Increased coagulation factors V, VIII, IX, and homocysteine in Mexican patients with Legg-Calvé-Perthes disease

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Research Article

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Abstract

Background: Legg-Calvé-Perthes disease (LCPD) is a pediatric disorder that occurs due to the avascular necrosis of the femoral head and affects the range of motion of the hip in various degrees. Its etiology is still unknown, although it has been associated with coagulation abnormalities. In Mexico, there are no reports describing hypercoagulability markers in patients with LCPD. Therefore, our objective is to determine coagulation factors, Von Willebrand factor (VWF), and Homocysteine (Hcy) in patients with LCPD and controls.

Results: Were tested 25 patients with LCPD and 50 controls, matched by sex and age. The results showed significant differences in patients with respect to controls: Prothrombin time ↓, FV ↑, FVIII ↑, FIX ↑, and Hcy ↑. These results indicate that there may be hypercoagulable states in patients, which can cause thrombotic events.

Conclusions: This is the first report in Mexico with a clinical and research focus regarding patients with LCPD. The increase in FV activity, FVIII, FIX, and Hcy concentration support the hypothesis that microthrombi formation in small-caliber vessels could be causing avascularity and femoral necrosis, which are traits of LCPD.

Background

Rare diseases are characterized by low incidence, and difficulties may arise in obtaining timely and accurate diagnoses [1–3]. Legg-Calvé-Perthes disease (LCPD) is a rare disease (RD). This self-limited microvascular disorder causes an occlusion of the femoral head blood supply, resulting in idiopathic avascular osteonecrosis of the developing femoral head [4, 5]. It occurs in children from 4 to 9 years, mainly men, in a 4:1 ratio. Incidence varies significantly within racial groups and is predominant in Caucasians. The etiology of LCPD is still unknown; it remains one of the most controversial conditions in pediatric orthopedics, and many aspects remain unclear, although it has been associated with hypercoagulable states (thrombophilia) and coagulation abnormalities [6, 7]. The diagnosis of the disease is initially determined through clinical studies, physical exams, and radiological studies [8–11].

There are no incidence data or reports in Mexico that assess hypercoagulability markers (thrombophilia) in patients suffering from LCPD. These markers would allow us to determine the possible presence of thrombophilia in said patients and alert families regarding the possibility of developing diseases related to thrombophilia.

Moreover, due to the ambiguity of the existing reports, it has not been feasible to reach a consensus concerning if hypercoagulable states (thrombophilia) and coagulation abnormalities trigger LCPD or not. Most of the studies in the literature reference the Caucasian population, which highlights the need for research in Latin American populations [12, 13].

For the reasons above, this study aims to continue spreading awareness of this disease as well as to assist in solving the existing controversy regarding the etiology of LCPD and hypercoagulable states by analyzing thrombophilia risk markers in a population of Mexican patients of Instituto Nacional de Rehabilitación Luis Guillermo Ibarra Ibarra (INR-LGII).

Methods

This is a case-control study. The cases were first-time or recurrent patients of all ages, of both sexes, with a clinical and radiological diagnosis of LCPD, without other bone diseases or diseases related to coagulation abnormalities, and without any pharmacological treatment. There were also healthy controls, matched with the patients in a 2:1 proportion by age and sex, with radiological studies showing that they did not present any alteration in the femur or hip. In addition, they had no history of hematological or thrombotic pathologies and were not receiving any pharmacological treatment. All patients were recruited from the Orthopedic Service at INR-LGII in Mexico City. In addition, we obtained weight (kg), height (m), and body mass index (BMI). Both groups were selected under the guidelines of the Norma Oficial Mexicana NOM-253-SSA1-2012 for blood banks.

A blood sample from each participant was taken in a tube with 3.8% sodium citrate. Blood parameters were analyzed using Mindray Hematology Analyzer BC6800. The plasma was detached, and the samples were analyzed using commercial kits (HemosIL™) in a coagulation analyzer (coagulometer) IL ACL Elite / Pro. Coagulation times: Thrombin Time – 0009758515; Prothrombin time PT RecombiPlasTin 2G-0020002950; Activated partial thromboplastin times APTT-SP (liquid) – 0020006300. Coagulation factors: Factor I (Fibrinogen) HS PLUS 0008469810; Factor II (Prothrombin) 0008466050; Factor V (Proacelerin) 0020011500; Factor VIII (Antihemophilic A) 0020011800; Factor IX (Antihemophilic B) 0020011900; Factor X (Stuart-Prower Factor) 0020010000; Factor XI (Antihemophilic C) 0020011300; Factor XII (Hageman factor) 0020201200, Von Willebrand factor 0020002300 (VWF) and Homocysteine 0020007800 (Hcy) (Instrumentation Laboratory SpA-V.le Monza 338–20128 Milano, Italy) [14].

