Protein C Levels in Patients With Legg-Calve-Perthes Disease

Is It a True Deficiency?

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Abstract: A hyper-coagulable state due to protein C deficiency has been postulated to be the cause of avascular necrosis of the capital femoral physes in Legg-Calve-Perthes disease (LCPD). In order to test this hypothesis, plasma protein C levels were analyzed from 51 unselected cases of LCPD. These were compared with a control group. Our findings showed that the levels were less than the mean for age in 38 (74.5%) of the cases, though were within the normal range. We conclude that clinical thrombosis could be triggered off in these susceptible individuals by prothrombotic insults such as passive smoking, ultimately leading to LCPD.

Key Words: Legg-Calve-Perthes disease (LCPD), protein C deficiency, passive smoking

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It has been suggested that local thrombotic episodes may be the cause of some cases of avascular necrosis in children. There have been reports of antiphospholipid antibodies in a patient with multiple thromboses and avascular necrosis[1] and anticardiolipin antibodies[2] in patients with nontraumatic osteonecrosis of the hip. A hypercoagulable state can lead to a single or multiple occlusions that can take place over a period ranging from hours to weeks.

Vascular thrombosis is uncommon at a young age; if it occurs, it is related to a genetic defect.[3] The most common genetic defect in 50% of the inherited hypercoagulable states is a resistance to activated protein C.[4] Protein C was identified in 1976 by Stenflo and is a vitamin K-dependent protein.[5] The concentration is the same in both sexes, and a small but significant increase is noted with age (4% per decade). There is a maturational delay, normal adult values being reached in the late teens[6] (Table 1). Its anticoagulant effects are by virtue of its ability to proteolytically degrade the active forms of the coagulant cofactors factor V and factor VIII. The net effect of this activation is to curtail the potential formation of the procoagulant enzymes factor Xa and thrombin. Protein C deficiency has been reported to cause thrombosis in small-caliber veins such as cerebral, retinal, renal, and cutaneous veins. Several orthopaedic manifestations have been reported, with the common theme of ischemia of the bone and cartilage.[7] The vascular anatomy of the proximal femur makes it particularly vulnerable to thrombotic events. Vascular flow abnormalities are reported on the venous side.[8]

Catterall postulated a "pre-existing condition rendering some children susceptible to Perthes disease."[9] The search for this systemic cause has included investigations of thyroid function,[10] blood viscosity,[11] the effects of passive smoking,[12,13] and hypercoagulable states.[14–28] We have investigated protein C deficiency in patients with Legg-Calve-Perthes disease (LCPD) as a possible cause of this hypercoagulable state.

METHODS

Study Design

This was an observational study approved by the medical ethics committee.

Patient Profile

Fifty-one cases of LCPD were identified from the hospital records of five District General Hospitals in Southeast England. The mean age was 14.3 years (range 4–31 years). Males outnumbered females, in keeping with the natural history of this disease (44 males, 7 females). At the time of the study 27 patients were under observation with active disease and 24 patients had completed skeletal growth and did not have active disease.

Control Group

We recruited a control group comprising 36 children and young adults from the fracture clinics. This group was well matched with our disease group for age and sex. They did not have any hip pathology or a coagulation disorder.

Protein C Levels in the Normal Population

Compared with the other coagulation factors, protein C demonstrates a delay in attaining normal adult values. This maturational delay has been studied and the levels at different ages have been established[6] (see Table 1). We used these data to compare the expected age-matched values of the protein C levels in the LCPD cohort and compared it to the observed values.

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TABLE 1. Changes in Protein C Levels in the Normal Population

Age (yr)	Mean	Minimum	Maximum
1–5	66	40	92
6–10	69	45	93
11–16	83	55	111
>16	96	64	128

Values are given as IU/dL.
Data from reference 6.

Assessments

The patients (with their parents when appropriate) were seen in an outpatient clinic. The purpose and nature of the study were explained and written information was provided. The history of smoking in either parent was noted. Twenty milliliters of venous blood was drawn for protein C levels. All the samples were analyzed at the Hemophilia Center at the Kent and Canterbury Hospital. The samples that were collected outside the Kent and Canterbury Hospital were frozen and the frozen plasma was transported to the Haemophilia Centre.

Analyses

The data are expressed as mean ± SE of mean and the upper and lower limits of the 95% confidence intervals (Table 2). We performed the statistical analyses using the two-sample *t* tests for significance and the correlation assessments using the Pearson rank correlation coefficient with a two-tailed level of significance at alpha = 0.05.

RESULTS

Protein C Levels in the LCPD Cohort

The mean protein C levels in patients with LCPD were 70.2 ± 2.01 IU/dL (95% CI 66.16–74.23). Because protein C levels are age-dependent and the levels plateau into the adult levels in the late teens, we computed the expected adjusted mean and the minimum levels of protein C for the given age of the patients. The age-adjusted mean levels of protein C expected in the cohort were 82.9 ± 1.45 IU/dL (95% CI 80.01–85.84). The age-adjusted minimum levels expected in the cohort were 54.8 ± 1.04 IU/dL (95% CI 52.72–56.89) (Figs. 1 and 2).

