Evaluation of Protein Biomarkers in Serum and Urine For Canine Hip Dysplasia in Adult Dogs

Cruz CN, Ahner CE, Stoker AM, Cook JL

Comparative Orthopaedic Laboratory, University of Missouri, Columbia, Missouri

www.columc.missouri.edu

Veterinary Research Scholars Program
University of Missouri

Introduction

Canine hip dysplasia (CHD) is a multifactorial trait that is one of the most common developmental orthopedic disorders in medium and large-breed dogs worldwide. According to the Orthopedic Foundation of Animals (OFA), prevalence of CHD in the United States is as high as 72% of medium-large breed dogs. CHD is characterized by the laxity of the coxofemoral joint with progression to secondary osteoarthritis (OA) causing pain, stiffness and lameness. Current diagnosis is typically subjective radiographic hip evaluation at the irreversible stage of the disorder. However, radiographic selection for good hips has led to only a modest reduction in CHD prevalence. Thus, there is a need for an improved method of diagnosis for canine hip dysplasia..

Objective: To determine which protein biomarkers have the highest discriminatory capabilities to differentiate dysplastic adult dogs from dogs without hip dysplasia.

Hypothesis: A panel of protein biomarkers in serum and/or urine will have high discriminatory capability for differentiating between adult dogs with dysplastic hips from adult dogs with normal hips.

Methods

Animals

Informed client consent was obtained for all adult dogs, greater than 2 years old, recruited across the United States. Seventy-two enrolled dogs were radiographically evaluated. The dogs were divided into two groups based on the OFA grading criteria, dogs with 'excellent hip' grade (n=20) and 'good hip' grade (n=28) were placed in the normal hips cohort, while dogs with 'mild', 'moderate', or 'severe' hip grades (n=24) were placed in the dysplastic cohort. A one time blood and urine collection was obtained from each dog on the same day, then shipped to our laboratory overnight on ice. For processing, 5-10 mL of coagulated blood samples were centrifuged at 20,000 rpm for 20 min to obtain the serum. The serum and urine were collected, transferred to separate Eppendorf tubes, and then stored in -80˚ C until they were used for the assays. All dogs used in this study are client-owned and were approved by ACUC protocol no. 7471.

Biomarker Assays

Cartilage oligomeric matrix protein (COMP), cross linked C-telopeptide of type I,II collagen (CTX-I, CTX-II), nuclear factor κ B-ligand (RANK-L), tissue inhibitor of metalloproteinase 1 (TIMP-1), N-terminal propeptide of procollagen type I (PINP) (Neoscientific, Cambridge, MA), collagenase-generated cleavage epitope of type II collagen (C2C) and C-propeptide of type II procollagen (PIICP) (MyBioSource Inc., San Diego, CA) concentrations in serum and urine were measured using commercially available enzyme-linked immunosorbent assays (ELISA) according to the manufacturer's instructions.

Multiplex human immunoassays were also used to analyze matrix metalloproteinase-1, 2, 3 and 9 (MMP-1, MMP-2, MMP-3, MMP-9) (R&D Systems, Minneapolis, MN) in serum and urine. This MMP assay has been previously validated in our laboratory to cross-react with samples of canine origin.

The urine creatinine concentration was measured with a creatinine colorimetric assay (Cayman Chemical Company, Ann Arbor, MI), and was used to standardize the urinary concentrations obtained for the other assays.

Statistical Analysis

Comparisons between CHD-affected dogs and excellent-hip and/or good-hip individuals were performed with a 1-way ANOVA and unpaired *t*-tests or Mann-Whitney rank-sum tests if the data failed normality testing. All statistical analyses were conducted using a commercially available computer software with significance set at $p < 0.05$ (SigmaPlot 12.3; Systat Software, Inc., San Jose, CA).

