

Clinicopathological, Radiological and Synovial Fluid Evaluations in Common Musculoskeletal Affections in Horses

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Abstract: The current work was conducted on 105 horses at Cairo, Egypt and Pennsylvania, USA during the period between 2007 and 2009. Horses were examined clinically to investigate prevalence of different musculoskeletal affections and then were subjected to hematological, serum biochemical, cytological and radiological examinations. Clinical investigation revealed that arthritis was the dominant, followed by tendinitis and then myopathy. Clinical signs included lameness, stiffness gait and off food (Mainly), followed by fever and pain as well as swelling. Horses showed X-ray and ultrasound abnormalities with different degrees. Hematological examination of horses with musculoskeletal affections showed significant macrocytic normochromic anemia in the myopathy group and insignificant decreases of erythrocytes' parameters in other groups, a significant leukocytosis in all affected animals, neutrophilia in acute arthritis group, monocytosis in chronic arthritis group, esinopenia in all affected groups except the tendinitis group. Serum biochemical analysis revealed that examined horses showed significant hyperproteinemia, hyperalbuminemia (Except acute arthritis group) as well as hyperglobulinemia. Significant increases of serum creatinine, blood urea nitrogen and hyperglycemia were seen in horses with myopathy. Serum enzyme activities revealed significant elevation in alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and creatine kinase (CK) activities in different groups with higher increase in the acute arthritis and myopathy groups. Insignificant changes were observed in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Except elevated AST in myopathy group). Examination of the synovial fluid of arthritic horses revealed physical, cytological, as well as chemical changes.

Key words: Horse • Arthritis • Tendinitis • Myopathy • X-Ray • Hemogram • Serum Biochemistry • Synovial Fluid Analysis

INTRODUCTION

The musculoskeletal system consists of bones, cartilages, muscles, ligaments and tendons. It primarily functions to support the body, provide motion and protect the vital organs. Muscular disorders are a common cause of disability in affected horses. Recently metabolic, inflammatory, dystrophic and other inherited muscle diseases have been described in horses [1].

Musculoskeletal injuries (MSIs) are degenerative diseases and inflammatory conditions that cause pain and impair normal activities [2], musculoskeletal injuries are commonly faced in racehorses worldwide their prevalence

tends to exceed that of cardiac problems and epistaxis, which contribute significantly to the burden of health problems for racehorses [3].

Musculoskeletal injuries involving the distal limb joints remain the greatest cause of loss of athletic performance in racehorses industry. Among all, joint injuries and diseases are most frequently encountered and represent a major part of the caseload for equine clinicians [4].

Lameness in the horse represented the most common cause of impaired athletic performance in the horse [5]. Majority of lameness's cases are localized to areas within the distal limb; however, the sources, the causes and the

locations of lameness are diverse. Lameness can be caused by numerous and diverse conditions, as wear-and-tear, overuse and trauma [6].

Early detection of musculoskeletal disease is a key factor for prevention of further injuries and to increase the likelihood of successful treatment of equine athletes [7].

Clinical examination is still critical. Imaging techniques include radiography [8], nuclear imaging [9], computed tomography (CT) [10], magnetic resonance imaging (MRI) [11] and ultrasonographic examination [12]. Conventional synovial fluid analysis is still used to define infection [13], together with serum biomarkers offer real potential for diagnosis of early change in cartilage and bone. Arthroscopy still is the gold standard for diagnosis of cartilage defects [14].

The present work aimed to investigate the musculoskeletal affections in surveyed horses by laboratory means including hematological, serum biochemical and cytological analysis as well as radiological examination.

MATERIALS AND METHODS

Animals: The current work was carried out on horses suffered from musculoskeletal affections presented at the equine clinics or admitted to the veterinary clinics in Cairo, Egypt and Fox-Run Equine center, Pennsylvania, USA during the period extended from 2007 to 2009.

A total number of 105 horses were studied and divided into four main groups as follow:

Group A: Composed of apparently normal 20 horses and was used as a control group.

Group B: Composed of 60 horses suffered from arthritis, which were then divided into 2 subgroups:

Sub Group B1: Composed of 40 horses suffered from acute arthritis, in one or more joints.

Sub Group B2: Composed of 20 horses suffered from chronic arthritis, in one or more joints.

Group C: Composed of 16 horses suffered from tendinitis.

Group D: Composed of 9 horses suffered from myopathy.

Sampling

Blood Samples: Blood samples were collected from the jugular vein. A part of the collected blood was received on dipotassium EDTA and used for hematological

studies. Another part of the blood was collected in plain tubes, allowed to clot, centrifuged at 3000 rpm for 10 minutes for serum separation. The clear nonhemolysed supernatant sera were harvested for biochemical examination.

