermore, the PC activity was measured using the chromogenic assay (STA-STACHROM® Protein C, Diagnostica Stago, France). The AT-III activity was assessed as anti-factor activity (IL Test™ Antithrombin, Instrumentation Laboratory, Lexington, MA). In the PC and AT-III assay, the coefficient of variation (CV) of intravariation was 1.6%, 1.8% and 1.8%, and intervariation was 3.0%, 3.6% and 3.6% respectively.

Statistical analysis

Descriptive statistics for continuous variables were listed as mean and standard deviation. For categorical variables, the number and percentage of patients are provided. Differences between means were assessed with Least Significant Difference (LSD) test or 2-tails Student's unpaired t-test for independent samples for variables with a normal distribution. Categorical variables (age, gender, hypertension, diabetes mellitus) were evaluated with Chi-Square test or Fisher exact test. A probability less than 0.05 was considered significant.

RESULTS

A total of 32 cerebral ischemia patients with cutis marmorata (CMCI), 103 cerebral ischemia patients without cutis marmorata (non-CMCI), and 35 healthy subjects were enrolled in this study. CMCI patients included 23 men and 9 women, ranging in age from 45 to 84 years, with the average of 61.6 years. Non-CMCI patients included 67 men and 33 women, ranging in age from 32 to 89 years, with the average of 61.7 years. Healthy controls included 21 men and 14 women, ranging in age from 47-70 years, with the average of 60.5 years.

Cutis marmorata could be found in multiple sites, and was mainly found on the thigh (16 patients), followed by knee (12 patients), chest (3 patients), abdomen (3 patients), upper limb (2 patients) and buttock (1 patient) in our CMCI patients in symmetry. Raynaud's phenomenon was not found. No CMCI patients had experienced gangrene or skin ulcer before.

The frequency of hypertension (defined as: Systolic pressure > 140 mmHg and/or diastolic pressure > 90 mmHg in three occasional measurements or if the patients were under antihypertensive treatment) and male gender was similar between the CMCI and non-CMCI patients. However, diabetes (defined as: An increase of fasting blood sugar > 150 mg/dl in two occasions and glycohemoglobin > 6.2% or under antidiabetic treatment) was more frequent in CMCI than non-CMCI patients (Chi-Square test= 4.57, $p< 0.05$) (Table 1).

Serum fibrinogen, albumin, aminotransferases, creatinine, platelet count and frequency of abnormal antiphospholipid antibody level were similar among the three groups of subjects (LSD test, $p>0.05$) (data not shown). Furthermore, PC, PS and AT-III activity did not differ among CMCI patients, non-CM patients and controls (LSD test, $p>0.05$) (Table 2). However, PC/AT-III decreased significantly in CMCI patients (LSD test, $F=3.264$, $p=0.041$) compared to non-CMCI patients ($p=0.048$) or controls ($p=0.013$). No difference in PC/AT-III activity was observed between non-CMCI patients and controls ($p=0.289$). This decrease did not result from a decrease in PC or an increase in AT-III, suggesting an uncoupling of PC and AT-III in CMCI

Table 1. *Demography data*

Items	CMCI patients (n=32)	non-CMCI patients (n=103)	Controls (n=35)
Age, mean (years)	61.59	61.65	60.50
Age, range (years)	45 ~ 84	32 ~ 89	47 ~ 70
Gender, men, n (%)	23, 71.9 %	67, 65.1 %	24, 68.6%
Hypertension, n (%)	26, 81.3 %	72, 69.9 %	0, 0.0%
Diabetics, n (%)	14, 43.4 %	25, 24.3 %*	0, 0.0%
Non-HTN/DM, n (%)	5, 15.6 %	24, 23.3 %	35, 100%

CMCI: cutis marmorata and cerebral ischemia; Non-HTN/DM: no hypertension or diabetics; *Chi-Square test= 4.57, p< 0.05.

Table 2. *The protein C (PC), protein S (PS) and antithrombin III (AT-III) activity and their ratios in patients and controls*

Variables/Subjects	CMCI patients	non-CMCI patients	Controls
PC (%)	91.38±25.85	95.48±21.04	99.83±14.50
PS (%)	77.00±13.91	77.87±19.48	83.86±13.91
AT-III (%)	128.69±39.57	119.60±36.19	115.26±28.91
PC/PS	1.260±0.539	1.267±0.308	1.238±0.227
PC/AT-III	0.753±0.253*	0.867±0.311	0.926±0.217
PS/AT-III	0.713±0.254	0.713±0.287	0.767±0.217

The value is given as mean±1SD. CMCI: cutis marmorata and cerebral ischemia; *PC/AT-III activity: CMCI patients vs non-CMCI patients or healthy controls (p=0.041, LSD).

patients. None of the CMCI patients tested positive for VDRL and ANA.

