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Short Communication

Erythrocyte Sedimentation Rates and Leukogram Changes in Canine Model of Osteoarthritis

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Summary: Inflammatory markers such as erythrocyte sedimentation rates (ESR) have been evaluated in humans with osteoarthritis (OA). However, there has been no record of evaluation of ESR during OA in dogs. Changes in erythrocyte sedimentation rates (ESR) and leukogram functions were evaluated following experimental knee osteoarthritis (OA).Ten dogs of both sexes with (mean weight = 12.4 ± 1.8kg) were used. Experimental OA was induced in the right knee, using the groove model and confirmed radiographically using evidence of joint space narrowing and presence of osteophytes. Gait was assessed subjectively and scores (GAS) were assigned. Blood was obtained fortnightly for the determination of ESR, total white blood cell (tWBC), neutrophil and lymphocyte counts, while knee radiographs were obtained fortnightly for twelve weeks. Radiographic scores (RAS), GAS, ESR and leukocyte parameters between the different time points were compared with ANOVA. Correlation between parameters was evaluated using Pearson's correlation. A "P" value less than 0.05 was considered significant. Both ESR and neutrophil/lymphocyte (N/L) ratio increased from week 0 to week 12 of OA. However, tWBC, neutrophil and lymphocyte counts did not differ significantly. Both GAS and RAS increased up to week 4 and 6 of OA respectively. Erythrocyte sedimentation rates was significantly (p= 0.033) and positively correlated (r=0.793) with N/L ratio, but negatively and slightly correlated (r= -0.843) with GAS. There was no significant correlation between ESR and RAS. It was concluded that both ESR and N/L ratio might be useful in monitoring progression of OA in dogs.

Keywords*:* Canine, Osteoarthritis, ESR, Neutrophils.

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INTRODUCTION

Osteoarthritis (OA) is a degenerative joint disease characterized by damage to the articular cartilage and changes in the subchondral bone (Lepine and Hayek 2001).It is frequently accompanied by inflammation of the synovium (Marijnissen *et al.* 2002). Osteoarthritis is more frequent in dogs than in cats. The first change in OA is probably biomechanical stress that feeds back onto the cartilage surfaces and later subchondral bone (Marijnissen *et al.* 2002). This leads to biochemical changes in the joint tissues. Once an injury occurs in the joint; there is an attempt to repair the injured part. This repair may be an inflammatory response with mononuclear cellular infiltrate or a fibroblastic response with the formation of fibrocartilage (Solomon 1997). Other definitive changes such as subchondral bone sclerosis, osteophytic proliferation and cartilage loss occur later in the course of the disease (Frost- Christensen *et al.* 2008).

Clinical parameters of OA such as pain, stiffness and functional ability can be measured relatively simple. However, evaluation of structural changes demands much greater effort. Imaging technique and biomarker analyses are done to obtain information on structural changes in OA joints (Frost-Christensen et al. 2008). The ideal molecular marker should not only relate to the nature of the disease, but also the stage of degradation either directly or in proportion to the degeneration (Rorvik and Grondahl 1995). Inflammatory markers such as ESR have been evaluated in humans with osteoarthritis and have been shown to be mildly elevated in osteoarthritic patients (Dieppe and Lim 1998), however it has not been evaluated in dogs. Most inflammatory OA markers evaluated in dogs such as C- reactive proteins and interleukins are relatively expensive. Till now, there appears to be no simple biochemical test that can be used for the confirmation and /or monitoring the progression and severity of osteoarthritis in dogs. The aim of this study therefore was to evaluate the changes in ESR and leukogram during experimental knee osteoarthritis in dogs, and to determine if these parameters can be used as a biomarker for monitoring the progression of osteoarthritis.

MATERIALS AND METHODS

Ten adult local dogs of both sexes with mean weight age ranging from 1-3 years and mean weight of 12.4 ± 1.8 years were used. They were adjudged to be free of any musculoskeletal disease based on the visual assessment of gait and radiographic evaluation of the joints. The dogs were housed individually in concrete -floored kennels and were fed once daily on cooked rice supplemented with sufficient amount of fish and palm oil, while water was provided *ad-libitum*. Ethical approval for this study was obtained from the Research Ethics Committee, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Ogun State.

Dogs were premedicated with intramuscular injections of 0.04mg/kg Atropine (Amopin®, Yanzhou Pharmaceuticals, China) and 1mg/kg Xylazine (XYL-M2®, V.M.D, Germany). Fifteen minutes later, anaesthesia was induced with intravenous injection of 0.5mg/kg Diazepam (Calmpose® Ranbaxy, India) and 15mg/kg Ketamine (Ketamin hydrochloride USP®, Rotex Medica, Germany). Following induction of anaesthesia, the right knee was prepared aseptically. Experimental OA was induced as described by Frost-Christensen et al. 2006.Thereafter, the incision was then closed in three layers. The dogs were allowed to recuperate for two weeks. During this period, the dogs were treated with Penicillin-Streptomycin (PenStrep®, Kepro, Holland) and pain was controlled using a combination of Dipyrone and Sodium salicylate (Febralgina®, Agrovet, Peru) administered for three days.