Statistics

The Kolmogorov-Smirnov test was used to verify if the data distribution was consistent with a normal distribution curve. However, when the data were analyzed, some did not follow a normal distribution: specifically, the median, the 25 and 75 percentiles, the mean, and the 95% confidence interval. The age group and gender analysis were performed independently. A one-way variance analysis was performed to determine any significant differences between gender and age groups. A Mann-Whitney *U* test or Student's *t*-test was applied in the comparative analysis.

Ethical Aspects

All participants received oral and written information about the study and signed a letter of consent. The INR-LGII Research and Ethics Committees reviewed and approved the study protocol.

Results

25 patients (23 men and 2 women) and two controls for each patient (46 men and 4 women) were recruited and matched by sex and age (Table 1) (Fig. 1). The patients had a mean age of 16.3 years and a mean age at diagnosis of 5.6 years, the mean age for the control group was 15.8 years (Table 1). No significant differences were found in average weight and height. The average height in patients was 1.48 ± 0.23 m and 1.49 ± 0.23 m in controls, while the average weight was 46.21 ± 15.02 kg in patients and 53.83 ± 24.06 kg in controls. In addition, body mass index (BMI) is shown in Table 1.

Table 1
Population characteristics

	Patients	Controls	p
Sex	Males: 23 Females: 2	Males: 46 Females: 4	N/A
Age	16.3 years	15.8 years	0.80
Weight	45.3 kilos	52.3 kilos	0.17
Height	1.5 meters	1.5 meters	0.90
Body Mass Index	20.4	22.8	0.90
Probability: $p \leq 0.05$			

In 17 patients, the left extremity was affected; 5 patients showed an affected right side, and 3 cases were bilateral. Most of the patients belonged to blood group O (18 patients), followed to a lesser extent by group A (4 patients), and only 3 patients belonged to group B. There were no cases of group AB. The entire population was type RH positive (Fig. 1).

Blood groups, hemoglobin, and leukocytes presented a similar distribution to that reported in Latin American populations, also without significant differences between patients and controls. Regarding platelets and coagulation times, Thrombin Time (TT) and activated Partial Thromboplastin Time (aPTT) did not present significant differences between both groups, nor did they exceed the reference values in any of the parameters analyzed. Only Prothrombin Time (PT) was lower in patients and presented a significant difference without leaving the reference values (Table 2 and Fig. 2).

Table 2
Platelet values, coagulation time and factors, antithrombotic proteins, Von Willebrand factor, and homocysteine.

Parameter (reference values, units)	Patients (N = 25)	Controls (N = 50)	P
Leukocytes	6.1 ± 1.3	6.6 ± 1.4	0.17
Hemoglobin	16.1 ± 1.9	15.4 ± 1.8	0.10
PLTs (150–450 mm³)	258 (219.0–306.0)	271.5 (227.5–326.0)	0.25
TT (15.8–24.9 s)	17.9 (17.2–18.1)	17.4 (15.7–18.33)	0.52
PT (0–13.7 s)	12.2 ± 0.85	13.0 ± 0.83	0.0002*
aPTT (26–40.2 s)	31.50 (29.80–34.08)	30.1 (29.0–32.5)	0.40
Factor I (80–700 mg/dL)	354.0 (288.5–409.5)	304.0 (249.0–380.5)	0.20
Factor II (50–150%)	108.0 (97.5–118.0)	110.0 (97.4–125.5)	0.17
Factor V (50–150%)	134.4 ± 25.7	96.0 ± 38.6	0.0001*
Factor VII (50–129%)	111.6 ± 21.8	115.2 ± 30.3	0.60
Factor VIII (50–150%)	99.0 (92.2–114.5)	78.9 (50.9–116.3)	0.02*
Factor IX (65–150%)	130.1 ± 21.3	92.6 ± 21.8	< 0.001*
Factor X (77–133%)	117.2 ± 18.4	123.6 ± 26.7	0.30
Factor XI (65–150%)	109.0 ± 19.5	106.1 ± 42.0	0.75
Factor XII (50–150%)	93.1 (79.1–115.0)	87.0 (59.3–111.3)	0.40
VWF (66–170%)	86.7 ± 29.5	97.2 ± 35.3	0.20
Hcy (Girls 4.9–11.9 µmol/L) and Boys 5.5–13.4 µmol/L)	8.3 (7.0–9.7)	7.1 (5.1–8.4)	0.02*
The parameters in bold text are the parametric variables, for which the mean and the standard deviation are presented [X (± SD)]. The ones in plain text are the non-parametric variables, in which the median and the 25th and 75th percentiles are presented [Me (25th – 75th)]. *P ≤ 0.05.			
PLTs: platelets; TT: Thrombin Time; PT: Prothrombin Time; aPTT: activated Partial Thromboplastin Time; VWF: von Willebrand Factor; Hcy: Homocysteine.			