Hematological and Serum Biochemical Studies

Hematological Studies: Complete blood picture were carried out according to Feldman *et al.* [15].

Serum Biochemical Studies: Serum samples were prepared to assay serum total proteins according to Weichselbaum [16], serum albumin according to Dumas and Biggs [17], serum globulins were determined by subtracting value of the serum albumin from the value of serum total proteins concentration according to Grotty and Knottenbelt [18] and A/G ratio was obtained by subdividing values of the serum albumin by those of serum globulins. Serum creatinine according to Fabiny and Ertingshausen [19], blood urea nitrogen according to Searcy *et al.* [20] and serum glucose according to Howanitz and Howanitz [21]. Colorimetric determination of the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) according to Reitman and Frankel [22], alkaline phosphatase (ALP) according to Tietz [23], lactate dehydrogenase (LDH) and kinetic creatine kinase (CK) according to Kachmar and Moss [24]. Serum biochemical parameters were assayed using commercial diagnostic kits supplied by Stanbio Laboratory, Texas, USA.

Synovial Samples: About 10 ml. of synovia were collected from acute and chronic arthritic animals. The collected synovial samples were anti-coagulated with EDTA and subjected to physical, biochemical and microscopical examination. The physical examination included volume, viscosity, turbidity and pH, while the biochemical examination included determination of total proteins and glucose concentrations, as well as activities of AST, LDH and ALP. The microscopical examination included total and differential cell counts as indicated by Van Pelt [25].

Radiological and Sonographic Analysis: Radiological and sonographic analysis was performed for clinical cases according to the standard protocol.

Statistical Analysis: All numerical data were statistically evaluated for the mean and standard error for each group. The significance of the results was determined by conducting the least significance difference (LSD) between different times outlined by Snedecor and Cochran [26].

Table 1: Prevalence of musculoskeletal affections causing lameness in surveyed horse

	Group B				Total
	Group B1	Group B2	Group C	Group D	
Number	40	20	16	9	85
Prevalence	47.06%	23.53%	18.82%	10.59%	100%

Group (B1) represents horses suffered from acute arthritis.

Group (B2) represents horses suffered from chronic arthritis.

Group (C) represents horses suffered from tendinitis.

Group (D) represents horses suffered from myopathy.

Table 2: Clinical signs and radiological analysis of affected horses with musculoskeletal affections causing lameness

	Group B				Total
	Group B1	Group B2	Group C	Group D	
Lameness	36	18	14	9	77
Stiffness gait	36	9	12	8	65
Off food	35	8	8	9	60
Fever and pain	22	11	10	8	51
Swelling	11	10	13	7	41
X-ray abnormalities	15	16	4	-	35

Group (B1) represents horses suffered from acute arthritis.

Group (B2) represents horses suffered from chronic arthritis.

Group (C) represents horses suffered from tendinitis.

Group (D) represents horses suffered from myopathy.

RESULTS AND DISCUSSION

Prevalence of Musculoskeletal Affections: Clinical investigation of the examined 85 horses revealed that the most prevalent finding was arthritis, both acute and chronic, tendinitis and then myopathy (Table, 1). Similar findings were observed by Egenvall *et al.* [27], whom indicated that arthritis outnumbers ligament disorders and tendon injuries, while dissimilar findings were reported by Bertuglia *et al.* [28], whom indicated that the suspensory ligament injuries and superficial digital flexors tendonitis (SDFT) were the most prevalent exercise-related MSIs those occurred more frequently than traumatic fetlock arthropathy and bone fracture.

Clinical Signs, Radiological and Sonographic Analysis: Concerning the clinical signs, most of the affected horses with musculoskeletal affections exhibited lameness, stiff gait and off food followed by fever and pain as well as swelling. Horses of different groups showed X-ray and ultrasound abnormalities with different degrees (Table, 2 and Photos, 1-6). The observed lameness and joint swelling (In arthritic horses) could be attributed to the initiation of an inflammatory process that caused synovitis and capsulitis [29]. The observed signs were similar to those described by El-Deeb and El-Bahr [30].



Photo 1: Swelling in both the carpal and fetlock joints due to acute arthritis in thoroughbred horse



Photo 2: Inflammation of the fetlock region including fetlock joint.



Photo 3: Inflammation of the Bursa that's in front of the carpus and not of one of the carpus joints.