Thirty-eight patients (74.5%) had levels of protein C less than the expected mean for the age (difference of <5 IU/dL), and an additional 10 patients (94.1%) had levels 1 to 3 IU/dL higher than the mean expected levels for age. None of the patients had a true protein C deficiency.

A two-sample *t* test was performed for significance between the groups. The values of protein C compared with both the mean ($P < 0.0001$) and minimum age-adjusted values ($P < 0.0001$) showed statistical significance. Both sets of values showed a good correlation on performing the Pearson rank correlation test ($r = 0.62$; $P < 0.05$).

Protein C Levels in the Control Group

The mean protein C levels in the control group were 72 ± 2.2 IU/dL (95% CI 67.52–76.48). A two-sample *t* test failed to show statistical significance ($P > 0.05$) between the LCPD cohort and the controls. We calculated the expected mean values of the control group. The protein C levels of the controls were compared with the mean age-adjusted values with a two-sample *t* test, which was not statistically significant ($P > 0.05$, Pearson correlation coefficient $r = 0.2$, $P > 0.05$).

Protein C Levels in Passive Smokers and Nonsmokers

Thirty-eight patients with LCPD reported a history of passive smoking from either parent, eight did not, and information from five patients was inconclusive. The mean protein C levels in the passive smoking group were 70.2 ± 2.37 IU/dL (95% CI 65.39–74.98) and that in the nonsmoker group was 69.5 ± 5.05 IU/dL (95% CI 57.55–81.45). A two-sample *t* test comparing these two groups showed that the values between the two groups were not significant ($P > 0.05$; Pearson correlation coefficient $r = -0.14$; $P > 0.05$).

DISCUSSION

Our study focused on the hypothesis that protein C deficiency is the potential cause of LCPD. Protein C was chosen because it is the most common known factor that could influence coagulability in these patients, either by resistance to its activated form or as a result of a deficiency state.

Extensive work by Gleuck[12,15–17,24,29] has suggested that the pathologic findings in thrombophilia and other hypercoagulable states such as disseminated intravascular coagulation can result in orthopaedic manifestations such as adult nontraumatic osteonecrosis, alveolar osteonecrosis of the jaws, and LCPD.

Although the literature supports that various sites in the skeleton are affected, the predilection for the growing femoral

TABLE 2. Demographic Data for 51 LCPD Study Patients

	n	Mean (IU/dL)	SD	SEM	95% CI (IU/dL)	Range (IU/dL)
Perthes	51	70.2	14.34	2.0084	66.216–74.23	44–116
Age-adj. mean	51	82.9	10.4	1.45	80.01–85.84	66–96
Age-adj. minimum	51	54.8	7.4	1.04	52.72–56.89	40–64
Controls	36	72	13.23	2.21	67.52–76.48	39–95
Passive smokers	38	70.2	14.6	2.37	65.39–74.98	40–116
Nonsmokers	8	69.5	14.3	5.05	57.55–81.45	54–93

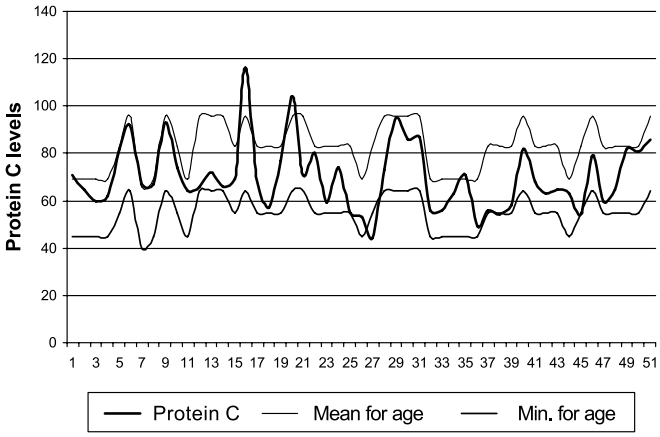

FIGURE 1. Protein C level means and minimum expected by age for 51 LCPD study patients.

head can be explained on the basis of certain specific anatomic features. Chung noted that the intra-articular rings were discontinuous, more often in male specimens, and that there were fewer ascending cervical arteries crossing the anterior and medial surface in mid-neck in 3- to 10-year-old white children than in younger or black children.[30] Although the arterial channels run a straight course, veins are seen to spiral around the arteries and run a tortuous course.[31,32] The veins are thin-walled and are less capacious in the lateral and superior part of the femoral head,[33,34] causing a rather sluggish flow in this region. This could explain the tendency for the femoral head to be prone to vascular stasis and consequent venous thrombosis.