As for coagulation factors (CF), von Willebrand Factor (VWF), and homocysteine (Hcy) (Table 2), the mean, median, quartiles, percentages, and standard deviation were calculated. It should be noted that both the mean and median for patients and controls do not exceed the reference values in any of the parameters analyzed.

Discussion

Despite its low incidence, LCPD represents a worldwide health problem, so its study and understanding are crucial (7). Unfortunately, it has not received the attention it needs in Latin American countries. LCPD research presents different challenges because its etiology is unknown. It is characterized by unilateral or bilateral idiopathic osteonecrosis of the proximal femoral epiphysis, in which the vascular system is highly compromised. It has been proposed that its etiology involves successive vascular occlusions, in which hypercoagulable states would play a central role. The proposed mechanism is that microthrombi would lodge in the vessels that nourish the femur, blocking the supply of blood, which would cause the characteristic necrosis of LCPD [15, 16]. Results in multiple studies have supported this theory; however, the lack of reproducibility in these results has given rise to controversies as to whether hemostasis is involved in the development and suffering of LCPD (12,17–20).

Hemostasis entails different physiological mechanisms involving the von Willebrand Factor (VWF) and CF, like vasoconstriction, cells that express tissue factor, and platelet aggregation. The PT measures the time required for coagulation to occur after the addition of tissue factor to citrated plasma. In this way, the formation of the clot is simulated, and its formation time can be measured (21). The normal range of the PT is between 0-13.7 seconds. Values above 13.7 seconds are related to hemorrhagic pathologies, while values close to 0 are linked to prothrombotic states. The results obtained in this work show that less time is required for the formation of the clot by the patient population, evidencing greater activity in hemostasis.

Alterations in coagulation factors have been described in different populations of patients with LCPD, including factor V, which functions as a cofactor that allows factor X to activate thrombin and continue with fibrin production (Fig. 3). Factor V Leiden is a mutation that is inherited in an autosomal and dominant manner that presents incomplete dominance and results in a variant of factor V that cannot be so easily inactivated by protein C (9). Since this mutation has been found in patients with LCPD in different studies, it could be involved in the disease (22–25). However, other studies have not found this mutation or alterations in factor V in patients with LCPD (26,27), thus raising the question of whether factor V is actually involved in the etiology of LCPD.

The main function of factor VIIIa, together with factor IXa and phospholipids in the presence of Ca^{+2} , consists of activating factor X, thus participating as a complex in clot formation (Fig. 3). This protein has been described with high values in patients with LCPD (28).

In this study, an increase in prothrombotic activity was observed in patients, and it was expressed as a significant increase in the activity of FV, FVIII, and FIX factors (Table 2 and Fig. 2). As mentioned, factor IX and FVIII participate in the formation of the clot by activating factor X, and the overactivity of this protease has been linked to venous thrombosis (29). It is important to note that this is the first time FIX is associated with LCPD, a topic which we suggest studying further. According to the International Federation of Clinical Chemistry (IFCC) and the Institute of Laboratory and Clinical Standards (CLSI),

each laboratory should establish its own RVs. In this case, we determined proposed reference values for children (PRVC); And it should be noted, that the FV, FVIII, and FIX are also significant increase, outperforming the PRVCs, which are listed below: Factor V (39–115%), Factor VIII (44–82%) and Factor IX (73–94%).

