

CHANGES IN BLOOD LEUKOCYTES, ERYTHROCYTE SEDIMENTATION RATE AND INTERLEUKIN-6 FOLLOWING KNEE ARTHROTOMY IN DOGS

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Summary

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Changes in leukocyte parameters, erythrocyte sedimentation rate (ESR) and interleukin-6 (IL-6) were determined in ten mongrel dogs following knee arthrotomy. About 5 mL of blood was collected from each dog before knee arthrotomy, 2 hours, 24 hours and 3 days after knee arthrotomy for determination of white blood cell counts (WBC), neutrophil counts (NEUT), lymphocyte counts (LYM), neutrophil-lymphocyte ratio (N/L), ESR and IL-6. The data were expressed as mean \pm standard deviation and differences compared using analysis of variance (ANOVA) at level of significance $P < 0.05$. The results of this study showed that following knee arthrotomy, WBC, NEUT and ESR were significantly ($P < 0.05$) increased 24 hours after surgery, while there were no significant change in LYM and N/L. Increase in ESR persisted up to three days following arthrotomy. In addition, IL-6 was significantly ($P < 0.05$) increased two hours after knee arthrotomy up to 24 hours after arthrotomy and then decreased significantly by the 3rd day. It was concluded that acute inflammation following knee surgery in dogs induced changes in leukocyte parameters, ESR and IL-6. These changes can be used to monitor prognosis following knee surgery in dogs.

Key words: arthrotomy, dog, erythrocyte sedimentation rate, interleukin, leukocyte, surgery

INTRODUCTION

Inflammation is a complex biological response of cells and tissues to noxious stimuli, such as pathogens, trauma or irritants (Gabay, 2006). The cardinal symptoms are all linked to changes in microcirculation. These microcirculatory responses are characterised by perfusion alterations, oedema formation and propagation of cell to cell interactions mostly manifested in activation and tissue invasion by polymorphonuclear leukocytes (Arndt *et*

al., 1995; Xing *et al.*, 1998). Changes in leukocytes and endothelial markers are indicative of increased inflammatory reactions. For instance, changes in the concentration of circulating neutrophils and lymphocytes have been reported to occur during surgical operations such as abdominal and orthopaedic procedures (Wilk, 2001).

Levels of acute-phase reactant proteins are elevated in response to stress or inflammatory states such as infection, in-

jury, surgery, trauma and other causes of tissue necrosis (Cha *et al.*, 2009). These proteins include alpha-1-antitrypsin, alpha-1-acid glycoprotein, haptoglobin, ceruloplasmin, complement protein, fibrinogen, C-reactive protein, and immunoglobulins (McPherson, 2007).

The erythrocyte sedimentation rate (ESR) is an indirect measurement of fibrinogen and is widely used in screening or monitoring patient with acute or chronic inflammatory diseases (Alao, 2010). ESR is probably the most popular laboratory marker of the activity of joint diseases in humans. A rise in ESR is one of the main hallmarks of inflammatory and non-inflammatory arthropathies (Punzi *et al.*, 2005). It has been proposed that ESR could also have an important prognostic importance in post surgical infections such as those associated with orthopaedic prosthesis, osteomyelitis and bacterial sepsis (Alao, 2010; Hughes *et al.*, 2010). However, its role in inflammation has not been properly investigated in dogs.

Inflammation is an important factor in the pathogenesis and clinical presentation of joint diseases in both animals and humans (de Graun *et al.*, 2009; Sutton *et al.*, 2009). Intra-articular inflammation and subsequently inflammatory or traumatic articular damage can therefore be studied in more detail by means of analysis of pro-inflammatory cytokines and inflammatory mediators. The aim of this study therefore was to evaluate the changes in leukocytes, active phase reactant protein (ESR) and pro-inflammatory cytokines (IL-6) in dogs following knee arthrotomy to determine their potential role in monitoring the risk of infection associated with knee surgery in dogs.

MATERIALS AND METHODS

Animals

Ten clinically healthy adult male mongrel dogs with mean body weight 12.7 ± 2.8 kg were used. They were aged between 2 and 4 years. The dogs were housed individually in concrete kennels at the Veterinary Teaching Hospital, Federal University of Agriculture, Abeokuta, Ogun State throughout the duration of the study. They were fed once daily with ration comprising of cooked rice and fish supplements, while water was provided as required. Also, the dogs were conditioned for the experiment for one week. During this period, they were dewormed with oral mebendazole (Vermox, Pfizer, Nigeria) at the dose of 22 mg/kg, while external parasites were treated by dipping in Diazintol (Animal Care, Nigeria) solution. In addition, the dogs were treated against blood protozoan parasites with intravenous injection of oxytetracycline (Oxytet®, Top-surf, Vancouver, Canada) at 10 mg/kg. Ethical approval for this study was obtained from the Research & Ethics Committee, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Ogun State.