The dogs were assessed radiographically, two weeks after arthrotomy of the right knee for confirmation of OA and then fortnightly, for twelve weeks. Similarly the gaits of the dog were assessed subjectively every two weeks up to twelve weeks of OA. In addition, 5 ml of blood was obtained from the cephalic vein before knee arthrotomy, immediately after radiographic confirmation of OA and fortnightly up to twelve weeks, for the determination of ESR, tWBC, and leukocyte differentials. The ESR was determined using the Wintrobe technique (Briend- Marchal et al. 2003), while the tWBC and the leukocyte differentials were determined with automated blood analyzer. Neutrophil lymphocyte ratio was taken as the ratio of the absolute counts of neutrophils and lymphocytes.

Statistical Analysis

Data were expressed as mean ± standard deviation. Gait assessment scores (GAS) and radiographic scores (RAS) were compared at six and twelve weeks using Wilcoxon sign rank test, while ESR and leukocyte parameters were compared between different time points with ANOVA. Correlation between parameters was evaluated using Pearson's correlation. A "P" value less than 0.05 was considered significant.

RESULTS

Radiographic signs of OA were first observed four weeks following knee arthrotomy and were characterized by joint space narrowing and presence of osteophytes. The radiographic scores of the dog increased progressively up to week 6 of experimental OA (Fig. 1a). However, there was no significant difference in the radiographic scores of the dog between week 6 and week 12 of OA. The gait assessment scores also increased progressively up to week 4 of experimental knee OA (Fig. 1b). Similarly, the gait assessment did not differ significantly between week 6 and week 12 of OA.

The mean ESR of the dogs increased from week 4 up to week 12 of experimental knee OA (Fig. 2a). The WBC decreased following knee arthrotomy in the dogs and up to ten weeks of experimental knee OA (Fig. 2b). However, the absolute neutrophil counts (ANC) increased gradually up to twelve week following experimental knee OA. The lymphocyte counts increased gradually up to four weeks after experimental knee OA, and then decreased up to ten weeks after experimental OA. The N/L ratio of the

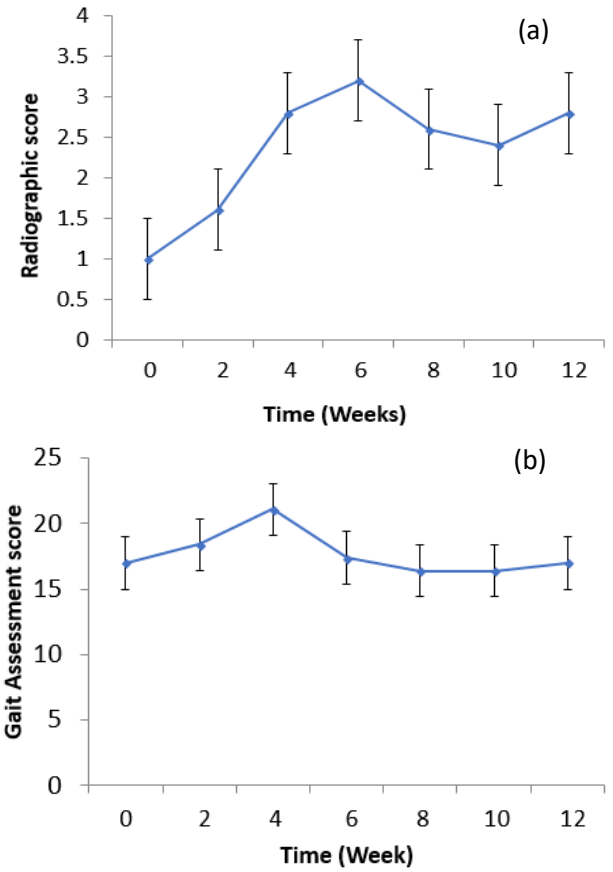

Fig. 1: (a) Radiographic scores and (b) Gait assessment scores of dogs immediately after confirmation of osteoarthritis (Week 0) and at two weeks interval up to a period of twelve weeks.