Photo 4: Anterior posterior X-ray image of normal carpal joint



Photo 5: Anterior posterior X-ray image of carpal joint showing osteoperiosteal reaction (Chronic arthritis).

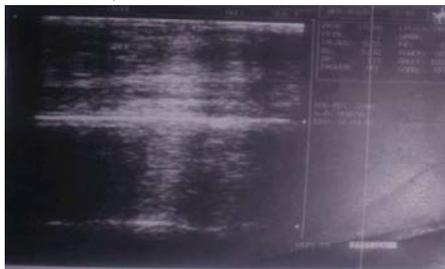


Photo 6: Enlarged SDFT thickness with hyper-echonic striations indicating old fibrous tissue formed in the thickness of tendon is 26 mm.

Clinicopathological Findings

The Hemogram: The mean values of erythrogram in different groups showed significant decreases in the circulating erythrocytes, packed cell volume and hemoglobin concentration with significant increase in MCV and normal MCHC in the myopathy group (D) in comparison to those of the control group (A) (Table 3). The detected macrocytic normochromic anemia could be attributed to the vitamin B12 deficiency [15]. These findings disagreed with Van Galen *et al.* [31], whom reported normal to increased PCV values in horses with atypical myopathy.

Compared to the control group, results of leukogram showed significant leukocytosis in all affected animals, significant neutrophilia in the acute arthritis group (B1), significant monocytosis in chronic arthritis group (B2), significant esinopenia in all affected groups except the tendinitis group (C) and nonsignificant changes in the mean values of lymphocytes and basophils in all groups (Table 3). The observed leucocytic response pattern might be secondary to endogenous corticosteroid release, due to stress or inflammation [15]. The recorded leukocytosis in the acute arthritis group (B1), because of the significant neutrophilia, agreed with Newquist and Baxter [32], while disagreed with Singh *et al.* [33], whom reported a slight rise in TLC, insignificant neutrophilia and monocytopenia.

Serum Biochemical Analysis: Statistical analysis of different serum biochemical parameters of different experimental groups is illustrated in Table 4.

Concerning the serum proteins profile, horses of the acute arthritis, tendinitis and myopathy groups showed significant hyperproteinemia, hyperalbuminemia (Except acute arthritis group) as well as hyperglobulinemia when compared with the control group. Horses with chronic arthritis showed nonsignificant changes in the mean values of serum proteins. Calculated A/G ratio revealed significant decrease in both acute arthritis and myopathy groups and nonsignificant changes in both chronic arthritis and tendinitis groups (Table 4). The observed serum proteins changes could probably be attributed to the local inflammatory process that was sufficiently strong to elicit acute phase response; systemic inflammatory response [34]. The observed hyperalbuminemia may be associated with high protein diets [35]. These findings disagree with El-Deeb and El-Bahr [36] whom reported insignificantly changed total protein, albumin and globulin values.

Table 3: Hemogram of control and affected horses with musculoskeletal affections causing lameness (Means ± SE)

	Group B				
	Group A	Group B1	Group B2	Group C	Group D
RBCs ($\times 10^6/\mu\text{l}$)	6.82±0.80	6.51±0.56	5.59±0.40	5.71±0.71	4.72±0.33*
PCV (%)	47.20±4.20	44.91±2.30	39.66±3.40	39.57±3.20	36.00±2.70*
Hb (g/dl)	12.61±1.20	11.32±0.82	10.72±0.96	10.30±0.76	9.35±0.56*
MCV (fL)	69.19±5.20	69.01±5.40	70.94±8.10	69.57±3.20	76.55±3.10*
MCH (pg)	18.41±1.40	17.88±1.70	19.77±1.10	18.10±2.09	20.13±1.60
MCHC (g/dl)	26.69±1.10	25.5±1.20	27.58±1.40	26.01±1.80	25.62±2.10
WBCs ($\times 10^3/\mu\text{l}$)	8.27±1.20	12.81±1.16*	10.60±1.80*	9.13±0.90*	10.8±0.93*
Neutrophils ($\times 10^3/\mu\text{l}$)	4.21±0.50	8.10±1.01*	5.49±0.67	4.77±0.56	5.42±0.34
Lymphocytes ($\times 10^3/\mu\text{l}$)	3.55±0.12	3.79±0.70	3.71±0.75	3.53±0.12	4.67±0.23
Monocytes ($\times 10^3/\mu\text{l}$)	0.41±0.09	0.62±0.12	1.09±0.17*	0.63±0.12	0.49±0.07
Eosinophils ($\times 10^3/\mu\text{l}$)	0.83±0.11	0.13±0.01*	0.21±0.03*	0.82±0.11	0.21±0.11*
Basophils ($\times 10^3/\mu\text{l}$)	0.00±0.00	0.00±0.00	0.11±0.03	0.00±0.00	0.00±0.00

*Significantly different from normal control, $P \leq 0.05$

Group (A) represents apparently normal control horses.