Results

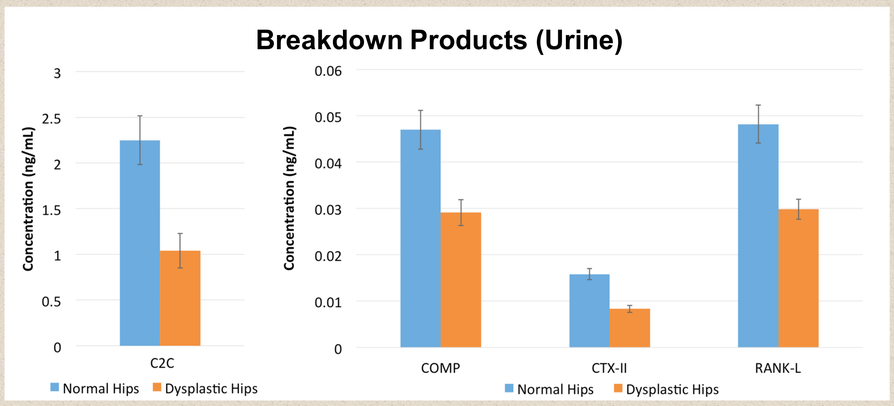

Figure 1. Mean±SE of urine protein concentration (ng/mL/creatinine) for collagenase-generated cleavage epitope of type II collagen (C2C) (*p=0.013*), cartilage oligomeric matrix protein (COMP) (*p=0.026*), cross linked C-telopeptide of type II collagen (CTX-II) (*p<0.001*), and nuclear factor κ B-ligand (RANK-L) (*p=0.005*) for normal-hip grade dogs (n=46) and dysplastic dogs (n=22).

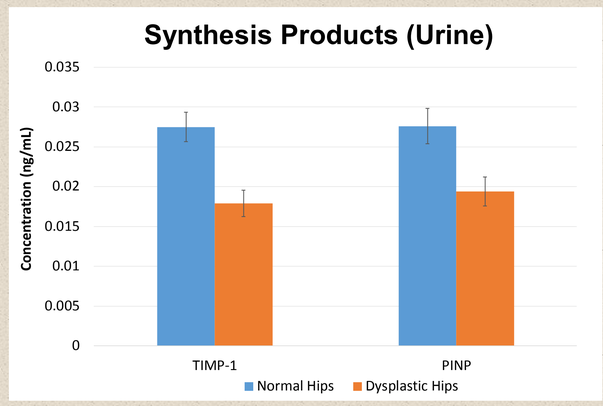

Figure 2. Mean±SE of urine protein concentration (ng/mL/creatinine) for tissue inhibitor of metalloproteinase 1 (TIMP-1) (*p=0.002*) and N-terminal propeptide of procollagen type I (PINP) (*p=0.011*) for normal-hip grade dogs (n=46) and hip dysplastic dogs (n=22).

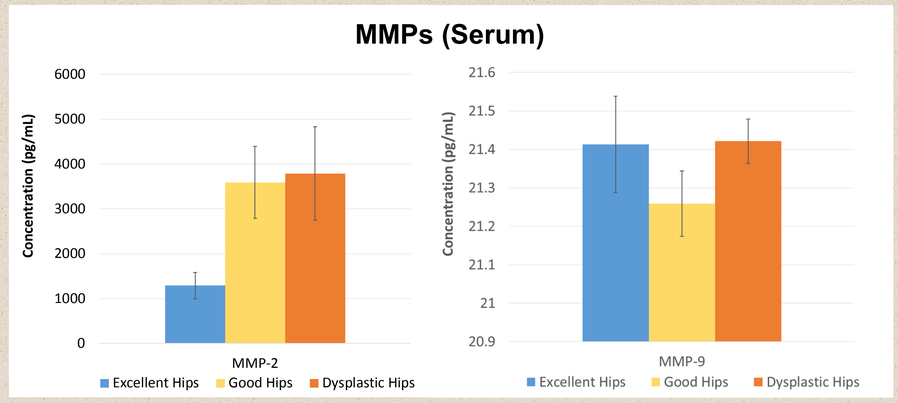

Figure 3. Mean±SE serum concentration (pg/ml) for matrix metalloproteinase-2 (MMP-2) and MMP-9 for excellent-hip status dogs (*n=20*), good-hip grade dogs (n=28) and hip dysplastic dogs (n=24). The MMP-2 concentration in the excellent hips group was statistically different than the concentration in the dysplastic hips cohort (*p=0.032*) and the good-hip dogs (*p=0.030*). MMP-9 serum levels between good hips and dysplastic hips were also significantly different (*p*=0.032), but not between excellent hips and dysplastic hips (*p*=0.328).