Hcy is an intermediate product of methionine and cysteine metabolism. In plasma, approximately 1% circulates as free, 70 to 80% bound to proteins, and 20 to 30% as Hcy dimers. Plasma free Hcy values are 5.0 to 15.0 $\mu\text{mol/L}$ in adults (30). In children: 5.5–13.4 $\mu\text{mol/L}$ for men, and 4.9–11.9 $\mu\text{mol/L}$ for women (31). Hcy and especially hyperhomocysteinemia (HHcy) have significant effects on bone remodeling, blood flow, and hemostasis (Fig. 3), systems that are altered in LCPD. In addition, HHcy has been linked to osteoporosis and ischemic heart disease, among others. Hcy at a concentration of 10 $\mu\text{mol/L}$ or greater is considered a risk factor in the development of cardiovascular diseases and ischemic heart disease (32–36). In this study, several patients exceeded that value. Some studies stating those high values of Hcy are present in patients with LCPD (27,37).

Conclusion

Our results show an increase in the activity of factors V, VIII, and, for the first time, an increase in the activity of factor IX in LCPD patients, which are related to greater activity in hemostasis. On the other hand, we also found high concentrations of Hcy, related to LCPD, thrombosis, and multiple bone diseases. It is likely that this has a significant part in the etiology of LCPD, affecting the bone, vascular system, or hemostasis by various mechanisms. The increase in the activity of some CF and the concentration of Hcy supports the theory that hemostatic alterations such as thrombophilia may play a central role in the etiology of LCPD (Fig. 3).

Abbreviations

LCPD

Legg-Calvé-Perthes disease.

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VWF

Von Willebrand factor.

Hcy

Homocysteine.

NOM

Norma Oficial Mexicana.

BMI

Body mass index.

TT

Thrombin Time.

aPTT
activated Partial Thromboplastin Time.
PT
Prothrombin Time.
CF
Coagulation factors.
PLTs
platelets.
MTHFR
methylenetetrahydrofolate reductase

Declarations

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Contributions

EHZ, AORO, and ERM conceived and designed the experiments. ERC, CZH, AORO and EHZ collected blood samples and clinical data. ERC and AORO performed the experiments. EHZ, AORO, and ERM wrote the paper. All authors read and approved the final manuscript.

Ethics declarations

All participants received oral and written information about the study and signed a letter of consent. The study protocol was reviewed and approved by the INR-LGII Research and Ethics Committees.

Consent for publication

All authors consent to publish.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All relevant data used in this study have been included in the manuscript. The corresponding author can be contacted if any further information is needed.

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Figures

Figure 1

Graphs showing the distribution of A) sex, B) affected limb, and C) blood type in the group of patients. There were no cases of blood group AB.

Figure 2

Comparison of patients (n = 25) versus controls (n = 50). Tests: A) Prothrombin time; B) Factor V; C) Factor VIII; D) Factor IX; E) Homocysteine.

Mustache graphs showing the median as well as the 10-90 percentile and out-of-range values. (•, •, ■, ■) Outliers are represented with circles outside the mentioned percentiles. The p-value comparing both groups (patients and controls) is also included. Stressed with an asterisk (*) are samples whose comparisons presented a significant difference during the comparative analysis when applying a Mann-Whitney U test or a Student's t-test, depending on the case. (* $P \leq 0.05$).

Activity (%) = percentage of activity, $\mu\text{mol} / \text{L}$ = micromoles per liter.

Figure 3

The top represents the evolution of femoral head deformity following ischemic necrosis in LCPD. On the bottom is the cell-based model of coagulation and fibrin formation. In this model, thrombin generation occurs in three phases: initiation, amplification, and propagation. In this model, the increase of Hcy and of the factors V, VIII, and IX are hemostatic alterations that favor hypercoagulable states (thrombophilia) that may play a central role in the etiology of LCPD.

Hcy: Homocysteine. Coagulation factors: II, V, VII, VIII, IX y X. VWF: von Willebrand factor. PT: Prothrombin time.