Group (B1) represents horses suffered from acute arthritis.

Group (B2) represents horses suffered from chronic arthritis.

Group (C) represents horses suffered from tendinitis.

Group (D) represents horses suffered from myopathy.

Table 4: Serum biochemical analysis of control and affected horses with musculoskeletal affections causing lameness (Means ± SE)

	Group B				
	Group A	Group B1	Group B2	Group C	Group D
Total protein (g/dL)	8.46±0.35	10.95±1.08*	8.79±0.90	10.27±0.81*	11.22±1.33*
Albumin (g/dL)	4.31±0.22	4.66±0.21	4.72±0.15	5.11±0.17*	4.97±0.13*
Globulin (g/dL)	4.25±0.12	6.32±1.01*	4.13±0.61	5.26±0.67*	6.43±0.48*
A/G ratio	1.07±0.04	0.75±0.02*	1.12±0.01	0.97±0.01	0.77±0.10*
Creatinine (mg/dL)	1.60±0.30	1.42±0.12	1.42±0.17	1.57±0.30	2.70±0.20*
BUN (mg/dL)	23.40±3.20	29.10±2.70	27.00±4.20	23.30±2.20	38.20±3.20*
Glucose (mg/dL)	86.00±5.10	73.20±8.10	82.80±7.20	85.10±12.40	108.20±12.30*
ALT (U/L)	16.12±2.10	30.90±3.70	28.23±2.50	25.60±2.40	18.50±2.10
AST (U/L)	93.26±11.00	105.8±12.40	97.11±9.70	75.30±12.10	118.20±10.10*
ALP (U/L)	68.52±5.40	129.90±17.10*	108.12±8.90*	87.90±13.20	137.80±11.10*
LDH (U/L)	109.21±11.00	319.50±22.80*	271.11±12.40*	183.70±17.40*	364.10±33.40*
CK (U/L)	148.61±9.30	327.1±45.20*	207.12±21.40*	193.10±12.20*	397.10±43.10*

*Significantly different from normal control, $P \leq 0.05$

Group (A) represents apparently normal control horses.

Group (B1) represents horses suffered from acute arthritis.

Group (B2) represents horses suffered from chronic arthritis.

Group (C) represents horses suffered from tendinitis.

Group (D) represents horses suffered from myopathy.

Results revealed significant increases of serum creatinine, blood urea nitrogen and significant hyperglycemia in horses of the myopathy group (D), as well as nonsignificant alterations in the other three groups, when compared with the control one (Table 4). The significant increase in serum creatinine and blood urea nitrogen could be due to acute kidney injury resulted from the exaggerated oxidative stress associated with increased muscle membrane leakage [37], or to the release of muscle fiber contents into the blood, which are

harmful to the kidney and often cause kidney damage [38]. The detected significant hyperglycemia could be as a result of a decrease in glycogen accumulation in skeletal muscle of these horses [39]. In addition to stress induced release of catecholamines may have aggravated the hyperglycemia or reduced glucose utilization in peripheral tissues [40]. These findings agree with El-Deeb and El-Bahr [36] who reported increased values of creatinine, urea and glucose in rhabdomyolysis-diseased Arabian horses.

Table 5: Physical, cytological and biochemical analysis of synovial fluid associated with acute and chronic arthritis (Means ± SE)

Parameters	Normal	Acute Arthritis	Chronic Arthritis
Synovial volume (ml)	1.12±0.07	4.04±0.32*	2.17± 0.12
Viscosity	Viscous	Watery	Viscous
Turbidity	Clear	Turbid	Turbid
pH	7.34±0.12	7.14±0.23	7.24±0.11
TLC (10 ³ /µl synovia)	2.10±0.12	18.70±0.23*	3.40±0.11
Neutrophil (%)	51.30±1.10	61.20±2.10*	48.50±1.90
Lymphocyte (%)	48.30±1.40	34.60±0.90	44.30±1.70
Monocyte (%)	1.40±0.40	3.20±0.20*	6.20±0.50*
Eosinophil (%)	1.00±0.00	1.00±0.00	1.00±0.00
Total proteins (g/dl)	4.73±0.82	8.92±1.54*	7.46±1.28*
Glucose (mg/dl)	67.22±3.11	101.55±4.65*	98.31±3.75*
AST (U/L)	62.34±3.20	119.32±12.10*	139.21±11.20*
ALP (U/L)	39.32±8.34	192.98±12.20*	243.12± 22.20*
LDH (U/L)	179.23±12.90	214.61±33.20*	318.12±36.10*

*Significantly different from normal control, P ≤ 0.05

Group (B1) represents horses suffered from acute arthritis.

