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Physical and biochemical evaluation of synovial fluid in equine osteoarthritis

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Abstract

Synovial fluid is an ultrafiltrate of blood plasma, supplemented with hyaluronic acid and other products and supplies nutrients to the articular cartilage. Horses presented with lameness and showed signs of osteoarthritis were taken for this study. A total of 12 osteoarthritic joints fluid were physically evaluated for appearance, colour, volume, viscosity, clot formation and mucin clot test. Both serum and synovial fluid were evaluated for alkaline phosphatase (ALP), alanine aminotransaminase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH). This study found that viscosity and mucin clot test were useful in diagnosing osteoarthritis and biochemical values of synovial fluid had some diagnostic value but serum biochemical values did not have any role in diagnosing osteoarthritis.

Keywords: equine, synovial fluid, osteoarthritis, lactate dehydrogenase

Introduction

Osteoarthritis (OA) is a slowly progressive disease characterized by cartilage degeneration, subchondral bone sclerosis, osteophyte formation, varying degrees of synovial inflammation and periarticular tissue fibrosis [1]. Increased cellularity resulted in a turbid synovial fluid which was indicative of inflammation and turbid or flocculent, cloudy and non-viscous would indicate septic synovial fluid [6]. Protein leaks from damaged vessels increased total protein concentration in joint trauma and inflammatory conditions [6]. So total protein would increase with joint inflammation and this would increase the synovial fluid protein to that of serum level. Hyaluronan was depolymerized in untreated inflammatory arthritis and this was considered to be the basis for reduction in viscosity. Hemorrhage due to needle puncture appears as streaks of blood in the aspirate and uniformly diffused hemorrhage represents an acute trauma, whereas dark yellow or pale amber (xanthochromic) samples represent previous hemorrhage. Intense synovitis associated with infective arthritis results in a serofibrinous or fibrinopurulent sample. Bloody fluid could be observed due to hemorrhage from a severely infected joint [3]. Articular cartilage damage could be differentiated through the level of LDH isoenzyme in equine synovial fluid. The content of LDH4 and LDH5 are high in the articular cartilage. Hence, the increases in these isoenzymes are the characteristic features of cartilage damage [5].

Materials and methods

Horses with a history of lameness and corresponding clinical signs, suggestive of osteoarthritis were identified and confirmed with radiography and ultrasonography. A total of 12 osteoarthritic joints involving knee (3), fetlock (3), hock (3), and coffin joint (3) were included in this study.

Collection

Synovial fluid (2ml) was collected in both EDTA and plain tubes and analyzed within one hour of collection (figure 6).

Appearance

Colour and turbidity of the synovial fluid was evaluated at the time of collection.

Viscosity

Viscosity was analyzed by placing a drop of synovial fluid between the thumb and forefinger, slowly pulling the fingers apart and observing the strand of synovial fluid that forms between fingers.

Mucin clot test

This test was performed by mixing about 1 part of the supernatant from centrifuged synovial fluid with 4 parts of 2.5% glacial acetic acid. After gently mixing, the clumped mucin was observed. A four level subjective grading scheme was used for recording results of mucin clot test [2, 3].

Biochemical Analysis

Alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransaminase (ALT), lactate dehydrogenase (LDH) and total protein (TP) were analyzed in A15 Bio-systems analyzer by using readymade diagnostic reagent kits supplied by M/s. Agappe Diagnostics Limited, Kerala, India.

Results and Discussion

Appearance

The colors were categorized as pale yellow, straw yellow, dark yellow and bloody yellow. Seven joint fluids were pale yellow in color (58%). Straw yellow color was found in one joint (8%). Both dark yellow and bloody yellow was observed in each two joints (17% each).

Fifty eight percent of the joints had pale yellow colored synovial fluid which is considered normal colour. The aspirate from two joints had streaks of blood which could possibly be due to hemorrhage at the time of needle puncture [2, 3, 7].

Volume

The collected synovial fluid volume varied from 1 to 2 ml. Four horses showed apparently increased volume of synovial fluid and one joint yielded proportionately smaller volume.

The volume of synovial fluid collected varied in direct proportion to the size of the joints [7].

In the present study, four horses showed apparently increased volume of synovial fluid which could be due to acute synovitis correlating with history of single event trauma. One joint yielded proportionately smaller volume which could be due to chronic OA [3].