Experimental arthrotomy

The dogs were premedicated with intramuscular injections of atropine sulphate (Atocan®, Sishui Xierkang Pharma, China) at 0.03 mg/kg and xylazine hydrochloride (XYL-M2®, VMD, Belgium) at 1.0 mg/kg. Following premedication, size 21 gauge scalp vein needle was inserted into the cephalic vein to secure the venous access and the cardiovascular system maintained with lactated Ringer's solution at the rate of 0.2 mL/kg/h. Thereafter, anaesthesia was induced and maintained with a combination of diazepam (Calm-

pose[®], Ranbaxy, Dewas, India) at the rate of 0.3 mg/kg and ketamine hydrochloride (Rotexmedica[®], Trittau, Germany) at the rate of 10 mg/kg. The right knee was then prepared aseptically. About 5 cm parapatellar incision was made. Bleeding was controlled and soft tissue damage was kept to the minimum. The joint capsule was incised with a scalpel and then lengthened with a pair of scissors. Thereafter, the femoral condyles were exposed by flexing the joint maximally. Cartilage of the lateral and medial femoral condyles were then grooved (0.5 mm depths) in utmost flexion. Longitudinal and diagonal grooves were made on the weight bearing parts of the femoral condyles without damaging the subchondral bone. The joint capsule, subcutis and the skin were then closed separately. Following recovery, the dog were treated with penicillin/streptomycin injection (Strepcillin[®], Mobedco-Vet, Jordan) for five days and pain was controlled using tramadol injection (Ama-dol[®], Union Korea Pharma, Korea) at 3 mg/kg.

Blood sampling and analysis

About 5 mL of blood was collected from the cephalic vein of the dogs. Blood was obtained immediately before surgery, two hours, 24 hours and three days after surgery. Blood was collected in lithium heparin bottles and analysed for leukocyte parameters and ESR immediately after collection. The remaining blood was centrifuged for 20 min at 2000×g to obtain the plasma. The plasma was then stored at -4 °C until the IL-6 assay.

The following parameters were determined: total white cell counts, absolute neutrophil and lymphocyte counts on an automated blood analyzer; the neutrophil-lymphocyte ratio was calculated by dividing the absolute neutrophil counts with the

absolute lymphocyte counts. ESR was determined using the Wintrobe method (Cha *et al.*, 2009). The plasma levels of interleukin-6 (IL-6) were assayed by quantitative sandwich enzyme immunoassay technique using canine IL-6 enzyme linked immunosorbent assay (ELISA) kit (Quantikine[®], R&D Systems, McKinley Place, Minneapolis, USA). The intra-assay coefficient of variation (CV) for IL-6 was less than 3.3%, while the inter-assay CV was less than 7%. Average recovery of IL-6 in the canine plasma sample was 97%.

Statistical analysis

Data was expressed as mean ± standard deviation. Differences in the measured parameters were compared using analysis of variance (ANOVA). Association between ESR changes, IL-6 and leukocyte parameters was compared using Pearson's correlation coefficient. P values less or equal to 0.05 were considered significant.

RESULTS

The changes in the leukocytes parameters following arthrotomy in dogs are shown in Table 1. Both the white blood cell (WBC) and the absolute neutrophil counts (NEU) were significantly ($P<0.05$) higher 24 hours following arthrotomy, however, there was no significant change in the absolute lymphocyte count up to 3 days following arthrotomy. The WBC and NEU decreased, though not significantly, 3 days after knee arthrotomy, while the lymphocyte counts increased insignificantly during the same period. Similarly, the N/L ratio was significantly ($P<0.05$) increased 24 hours following arthrotomy and thereafter decreased significantly ($P<0.05$) by the 3rd day after knee arthrotomy. In addition, the ESR significantly increased from the basal value up to 3

Table 1. Changes in total white blood cell counts (WBC), neutrophil (NEUT) and lymphocyte (LYM) counts, neutrophil-lymphocyte ratio (N/L), erythrocyte sedimentation rate (ESR) and interleukin 6 (IL-6) following experimental arthrotomy in adult dogs. Data are presented as mean±SD, n=10)