DISCUSSION

Unlike livedo reticularis in Sneddon syndrome[2,10], no relationship between cutis marmorata and cerebral ischemia has been noted. Whereas the variable magnitude of endothelial cell proliferation and intravascular occlusion in small to medium size arteries resembling autoimmune vasculopathy is a cardinal feature of livedo reticularis[11], the histology of cutis marmorata is generally normal, except for minimal lymphocytic perivascular infiltrates, dilatation of capillaries in the deep dermis, dilatation of veins or venous lakes, or swelling of endotheliocytes may be found on some occasions[3]. Immunoglobulin deposition is infrequent. These distinctions may purport vasohemostatic diversity between cutis marmorata and livedo reticularis.

In our CMCI patients, cutis marmorata mostly occurred on the thigh, followed by the knee, chest and abdomen. Symmetric distribution was the rule. This pattern resembles livedo reticularis. However, they have not experienced gangrene or skin ulcer before. This difference may result from variation in hemostatic effect.

Cutis marmorata can occur in a few congenital disorders associated with cerebrovascular anomalies including Sturge-Weber syndrome, Cornelia De Lange syndrome or Dubowitz syndrome. However, cerebral ischemia is rarely mentioned in these conditions. Cutis marmorata telangiectatica congenital, a common variant of cutis marmorata which is pronounced starting in childhood, is sometimes associated with cerebral ischemia[12]. These congenital disorders were not found in our patients.

Sneddon syndrome has been reported with a variety of autoimmune and coagulation disorders including

antiphospholipid antibody syndrome, factor V Leiden mutation, protein C or protein S hypoactivity, prothrombin G20210A gene mutation, homocysteinemia[4], antimitochondrial antibody[13], factor VIII disorder[14], and dysfibrinogenemia[15]. On the other hand, concomitant autoimmunty or thrombophilia is rare in cutis marmorata in adults, although lupus[16], lymphohistocytosis[17] and abnormal increase in antinuclear antibody titers[18] have been reported in cutis marmorata telangiectatica congenital neonates. In this series, a similar frequency of abnormal increase in antiphospholipid antibody titers, decreased activity of PC, PS and AT-III, thrombocytosis or dysfibrinogenemia was noted between the CMCI and non-CMCI patients. Uncoupling PC and AT-III activity did not simply result from a decrease of either anticoagulant. Therefore, a dissociative interaction of natural anticoagulants may be vulnerable for promoting coagulation in CMCI.

Uncoupling of PC and AT-III has been reported in sickle cell disease, polytransfused thalassemia, cancer[19] or abnormal increase in blood anti-beta2-glycoprotein I antibody titers[20] known for procoagulation. PC and AT-III share a common source of synthesis and ultimately reduce thrombin generation via different pathways; AT-III inactivates thrombin whereas PC inactivates the activated factor V and VIII. PC and AT-III are closely correlated. Uncoupling of PC and AT-III can theoretically protect individuals from thrombosis when either one decreases. However, insufficient data exists to support a compensatory increase of another anticoagulant protease being sufficient for anticoagulation[21]. Therefore, the uncoupling of PC and AT-III, besides not being associated with compensatory increase of coupled protease, is a likely mechanism which increases the risk of cerebral ischemia in cutis marmorata patients.

The interleukin (IL)-6, IL-1β and tumor necrosis factor-α (TNFα)[22-25] could independently or synergistically influence PC and AT-III. For example, IL-6 is released in response to stimulation by the monocyte-derived cytokines IL-1β and TNFα. Unfortunately, we do not understand the role of cytokines in cutis marmorata. Nevertheless, it is known that PC and AT-III possess comparable anti-inflammatory effects counteracting the actions of IL-6, IL-8, IL-1β and TNFα[24,25]. Lower PC/AT-III ratio may reflect an imbalance in the immunocoagulation to maintain hemostasis in CMCI patients.

In this series, diabetes mellitus occurred more frequently in CMCI patients than non-CMCI patients, but did not occur in patients with cutis marmorata or livedo reticularis. Since the PC/AT-III coupling was similar between the diabetic and nondiabetic patients, diabetes mellitus was not considered as a contributing factor to PC/AT-III uncoupling in our patients. Nevertheless, our study is still limited by a small case series which may influence the final statistical result.

In conclusion, cutis marmorata is not uncommon in patients with cerebral ischemia in our stroke patient population. Altered hemostasis, such as an uncoupling of PC and AT-III activity, may account for the occlusive calamity. Further investigation is warranted to confirm that cutis marmorata is not uncommon among stroke patients seen in other medical centers in Taiwan or other countries.

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