Viscosity

A total of nine joint fluids had high viscosity (75%) (figure. 1) and three joint fluids had low viscosity (25%) (figure. 2). Of these, two joints were associated with recent trauma and were highly swollen. Osteoarthritis decreased the synovial fluid content and polymerization of HA resulting in thinner, less viscous synovial fluid.

Poor viscosity generally indicates the presence of inflammation [1] and the viscosity of synovial fluid was directly related to the quantity and degree of polymerization of HA [4].

Mucin clot test

Mucin clot test was graded as good for seven joint synovial fluids (58%) (figure. 3). Two joints each had fair and poor clot formation (each 17%) (Figure. 4 and Figure. 3). One joint showed very poor clot formation (8%) (Figure. 5).

Mucin clot test was graded as good in seven joints, two joints were fair, two were poor and one was very poor. The two joints graded as poor and one joint graded as very poor were also poor in viscosity.

The present study showed that there was a directly proportionate correlation between viscosity and mucin clot test.

Bio-chemical parameters of serum and synovial fluid

Table 1: Mean \pm SE of ALP, ALT, AST LDH and TP values of serum and synovial fluid

Parameters	Serum	SF	t- Test	P- Value	Result
	MEAN \pm SE	MEAN \pm SE			
ALP(U/L)	304.92 \pm 39.82	177.17 \pm 40.86	2.24	0.0356	*
ALT(U/L)	30.92 \pm 5.36	19.17 \pm 5.03	1.60	0.1240	NS
AST(U/L)	166.92 \pm 32.63	126.92 \pm 23.16	1.00	0.3284	NS
LDH(U/L)	311.92 \pm 48.43	320.00 \pm 93.64	0.08	0.9396	NS
TP (g/dL)	6.95 \pm 0.20	2.30 \pm 0.23	15.43	0.000	**

(* Significant difference $P < 0.05$, ** Highly significant $P < 0.01$ and NS Non-significant $P > 0.05$)

The mean total protein of synovial fluid was 2.30 \pm 0.2 g/dL. This is slightly elevated from normal value of 1.37 \pm 0.09 g/dL [7]. The protein concentration in most degenerative and traumatic joint diseases varied from unchanged to slightly increased [2]. The mean ALP, ALT, AST and LDH values showed no significant difference between serum and synovial fluid parameters. The mean ALP, ALT, AST and LDH values showed no significant difference between serum and synovial fluid parameters. But it should have highly significant lower values of ALP, ALT, AST and LDH in synovial fluid when compared with serum values in normal condition [7]. The increased activity of enzyme in the synovial fluid was probably due to release of enzyme from leukocytes, necrotic and inflamed synovial tissue [3]. In this study the elevation in the biochemical parameters could be due to increased severity of disease progression [3, 7]. Two horses with high synovial fluid LDH value showed radiographic and ultrasonographic evidence of articular cartilage damage. This could be due to cartilage damage [5].

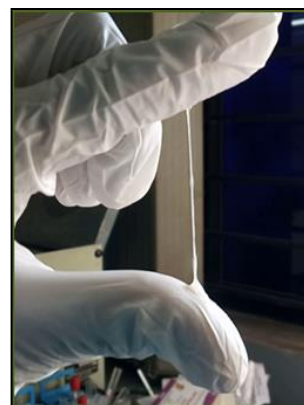


Fig 1: Synovial fluid - High Viscosity



Fig 2: Synovial fluid - Low Viscosity

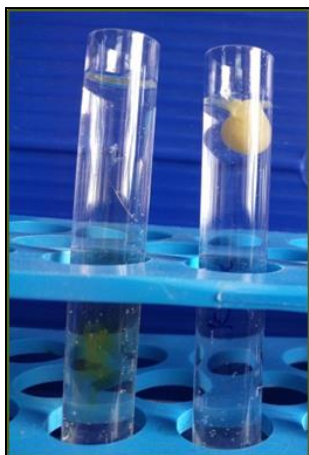


Fig 3: Mucin clot test a) Poor, b) Good



Fig 4: Mucin clot test – Fair



Fig 5: Mucin clot test – Very poor



Fig 6: Synovial fluid collection in Plain and EDTA tube

Conclusion

Viscosity and mucin clot test were useful in identifying synovitis and osteoarthritis. The serum biochemical values served no role in diagnosing the osteoarthritic condition. However, biochemical values of synovial fluid had some diagnostic value in identifying osteoarthritis.

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