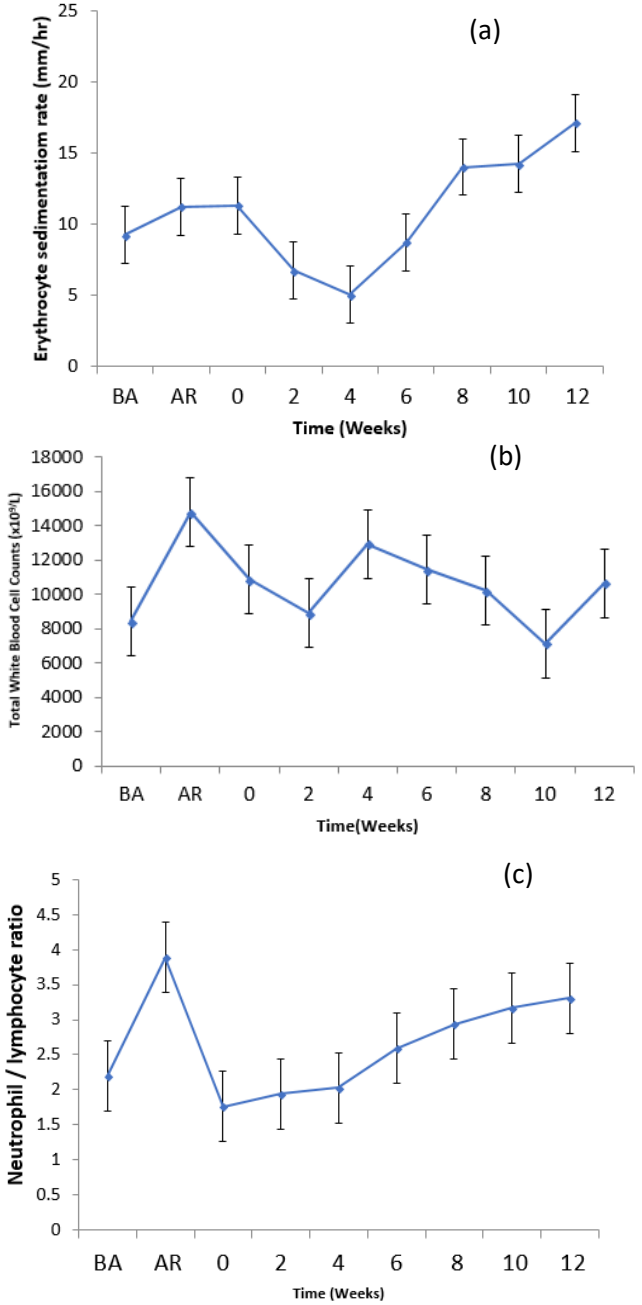

Fig. 2: ESR (a), WBC (b) and N/L ratio (c) of dogs before arthrotomy (BA), immediately after arthrotomy (AR), immediately after confirmation of osteoarthritis (Week 0) and at two weeks interval up to a period of twelve weeks.

dogs also increased from week 0 of experimental OA up to week 12 of osteoarthritis (Fig. 2c). The ESR was positively correlated to the neutrophil-lymphocyte ratio and negatively correlated to the gait assessment. The radiographic scores did not correlate with any of the measured parameters. Gait assessment scores and radiographic score were positively correlated but not significantly.

DISCUSSION

The results from this study showed that both the erythrocyte sedimentation rates (ESR) and neutrophil-lymphocyte (N/L) ratio increased progressively from the onset of osteoarthritis up to twelve weeks of osteoarthritis in an experimental model of knee osteoarthritis in dogs. In addition, there was a positive correlation between ESR and N/L ratio following experimental knee osteoarthritis in dogs. However, these parameters were negatively correlated to both gait assessment score (GAS) and radiographic scores (RAS) following OA in the dogs.

Involvement of phagocytic leukocytes during inflammatory response is an important aspect of the natural immune response (Hughes et al. 2010). Changes in leukocytes and endothelial markers are indicative of increased inflammatory reactions. It has been reported that the groove model of OA is characterized by minor inflammation and that the associated synovial inflammation tended to decrease from week 20 up to week 40 of OA (Marijnissen et al. 2002; Frost-Christensen et al. 2008). The progressive increase in the L/N ratio in this study up to week 12 of OA might be an indication of the progressive synovial inflammation secondary to the experimental OA.

ESR is probably the most widely used laboratory marker of the activity of joint diseases. A rise in ESR is one of the main hallmarks of inflammatory and non – inflammatory arthropathies (Punzi et al. 2005). In this study, ESR significantly increased following arthrotomy and then gradually declined up to week 0 of OA before it then progressively and steadily increased up to week 12 of OA. This changes were however, not significant. This finding further confirms that following the surgically induced inflammation which subsided thereafter, there was a mild component of synovial inflammation which accompanies the groove model of OA as earlier described (Frost-Christensen et al. 2008).

Although both ESR and N/L ratio progressively increased during the twelve week of OA, the lack of significant correlation between this parameters and radiographic and gait assessment scores makes it difficult to conclude that they are reliable indicators of OA in dogs. The lack of significant correlation further supports the previous findings that there is no association between the severity of OA and radiographic findings. This further confirms that OA is more than just damage to the cartilage and that complex mechanisms are involved in the response of cartilage to trauma during OA. In conclusion, although both ESR and N/L ratio may be useful to monitor the progression of knee OA in dogs, the lack of significant increase in their values would suggest further evaluation of their role as markers of inflammation in patients with OA.

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