Group (B2) represents horses suffered from chronic arthritis.

Results of serum enzyme activities exhibited nonsignificant changes in the mean values of both ALT and AST activities in different tested groups, except a significantly elevated AST activity in the myopathy group. ALP (Except in tendinitis), LDH and CK-total nearly behave the same manner, where they showed significantly elevated activities in the different tested groups; with higher elevations in the acute arthritis (B1) and myopathy (D) groups than in the chronic arthritis (B2) and tendinitis (C) groups (Table, 4). The observed significantly increased serum enzymatic activities might be correlated with muscular damage [41]. Similar enzymatic activities were previously reported by Sucre *et al.* [42]; ALT and AST during joint and muscle affections, Safadi *et al.* [43]; ALP in horses with myopathy, Valberg *et al.* [44]; LDH with musculokeletal affections and Annandale *et al.* [45]; CK in horses with myopathy. While Singh *et al.* [34] reported insignificantly changed AST and ALP activities.

Synovial Fluid Examination: Physical examination of the synovial fluid in arthritic group revealed increased synovial fluid volume (Significant in acute arthritis), decreased to normal viscosity (Acute arthritis and chronic arthritis, respectively) and turbidity, in the acute and chronic arthritic horses (Table 5). These physical changes of the synovial fluid are usually indicative of an inflammatory response [46]. The decreased viscosity of synovia seen in acute arthritis may be as a result of a dilution effect in the joint effusion, synthesis of a low quality hyaluronate by the inflamed synoviocytes [47] and the destruction of the hyaluronate molecule [48], or increased ALP activity or decreased synovial mucin [34].

Turbidity might change in relation to the presence of cells, cell types, hemorrhage and may be cartilaginous debris [49]. The observed physical changes agree with Tulamo *et al.* [50] whom indicated that the more a joint is inflamed the greater the synovial fluid volume, the higher the cell count and subsequent turbidity and the lower the viscosity.

Cytological analysis of the synovial fluid from acute arthritic horses revealed a highly significant elevated TLC, predominantly neutrophils, as well as significant monocytosis. While in chronic arthritis, insignificant increase of TLC with significant monocytosis was observed (Table 5). The observed leukocytosis, neutrophilia and monocytosis in acute arthritis could be due to the inflammatory response in the affected joints [51]. Chronic arthritis is not associated with signs that are seen with inflammatory joint disease, such as leukocytosis and its diagnosis cannot be made based upon synovial fluid cytology alone [52]. Leukocytosis together with neutrophilia in the synovial fluid was previously reported by de Grauw *et al.* [53]. Leukocytosis in arthritic horses was formerly observed by Meulyzer *et al.* [54].

Chemically, horses with acute and chronic arthritis showed highly significant increases in the synovial total protein concentration, synovial glucose, as well as AST, ALP and LDH activities (Table 5). The increased total protein content in the synovial fluid of inflamed joints, may possibly attributed to the increased capillary permeability, which permits the higher molecular weight protein fractions, mainly globulin, to enter the joint [55]. Similar results were reported by Ross *et al.* [56] in inflamed joints in equines. The increased synovial

glucose in the arthritic joints may be due to the lesser amounts of glucose that enter the joint as the severity of the inflammation increases, in contrast to the increased permeability of the synovial membrane to proteins in inflammation [57].

Regarding the synovial enzymatic activities, horses with acute and chronic arthritis showed significantly increased activities of AST, ALP and LDH (Table 5). The increased enzyme activity in the joint fluid may result from the release of enzymes from leukocytes and production and release of an increased amount of enzymes by necrotic or inflamed synovial tissue [34]. An observed positive correlation between the number of leukocytes and the enzyme levels is indirect evidence for the first possibility [58]. Similar results were reported by Mokbel *et al.* [29].

CONCLUSION

From the present study, it is concluded that, arthritis was the dominant MSI in examined horses, followed by tendinitis and myopathy. Examined horses showed related clinical signs, X-ray and ultrasound abnormalities, hematological changes, serum biochemical alterations and elevated serum enzyme activities of ALP, LDH and CK. Examination of the synovial fluid of arthritic horses revealed physical, cytological as well as chemical changes.

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