CLINICAL AND BIOCHEMICAL INVESTIGATION OF ARTHRITIS IN HORSES

Almujalli, A. M.; Fouda, T. A.; Eldeeb, W. M. and Eljalil, I. M.

Department Of Clinical Studies, College Of Veterinary Medicine And Animal Resources,

King Faisal University, Al-Ahsa 31982, Kingdom Of Saudi Arabia

ABSTRACT

Arthritis is a primary cause of lameness and loss of use in the equine industry. 25 diseased horses with arthritis with varying clinical signs were used in this study. The diseased horses showed the clinical signs of lameness, warmth of the affected joint, swelling and pain on flexion of the affected joint. In addition 15 clinically healthy horses were also used as control group. Blood samples were obtained from each animal and were subjected to both cellular and biochemical analysis.

The data presented in table 1 summarizing the mean values of the selected hematological parameters in healthy and diseased horses and revealed significant ($p \leq 0.05$) increase in WBCs count with significant ($p \leq 0.05$) increase in the neutrophils and monocytes count.

The mean values of the selected biochemical parameters were tabulated in Table 2, which revealed a significant ($p \leq 0.05$) increase in the activities of ALP and ALT, in addition to glucose, total protein, albumin, urea and creatinine levels when compared with their values in clinically healthy group. Moreover there was significant decrease in the activities of AST in diseased horses when compared with clinically healthy horses. Values of the other examined parameters remained unchanged significantly ($p \leq 0.05$) in disease horses when compared with control group.

Key words: *horses, arthritis, cellular, biochemical parameters, blood*

INTRODUCTION

Arthritis is a primary cause of lameness and loss of use in the equine industry. A study conducted by the USDA's National Animal Health Monitoring System (NAHMS) in 1998 estimated the total annual cost of lameness to be million to billion, with 66% attributed to loss of use **(Animal and Plant Health Inspection Service website, 2006)**. It has been suggested that 60% of lameness is due to osteoarthritis **(Caron and Genovese, 2009)**.

In horses, synovial inflammation (synovitis) often occurs in conjunction with osteoarthritis (OA) and may precede or promote degenerative changes within the articular cartilage **(Valentine, 2007)**. Serum biomarkers have proven useful in the diagnosis of equine bone and joint disease **(McIlwraith, 2005)**. This study aimed to evaluate serum biochemical

changes that will be helpful in diagnosis of arthritis in horses.

MATERIALS AND METHODS

Animals:

25 diseased horses with arthritis with varying clinical signs were used in this study. The diseased horses involved the clinical cases admitted to the Veterinary Teaching Hospital at KFU, in addition to the cases diagnosed clinically in field visits for several localities at Al-Ahsa governorates. In addition to 15 clinically healthy horses were used as control group. Physical examination of animals and recording of clinical signs was carried out.

Samples:

Two blood samples obtained on heparinized vacuumed tubes from each animal. The first blood sample used for cellular examination. The second blood samples obtained in plain vacuumed tube for obtaining clear non hemolysed sera for analysis of total plasma proteins and albumin. In addition to the blood concentration of muscle-derived enzymes were measured.

I. Cellular examination of blood:

Cellular examination of blood carried out determined by an automatic cell counter adjusted for animal cell counting.

II. Biochemical analysis :

Biochemical analysis of sera samples for measuring the concentration of CK, AST, ALT, ALP. glucose, total protein, albumen, urea, and creatinine carried out using the autoanalyzers.

Statistical analysis :

The data was presented as mean ± standard error (SE). A two-way repeated measures analysis of variance had been applied for statistical analysis. The level of significance was set at $P<0.05$. Significances between means were assessed using the least-significant-difference procedure according to Snedecor and Cochran (1982). All calculations performed using SPSS/PC software

RESULTS

Clinical signs :

The diseased horses showed the clinical signs of lameness, warmth of the affected joint, swelling and pain on flexion of the affected joint.

Selected hematological parameters in control and diseased horses :

The data presented in table 1 revealed significant ($p\leq 0.05$) increase in WBCs count with significant ($p\leq 0.05$) increase in the neutrophils and monocytes count.

Selected biochemical indicators in control and diseased horses :

The data summarized in Table 2 included the activities of CK, AST, ALT, and ALP. In addition to the values of glucose, total protein, albumen, urea, and creatinine in control and diseased horses. The present findings (Table 2) revealed a significant ($p\leq 0.05$) increase in the activities of ALP, ALT, glucose, total protein, albumin, urea and creatinine when compared with the control group. Moreover there was significant decrease in the levels of AST in diseased horses when compared with

clinically healthy horses. Values of the other examined parameters (Table 2) remained unchanged significantly ($p \leq 0.05$) in disease horses when compared with control group.

