Iranian journal of ruminants health research (2017), 2(1):31-37.
Doi: 10.22055/ijrhr.2017.14415

Comparative study of synovial fluid and serum protein electrophoretic pattern of river buffalo (*Bubalus bubalis*) in Ahvaz

Razi Jalali, M.*, Baniadam, A., Kaviani, F.

Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

****Correspondence****: jalali_m@scu.ac.ir*

Abstract

Electrophoresis is a meticulous method in para-clinical science but has received less attention. In this study, the protein profile of synovial fluid of carpal joint and blood serum in buffalo was detected. Sampling was performed by arthrocentesis of 80 left and right forelimbs and obtaining blood serums of 40 *Bubalus bubalis* in Ahvaz industrial slaughterhouse. Samples were stratified by gender, age and left and right forelimbs. At first, the health of samples was confirmed by measuring the physical parameters of synovial fluid such as color, viscosity, transparency, and quality of mucin clot. Then, total protein was measured by Biuret method. Separating of synovial fluid protein fractions (include: albumin, alpha globulin, beta globulin, gamma globulin) was performed by electrophoresis method with cellogel kit. Statistical analysis showed that total protein, albumin, alpha globulin, beta globulin, gamma globulin in synovial fluid were significantly lower than the serum (P<0.05). The statistical analysis showed that the measured parameters in relation to age, gender and the limbs had no significant difference. Although synovial fluid, like all other biological fluid, is structurally similar to serum but is slightly different following disorders and diseases, nature and quantity of some these proteins change and analysis of the fluid can be used to identify trends of disease, prediction of the development and evaluation of the treatment processes.

Keywords: Synovial fluid, Protein, Electrophoresis, River buffalo, Bubalus bubalis

Introduction

Buffalo is a native animal, particularly in rural areas, has a long history and is present in many countries of the world. Iran's buffalo is the best series buffalo of Asia, mainly is developing in the provinces of North West, North and South West in Iran (Borghese, 1987). Doing different studies in different areas among the analysis markers inserts in biological fluids can be valuable results towards a better understanding of the physiology and providing appropriate solutions in order to diagnose different diseases in buffalo. Limbs have an essential role in animal movements that are formed of joints, bones, ligaments, muscles, tendons, nerves and blood vessels. Damage to these organs finally appears lameness. In fact, lameness is not a sign of certain diseases, but generally represents disorders or injuries to joints, bones, ligaments, muscles, tendons and nerves, abnormal limbs defects are related to severe trauma, infectious diseases, and immunological disorders or is metabolic disturbances (Kumar *et al*., 2001; Stashak, 2002). Synovial fluid, which is produced by an internal layer of the joint capsule, is a lubricant and soft liquid and attracts the pressure in the joints. Any lesion in the joint capsule causes lameness and the devaluation of animals. In recent years, the synovial fluid analysis is important as an aid in the diagnosis, prediction and evaluation of joint disease and injury. But because of various causes is not practical; for example, the lack of access to laboratory equipment, the high costs of experiment and the long time to do the experiment.

,
Progress in clinical pathology and introduction of new procedures, these tests are increasingly common (Latimer *et al.*, 2003).

In the case of disease, natural exchange of material between the vascular system, the lymphatic and synovial fluid is impaired (Coles, 1986). Therefore, synovial fluid tests could provide useful information about changes that occurred within the joint. Among biochemical markers proteins that may be changed in different situations in the synovial fluid. The status of joint fluid compared to serum proteins could be useful in assessing joint health (Madison *et al.*, 1991). In this study, the electrophoretic pattern of carpal joint synovial fluid is compared with serum in apparently healthy buffaloes.

Materials and methods

In order to do this research, forty, apparently healthy, river buffaloes referred to Ahvaz slaughterhouse were used. Before slaughtering, the animals were examined for examination of the limb health. The sex of buffaloes was registered and maturity was detected according to teeth formula. Immediately after slaughtering, venous blood samples were transferred to a clean test tube without anticoagulant. Synovial fluid from both limbs (40 samples from forelimb and 40 samples from hind limb) were collected by arthrocentesis, using a 20 mL syringe and an 18 needle. Physical properties of synovial fluid samples, such as gross appearance, mucin clot test and viscosity were examined. The sera of the blood samples were isolated with the centrifuge (3000 RPM for 10 minutes) and stored in -20° C until the time of the serum biochemical analysis.

The concentration of total protein in synovial fluid and blood serum was measured by the Biuret method. The measurement of serum protein concentration is one of the most frequent routine analyses performed to investigate metabolic disorders, inflammatory or infectious diseases, colostrum intake, tumors, etc. Its determination is also a prerequisite of protein electrophoresis. The routine measurement of plasma total protein concentration (P-Protein) was commonly performed by the biuret method based on the formation of copper chelates by the ionized peptide bonds of proteins at alkaline pH.

Serum protein electrophoresis (SPEP) is an easy, inexpensive method of separating proteins based on their net charge, size, and shape. The two major types of protein present in the serum are albumin and the globulin proteins. Albumin is the major protein component of serum and represents the largest peak that lies closest to the positive electrode. Globulins comprise a much smaller fraction of the total serum protein but represent the primary focus of interpretation of serum protein electrophoresis. Bovine globulin categories are represented: alpha, beta and gamma, with the gamma fraction being closest to the negative electrode. Synovial fluid and blood serum fractions of protein were detected by electrophoresis. After calculating, various amounts of each protein fractions were compared by using statistical test (SPSS-24, T-test and ANOVA).