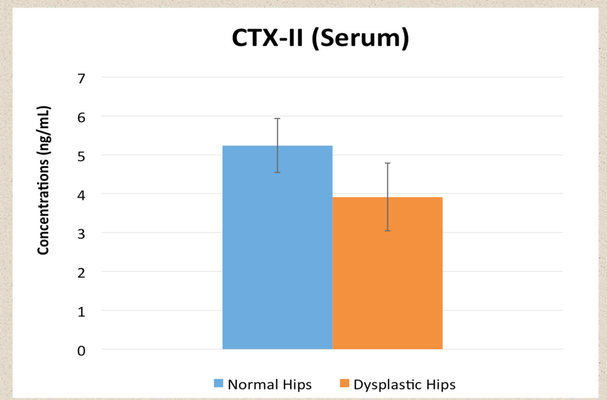

Figure 4. Mean±SE serum concentration (ng/ml) for cross linked C-telopeptide of type II (CTX-II) (*p=0.037*) collagen for normal-hip grade dogs (n=46) and hip dysplastic dogs (n=22).

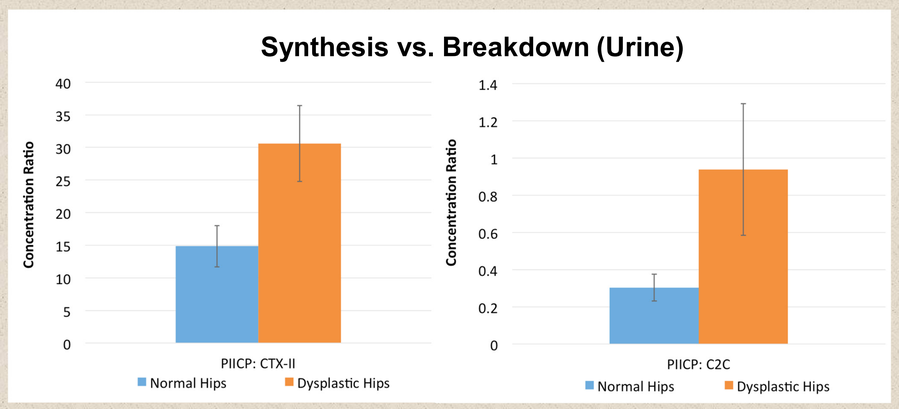

Figure 5. Mean±SE ratio of the urine concentration (ng/mL) of collagen synthesis (PIICP) to two collagen breakdown productions CTX-II (*p=0.002*) and C2C (*p=0.036*) for normal-hip grade dogs (n=46) and dysplastic hip dogs (n=22).

Discussion

The present study illustrates that dogs with CHD are associated with a significant decrease in urine concentrations of the breakdown products (C2C, COMP, CTX-II), intercellular signaling (RANK-L), bone synthesis (PINP), and proteinase inhibition (TIMP-1) compared to dogs with normal hips. Further, dysplastic dogs had a significantly altered ratio of the urinary concentrations of synthesis and breakdown products compared to dogs with normal hips. CTX-II serum concentrations also decreased in CHD-affected dogs, similar to the CTX-II trend in urine.

These biomarkers are primarily related to bone and cartilage turnover which shows a significantly different biological process in dysplastic dogs compared to dogs with normal hips. These data indicates that measurement of urinary biomarkers associated with cartilage and bone turnover could potentially be used to differentiate between dogs with normal and dysplastic hips clinically. Further study is required to determine if these biomarkers can be used to detect CHD prior to clinical presentation.

Significance

- The combination of urine biomarkers C2C, COMP, CTX-II, RANK-L, PINP, and TIMP-1 shows strong potential to discriminate between dogs with normal hips and dogs with dysplastic hips.
- These data provides a basis for establishing reference ranges for these biomarkers in normal and dysplastic dogs.
- The current study provides pilot data for future studies in the early diagnosis of hip dysplasia in puppies.

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