DISCUSSION

The diseased horses showed the clinical signs of lameness, warmth of the affected joint, swelling and pain on flexion of the affected joint. These results were in agreement with those obtained by **Singh et al. (1999)**.

The data presented in table 1 revealed significant ($p \leq 0.05$) increase in WBCs count with significant ($p \leq 0.05$) increase in the neutrophils and monocytes count. These changes could be attributed to the inflammatory conditions and systemic reaction of diseased horses. These results are in accordance with those obtained by **Singh et al. (1999)**.

The data summarized in Table 2 included the activities of CK, AST, ALT, ALP, values of glucose, total protein, albumen, urea, and creatinine in serum of healthy and diseased horses. The present findings (Table 2) revealed a significant ($p \leq 0.05$) increase in the activities of ALP, ALT, and mean values of glucose, total protein, albumin, urea and creatinine concentrations when compared with the control group. Moreover there was a significant decrease in the levels of AST in diseased horses when compared with clinically healthy horses. Values of the other examined parameters (Table 2) remained unchanged significantly ($p \leq 0.05$) in disease horses when compared with control group.

Elevated AST activities are also an indicator of muscle damage **(Skenderi et al., 2006; Valentine & Löhr 2007)**. Elevated activities of AST are also thought to be indicative of liver damage **(Nyblom et al., 2004)**. In the cell, ck is involved in formation of adenosine triphosphate (ATP) **(Peach et al., 2006)**.

Several plasma isoenzymes of alkaline phosphatase are recognized, including hepatic, osseous, intestinal, and placental forms; the relative proportions of these isoenzymes in plasma vary with species. ALP is widely distributed in tissues notably on the border membranes of the bile canaliculi and on the sinusoidal surfaces of the liver, the intestinal mucosa, the osteoblasts of bone, the renal proximal tubules, the placenta, and the mammary glands. In most species, age-related changes of osseous ALP are observed, reflecting bone growth in the neonatal and juvenile periods **(Syakalima et al., 1997)**. Although the diagnostic emphasis is mainly on increases of plasma ALP and hepatotoxicity, the enzyme may be reduced in hypothyroidism and pernicious anemia and may be changed in several non hepatic-related conditions **(Fernandez and Kidney, 2007)**. In the present study elevated levels of ALP in diseased horse could be attributed to the inflammatory changes in the affected joint.

In many species, the proportion of ALT in hepatic tissue is greater than in any other organs (e.g., rat and dog); in other species, the proportions of ALT in hepatic and cardiac tissue are similar (e.g., rabbit) **(Clampitt and Hart, 1978; Lindena et al., 1986)**. General texts often state that ALT is a cytosolic enzyme, although mitochondrial ALT is present in many tissues of some species including the

rat **(Ruscak et al., 1982)**. It is the relative proportions of cytosolic to mitochondrial forms and the variations between major organs that lead to such statements and, although these ALT isoenzymes exist, they are not widely measured in diagnostic enzymology at present. Again, in some texts, it is suggested that plasma ALT is specific for liver injury in the dog and, although the majority of observations of increased ALT activities are associated with liver changes in the dog, there are some examples where plasma ALT is increased in muscle necrosis **(Valentine et al., 1990)** or as a consequence of acute intestinal enteropathies in dogs **(Dodurka and Kraft,, 1995)** and other causes (Swenson **and Graves, 1997)**. In some species, such as the common marmoset, plasma ALT activity is low and may be less useful as a marker of hepatotoxicity **(Cowie and Evans, 1985)**.

In the present study elevated levels of ALT could be attributed to inflammation. The reported significant increase in glucose levels in diseased horses than that of the control perhaps attributed to increase the rate of glycogenolysis, glycolysis and lipogenesis, respectively or may attributed to stresses **(Westermann et al., 2007)**.

Creatinine is a product of the degradation of creatine and creatine phosphate, which are present mainly in muscle and in food. Plasma creatinine is dependent on muscle mass and can be lowered in severe myopathy. Although plasma levels are less affected by diet compared to urea, malnutrition may lower plasma creatinine **(Braun et al., 2003)**. Plasma creatinine is normally filtered from the plasma, and it is reabsorbed and secreted by the proximal tubules to a minor extent, although secretion is higher in rodents compared to humans. Elevated plasma Creatinine is a reliable indicator of impaired glomerular filtration or alterations in renal blood flow, but severe tubular dysfunction can also increase plasma creatinine.

