PLASMA LEVEL OF PROTEIN C, FIBRINOGEN CONCENTRATION, AND PLATELET NUMBER IN DOGS WITH LEGG-CALVE-PERTHES DISEASE AND OSTEOCHONDROSIS. PRELIMINARY STUDIES

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Abstract

The possible role of coagulatory disorders in pathogenesis of Legg-Calve-Perthes disease (LCPD) and osteochondrosis (OC) was examined. A decrease in protein C level in dogs with LCPD (94.31±4.74%) in comparison with healthy dogs (95.8±6.35%) was observed. Moreover, in OC affected animals, the value varied between 92.25±2.5% and 94.33±5.5%. The mean plasma fibrinogen level in control group was 2.69±0.65 mg/mL, whereas in OC groups significantly higher values were found. Platelets number varied between individuals but was within normal range in all groups. Taking into account a decrease in protein C plasma level and an increase in fibrinogen concentration, the relationship between developmental diseases and coagulation disorders was revealed in dogs.

Key words: dog, developmental bone diseases, coagulation.

Developmental bone diseases including Legg-Calve-Perthes disease (LCPD) and osteochondrosis (OC) are serious problems in veterinary practice. Both of them lead to chondroidal deformation and locomotory dysfunction in many breeds of dogs (3, 13).

LCPD, which is defined as avascular necrosis of femoral head is a developmental bone disorder that occurs in immature small dogs (17). Because of changes, which occur in articular cartilage, the disease has been described as the form of osteochondrosis (2). The cause of LCPD remains unknown, but several possible factors may be taken into account including: heredity, infection, hormonal imbalance, trauma, and coagulation abnormalities leading to the formation of infarction and obstruction of the venous drainage of the femoral head and neck. In human medicine, LCPD is defined as idiopathic avascular necrosis of the femoral capital epiphysis in childhood, which may lead to femoral head deformity and arthritis. According to the thrombophilia theory, it was proposed that thrombophilic disorders and hypercoaguable conditions such as protein C deficiency play an important role in pathogenesis of LCPD (5, 15).

Another developmental bone disease – OC includes osteochondrosis dissecans (OCD), fragmented coronoid process (FCP), and ununited acorneal process (UAP) (13). Although the main pathological process during OC is a disturbance of endochondral ossification, the cause of this status is still not clear. It was determined that conditions such as: rapid growth, hereditary, hormonal balance, trauma, overnutrition with high intake of calcium during growth are important aetiologic factors of OC in dogs, but except that there are some other unknown factors, including possible disorders of coagulation (13).

In both described disorders, possible markers could be protein C and fibrinogen concentrations. Protein C is an anticoagulant and a fibrinolytic agent in the blood-clotting cascade (11). Fibrinogen, in turn, as the acute phase protein is essential for blood coagulation and plays three roles in haemostasis: as a precursor to fibrin it acts to seal blood vessels and restrain haemorrhage, supplies a component of the fibrin structure, and controls the blood viscosity. The elevated fibrinogen concentration occurs in many medical conditions such as cardiovascular disease, arthritis, burn, surgery, trauma, inflammatory disorders, emotional stress, and infection (7, 12). It was suggested that platelets could take part in pathogenesis of LCPD in humans, whereas in dogs this problem was not highlighted (6, 16).
The recognition of the mechanisms involved in these disorders tends to increase efficiency of the diagnosis and therapy (8). In our study protein C level in plasma, fibrinogen concentration, and number of platelets were taken into account since they might play a role in pathogenesis of ossification disturbance (15) as in the case of human medicine, and could constitute possible markers of LCPD and OC in dogs.

**Material and Methods**

**Collection of blood samples.** Blood samples were collected from 16 dogs with LCPD, four dogs with OCD, four dogs with FCP, and three dogs with UAP. The dogs with LCPD have presented typical signs of the disease: lameness in one rear leg, pain in the hip joint, muscle atrophy, and restricted joint movement. Diagnosis was confirmed by radiographic examination of the hip joint. The dogs with OCD, FCP, and UAP (assembled all together in OC group) were diagnosed based on physical examination (lameness, muscle atrophy, pain during manipulation of the diseased joint) and radiographic examination. The control group comprised twenty healthy dogs. According to clinical data analysis, all dogs were unrelated and familial effect was excluded. Blood was drawn from the saphenous vein and collected in tubes containing 3.8% sodium citrate. Then haematological tests were done together with estimation of platelet number using the haematological analyser (MEDONIC CA 530). Remaining blood samples were centrifuged at 2,000 x g for 10 min at 4°C, and then plasma was stored at –70°C for further analysis of protein C level, and at –20°C for assay of fibrinogen concentration.

**Coagulation measurements.** To obtain a canine reference value of protein C level, the concentration of this protein was measured in plasma samples of 20 healthy young dogs of different breeds and sex. Plasma samples were pooled and used for standard dilution curve. The optical density of the undiluted pooled plasma was defined as 100%. The activity of protein C was measured using a colorimetric assay Asserachrom Protein C (Diagnostica Stago) at 492 nm wavelength. The optical density was read against the dilution curve with the unit of measurement estimated as the percentage of normal value.