Most of the components of the hemostatic system mature by age of 5 months, but protein C does not reach its maximum levels until the latter half of the second decade of life. A prospective cohort study from Ontario provides the reference ranges for all the factors and proteins involved in the hemostatic system for different age groups[6] (see Table 1). To ascertain whether a person exhibits a protein C deficiency, the values must be adjusted for age. We adjusted the values in our cohort to determine the mean and minimum expected for age. We found that these data were more reliable than our own control group.

Inherited protein C deficiency is rare. Meningococcal sepsis is the classic clinical model of acquired protein C deficiency. Venous thrombosis is seen in medium-caliber veins such as cerebral, retinal, renal, and cutaneous veins. On a similar theme, protein C deficiency-related coagulopathy can be postulated to cause thrombosis in the veins of the femoral head, which, as outlined earlier, is particularly vulnerable at the age when LCPD occurs.

Our findings point to a compromised state rather than a true deficiency. The values (70.2 IU/dL), though less than the mean expected for the age (82.9 IU/dL), are more than the minimum (54.8 IU/dL). Hence, they are in the normal range, though at the lower end of the spectrum. It could be postulated that this results in a potentially hypercoagulable state, though remaining subclinical. Clinical episodes of thrombosis may be precipitated by acquired prothrombotic insults in these predisposed individuals. These insults may be genetic, in the form of multigene interactions, or they may be acquired environmental factors such as passive smoking.

It has been suggested that passive smoking increases the risk of LCPD by more than five times.[13] Passive smoking

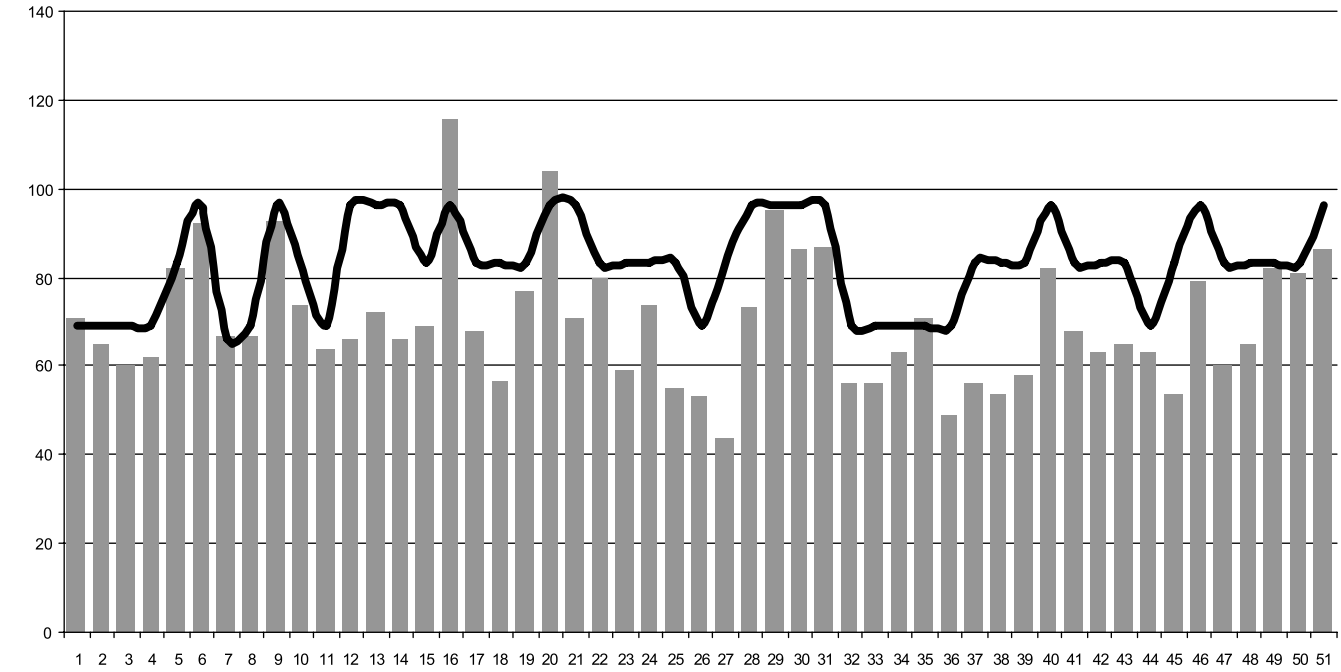

FIGURE 2. Comparison of protein C levels with mean age-adjusted values for 51 LCPD study patients.

has been inversely correlated with fibrinolysis and stimulated tissue plasminogen activator activity, but it is not connected with other coagulation parameters.[12] On comparing the protein C levels in the passive smoker and the nonsmoker groups, we did not detect any statistical difference. However, we observed that 74.5% of the patients with LCPD had a history of passive smoking.

In summary, our patient population showed that the protein C levels were in the lower end of the spectrum but did not represent a true protein C deficiency. The unique anatomic characteristics of the capital femoral epiphysis make it a likely target for venous thrombosis. Clinical thrombosis could be triggered in these susceptible individuals by prothrombotic insults such as passive smoking, ultimately leading to LCPD.

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