Plasma creatinine is a better marker of glomerular function than urea, and these two measurements are not always simultaneously increased or normal **(Prause and Grauer, 1998; Medaille et al., 2004)**. For measurements of plasma creatinine and urea to change, there has to be significant loss of renal function (i.e., a 50% loss of GFR capacity leads to a doubling of these plasma values), and these relationships are not linear. To a lesser extent, both plasma creatinine and urea show variation with age (**Corman et al., 1985; Goldstein, 1990**). The significant increase in urea and creatinine level in diseased horses (Table-2) indicated renal damage (**Radostits et al., 2007**).

From this study it could be concluded that selected blood biochemical indicators are of some importance in the diagnosis of arthritis in horses.

Table 1: Selected cellular parameters in the blood of healthy and diseased horses.

Parameters	Control	Diseased
Hb (g/dL)	13.04 ± 0.57	12.99 ± 0.53
WBC (x10^9/L)	8.56 ± 1.34	15.30 ± 0.58*
Neutrophils (x10^9/L)	5.3 ± 1.23	7.60 ± 1.39*
Monocytes (x10^9/L)	1.03 ± 0.03	6.08 ± 1.65*
Lymphocytes (x10^9/L)	4.33 ± 0.42	4.66 ± 0.42
Eosinophils (x10^9/L)	1.01 ± 0.01	1.02 ± 0.02

*Means are significantly different at the level ($p \leq 0.05$).

Table 2: Selected biochemical parameters in blood serum of healthy and diseased horses

Parameters	Control (n=10)	Diseased (n=20)
CK (IUL-1)	202.6±9.90	212.24 ± 9.20
AST (IU L-1)	275.3±6.60	207.47 ± 14.5*
Creatinine (µmol/L)	118.4±2.67	160 ± 0.33*
ALP (U/L)	131.83 ± 29.6	208.64 ± 6.50*
ALT (U/L)	31.9 ± 2.61	66.91 ± 5.13*
Glucose (mmol L-1)	5.6±1.20	9.91 ± 4.87*
Total Protein (g/dl)	6.8±1.45	7.9 ± 0.48*
Albumin (g/dl)	2.9±1.21	3.3 ± 0.29*
Urea (mmol/L)	7.3±0.52	21.15 ± 0.81*

*Means are significantly different at the level ($p \leq 0.05$).

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الملخص العربى

فحوصات إكلينيكية وبيوكيميائية عن التهاب المفاصل في الخيول

عبد العزيز محمد المجلي طه عبد المنعم فوده
وائل محمد الديب عصام محمد الجعلي

قسم الدراسات الإكلينيكية - كلية الطب البيطري والثروة الحيوانية - جامعة الملك فيصل

الإحساء -*31982* المملكة العربية السعودية

تعتبر التهابات المفاصل السبب الرئيس في حالات العرج مما يؤثر سلبا علي استخدام الخيول. في هذا البحث تم استخدام 25حصان مريض يعاني من التهابات المفاصل بمختلف علاماتها السريرية. وقد تمثلت هذه الأعراض السريرية في سخونة وتورم المفاصل المصابة بالإضافة إلي حالات العرج والألام عند ثني المفاصل.بالإضافة إلي ذلك تم استخدام 15حصان سليم ظاهريا وإكلينيكيا كمجموعة ضابطة للمجموعة المريضة.

تم الحصول علي عينات الدم من كل الخيول تحت الدراسة وذلك لإجراء الفحوصات الخلوية والتحاليل البيوكيميائية. هذا وقد أوضحت البيانات المتحصل عليه المتعلقة بالفحص الخلوي للدم (جدول - 1) حدوث زيادة معنوية في العدد الكلي لكرات الدم البيضاء، وكذلك العدد التصنيفي للخلايا المتعادلة (النيتروفيل) والخلايا الأحادية (المونوسايت) في دم الخيول المصابة عند مقارنتها بمعدلاتها في الخيول السليمة.

وفيما يتعلق بنتائج التحاليل البيوكيميائية (جدول- 2) فقد أظهرت النتائج حدوث زيادة معنوية في نشاط بعض الإنزيمات مثل ALT-ALP بالإضافة إلي حدوث زيادة معنوية في مستويات بعض العناصر وتشمل سكر الدم، البروتين الكلي الزلال، البوريا، والكرياتينين وذلك إذا ما قورنت بمستوياتها في الخيول السليمة في حين أوضحت النتائج حدوث انخفاض معنوي في نشاط AST في الخيل المصابة. بينما لم تحدث أية تغيرات معنوية في العناصر الأخرى في مصل الخيول المصابة عنها في الخيول السليمة.