Fibrinogen level was estimated by the heat precipitation method (4).

**Statistical analysis.** Statistical analyses were done using STATISTICA 5.0 (StatSoft, Poland) software package. All samples were run in duplicates. The statistical significance of differences between the mean values of the groups was compared using Student’s t-test. Values different to control at P<0.05 were considered as statistically significant. The relations between sex or age and protein C level, fibrinogen concentration, and platelet number were evaluated using regression coefficient.

**Results**

Among 16 dogs with LCPD, nine (56.25%) dogs were male and seven (43.75%) were female. All dogs represented the breeds predisposed (chihuahua, miniature pinscher, Pekinese, Yorkshire terrier, poodle), and small mongrels. The mean age of the dogs was 14 ±2 months.

The OCD group comprised St Bernard, Shar Pei, German shepherd, FCP group - German shepherds and mongrels, and UAP group - German shepherds. In OC group (including OCD, FCP, and UAP), there were five (45.45%) males and six (54.55%) females. Mean age of the dog was 6 ±1 months.

The examined haematological values were in normal range in all studied groups of dogs. Platelets number varied between individuals but was within normal range (from 150,000 to 400,000/ml) in all groups.

The range for protein C in control group was determined to be 87%–109% (mean 95.8 ±6.35%) of normal. In all studied groups with developmental disorders, a decrease in protein C level was observed (Fig. 1). Sixteen dogs with LCPD had levels of protein C ranging from 87% to 104% (mean 94.31 ±4.74%). Dogs in OCD group had protein C level between 91% and 96% (mean 92.25 ±2.5%). German shepherds with FCP had protein C level of 86%-100% (mean 93.75 ±7.3%), and in dogs with UAP protein C level ranged from 89% to 100% (mean 94.33 ±5.5%). The obtained values were not related to sex and age in all studied groups of animals.

The mean plasma fibrinogen level in the control group was 2.69 ±0.65 (2.4) mg/mL and in LCPD group - 3.25±2.22 (1.2-9.6) mg/mL. Within the group of dogs with OC, in OCD dogs, the estimated values were 7.05 ±3.07 (4.1-10) mg/mL, but without statistical significance, whereas in groups with FCP and UAP, significantly higher values of 4.6 ±0.65 (4-5.6) and 5.2 ±1.05 (4-6), respectively, (P<0.05 in comparison with control group) were observed (Fig. 2). No correlation between fibrinogen level and sex or age was observed in the dogs with developmental disorders.

**Discussion**

Protein C is a precursor of plasma vitamin K-dependent serine protease, which regulates blood-clotting cascade through proteolytic inactivation of the Va and VIIIa factors and therefore plays an important role as anticoagulant and fibrinolytic agent in the blood-clotting cascade (11). Over the last twenty years, many studies on the relationship between protein C levels and thrombophilia and LCPD in humans were performed. Some of them showed associations of LCPD with protein C deficiency (5); other did not confirm an aetiological role of protein C in the disease (10).
Low protein C level in humans is considered as a common cause of thrombophilia, which was proposed as the one of the possible cause of LCPD (5). Studies of Breing et al. (1) on a group of 18 dogs with LCPD demonstrated the normal activity of protein C with some statistically insignificant changes in comparison with healthy animals. There are no studies concerning the role of protein C level in development of OC in dogs. In our study we observed a decrease in protein C level in LCPD, OCD, FCP, and UAP dogs in comparison with the healthy dogs, but without statistically significance.

There are some discrepancies about the role of platelets in pathogenesis of LCPD. In human medicine Glueck et al. (6) found that in patients with LCPD, low level of protein C and inherited thrombophilia were diagnosed. According to Yilmaz et al. (16) in children, hereditary thrombophilia is one of the risk factors in LCPD. On the contrary, Seguin et al. (14) stated that osteonecrosis is not associated with thrombophilia. There are no suitable reports about platelet alterations in dogs with LCPD and OC. Our studies revealed that platelet number was not altered in dogs with LCPD and OC in all studied groups.
Elevated plasma fibrinogen is considered as a risk factor in many disorders including stroke and coronary and deep-vein thrombosis (7). The increased level of fibrinogen cleavage products could cause ICAM-1 upregulation on endothelial cells providing to leukocyte accumulation and extravasation. Fibrinogen cleavage by thrombin, neutrophil elastase, or plasmin releases potent chemotactic fragments, capable of mobilising leukocytes and fibroblasts to site of injury, thus potentiating the inflammatory response (9). Moreover, fibrinogen, as a positive acute phase protein and moderate indicator in dogs (2-10 fold increase), elevated during inflammation process in arthritis (7, 12).

Our study revealed increased fibrinogen level in all studied groups in comparison with control, and statistical significance was noted in FCP and UAP groups (P<0.05).

Taking into account a decrease in protein C plasma level and an increase in fibrinogen plasma level, (especially in OC group) our studies revealed relation between developmental diseases and coagulation system in dogs. In the future, other coagulatory factors should be investigated as possible markers of LCD and OC in dogs and the studies should be conducted on a greater population of dogs.

References