Parameters	Time of sample collection			
	Before knee arthrotomy	2 hours after knee arthrotomy	24 hours after knee arthrotomy	3 days after knee arthrotomy
WBC ($\times 10^9$)	19.3±6.4	21.2±8.6	28.7±10.7*	24.9±6.6
NEUT ($\times 10^9$)	12.6±5.8	14.8±8.1	21.8±9.2 *	15.6±6.1
LYM ($\times 10^9$)	5.5±1.3	5.3±0.7	5.3±1.8	8.0±3.2
N/L	2.4±1.2	2.8±1.5	4.2±1.6*	2.2±1.1
ESR (mm/h)	3.2±2.4	5.4±1.6*	11.0±4.32*	19.2±2.5*
IL-6 (pg/mL)	386.7±108.3	1252.0±234.9*	553.9±205.3	89.5±45.3

* P<0.05 vs baseline.

days following arthrotomy. The plasma IL-6 significantly ($P<0.05$) 2 hours after knee arthrotomy and then progressively decreased from the 24th hour up to the 3rd day after knee arthrotomy (Table 1).

The WBC and NEU were significantly ($P<0.05$) and positively correlated to plasma IL-6 ($r=0.215$ and 0.043) respectively. Similarly, the ESR was significantly ($P<0.05$) and positively correlated to plasma IL-6 ($r=0.293$). However, there was no significant ($P>0.05$) correlation between the absolute lymphocyte counts (LYM) and the plasma IL-6 ($r=0.845$).

DISCUSSION

The results of this study showed that the acute response of dogs to elective arthrotomy was characterised by elevated level of polymorphonuclear neutrophils (PMN) which was first noticed about 24 hours after surgically induced injury and persisted up to three days after arthrotomy. The PMN response is initiated by elevated plasma level of IL-6 which was noted 2 hours after arthrotomy. The values of IL-6 tended to decrease 24 hours after arthro-

tomy up to the 3rd day. This study also showed that IL-6 stimulated a rise in the level of ESR which was first noted 24 hours after arthrotomy and persisted up to 3 days following knee arthrotomy.

Inflammation is a complex defence mechanism in which leukocytes migrate from the vasculature into damaged tissues to destroy the agents that potentially can cause tissue injury. The results of this study confirmed that polymorphonuclear neutrophils are involved in the immediate response of the joint tissue to surgically induced trauma. This is evident by the increase in the number of the absolute neutrophil counts 24 hours after arthrotomy. Lymphocytic response to joint injury was delayed as shown by the lack of elevation in the absolute lymphocyte counts until about three days after arthrotomy when a slight insignificant increase was noticed.

Erythrocyte sedimentation rate (ESR) is an indirect measurement of fibrinogen, a positive acute phase protein and reflects a measure of local and systemic events that accompany inflammation such as neutrophil chemotaxis, vasodilation, platelet aggregation and release of lysosomal en-

zymes (Husain & Kim, 2002). In this study, ESR was elevated 24 hours after knee arthrotomy in the dogs and persisted up to 3 days after arthrotomy. This finding suggests that ESR may be a useful indicator of inflammation in joint diseases in dogs and can be used as a potential biomarker of inflammatory joint diseases of dogs as currently done in humans. ESR is relatively easy to determine compared with C-reactive proteins and thus can be used routinely to monitor the progression of osteoarthritis or inflammatory arthropathies in dogs.

IL-6 is the most important acute-phase protein inducer. In humans, IL-6 strongly stimulates hepatocytes to produce C-reactive protein, fibrinogen, haptoglobin and antichymotrypsin (Weinhold & Rutherford, 1997). IL-6 also acts synergistically with other cytokines, enhancing the proliferation of multipotential haematopoietic progenitors and promote the maturation of human megakaryocytes (Fonseca *et al.*, 2009). In this study, plasma IL-6 was elevated two hours after surgery and persisted up to 24 hours after knee arthrotomy. However, contrary to earlier reports, the plasma IL-6 level decreased significantly 3 days following arthrotomy. This decrease may be due to the administration of both systemic antibiotics and analgesics to dogs thus helping to control the progression of the joint inflammation.

In conclusion, the values of the IL-6, ESR and NEUT were positively correlated suggesting that changes in ESR and polymorphonuclear neutrophils can be used to monitor the prognosis of joint inflammation following surgery in dogs.

CONFLICT OF INTEREST

The authors do not have any conflict of interest.

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