Results

In this research, samples were taken from 40 river buffaloes (25 males and 15 females, 20 mature and 20 immature). Serum and synovial fluid samples obtained from both the right and left carpal joint for increasing the number of samples and make the data more valuable. At first, physical parameters of synovial fluids were studied in order to be confident of the health of carpal joints. Carpal joint synovial fluids were clear, colorless, had normal viscosity and mucin clot had good quality. In this study, comparison of the total protein of carpal joint fluid in both mature and immature buffaloes showed that

,
mean total protein were 1.09±0.10 and 0.88±0.08 gr/dl respectively, but the difference was not significant (Table 2). The statistical analysis showed that the difference in the amount of albumin, beta and gamma globulins between mature and immature groups were not significant (Table 2). The only fraction which showed a significant difference was alpha globulin (P<0.05). Also, carpal synovial fluid was tested in two groups of male and female. The amount of total protein in synovial fluid was measured 1.02±0.12 gr/dl in females and 0.78±0.08 gr/dl in males (Table 1), which the difference was not statistically significant (P>0.05). One of the main objectives this study away from identification of carpal joint fluid protein structure was comparing it with the blood serum. Based on physiological processes and according to joint structure, it was expected that the parameters, especially proteins that have a unique structure, be different between synovial fluid and serum.

The results of this study and previous studies have found that in buffalo, like most animals, sex and age had no significant effects on the total protein of synovial fluid. Statistical analysis showed that all parameters such as total protein, albumin, alpha globulins, beta globulins and gamma globulins in serum and carpal synovial fluid are different (P<0.05). Means of total protein in synovial fluid and serum of buffaloes were 0.99±0.67 and 6.94±0.16 gr/dl respectively (Table 3). In this study, total protein in both left and the right carpal joint was tested, the result showed no significant difference.

Table 1: Mean ± SE of protein fractions of both synovial fluid in male and female buffaloes

Protein / Sex	Total protein gr/dl	Albumin gr/dl	Alpha globulin gr/dl	Beta globulin gr/dl	Gama globulin gr/dl
Female	1.02± 0.12	0.77± 0.07	0.11±0.02	0.07± 0.02	0.09± 0.03
Male	0.78± 0.08	0.71± 0.06	0.08± 0.01	0,05± 0.01	0.05± 0.01

Table 2: Mean ± SE of protein fractions of both synovial fluid in mature and immature buffaloes

Protein / age	Total protein gr/dl	Albumin gr/dl	Alpha globulin gr/dl	Beta globulin gr/dl	Gama globulin gr/dl
Immature	0.88± 0.08	0.73± 0.06	0.06± 0.01	0.05± 0.01	0.04± 0.01
Mature	1.09± 0.10	0.85± 0.06	*0.12± 0.01	0.07± 0.01	0.09± 0.02

Table 3: Mean ± SE of protein fractions of synovial fluid in right and left limb of buffaloes

Protein / Fore limb	Total protein gr/dl	Albumin gr/dl	Alpha globulin gr/dl	Beta globulin gr/dl	Gama globulin gr/dl
Rright	1.02± 0.07	0.83± 0.05	0.08± 0.01	0.06± 0.01	0.05± 0.01
Left	0.97± 0.07	0.76± 0.05	0.09± 0.01	0.07± 0.01	0.07± 0.01

Table 4: Mean ± SE of protein fractions of both synovial fluid and serum of buffaloes

protein / sample	Total protein gr/dl	Albumin gr/dl	Alpha globulin gr/dl	Beta globulin gr/dl	Gama globulin gr/dl
Serum	*6.94± 0.16	*3.32± 0.10	*1.20± 0.04	*0.76± 0.03	*1.67± 0.08
Synovial fluid	0.99± 0.67	0.78± 0.04	0.09± 0.01	0.06± 0.01	0.06± 0.02

Discussion

,

Limb diseases are considered one of the most important topics in veterinary medicine. Different diseases that involve joints cause lameness. Synovial fluid analysis can provide valuable information on the cause of the disease, type of disease (inflammatory or non-inflammatory) and the way of treatment management (Watkins, 2000; Rohde, 2000). The synovial fluid has a high value in the pathological diagnosis (Swan *et al.*, 2002). Many experiments have been done to diagnose the healthy and unhealthy joints (Matsen, 2011). First reports were limited to the microbiology of synovial fluid and assessment of white blood cell count and pathogenic crystals. But today, further research is done on the biochemical examination and cytology (Swan et al., 2002). Electrophoresis, as a laboratory method, has a high value for the analysis of protein factors in biological fluids and has been used for many years (Smithies, 1959). Nowadays, great efforts have been made to find the relationship between synovial fluid and serum. Cell analysis of blood serum can be useful in the diagnosis of diseases and injuries in joints (Madison *et al.*, 1991). This study showed that the difference in the amount of total protein, albumin, beta and gamma globulins between mature and immature groups was not significant. The only fraction that showed a significant difference, was alpha globulin ($P<0.05$). In this study, synovial fluid was tested on two groups of male and female which total protein was different between two groups, but the difference was non-significant.

Other researchers have been studied the effect of age and gender on the quality of synovial fluid. Nasim *et al*. (2010) in a study about cellular and biochemical parameters of synovial fluid of metatarsophalangeal joint on Iranian fat-tailed sheep found that the concentration of total protein in the male was significantly higher than females. Van Pelt in 1974 reported similar results. In a study of cellular and biochemical properties of the joint fluid and blood serum in adult camels, there was no significant difference between male and female, and the amount of total protein of elbow was more in adults than the young camel. This difference is reported to be due to changes in the joints which occurs during aging (Nazifi *et al.*, 1998; Bani Ismail and Al-Rukibat, 2005). Mojabi *et al.* (1991) in experiments on synovial fluid in both male and female calves were observed no significant difference in mean of particular parameters especially total protein. Baniadam and Razi Jalali in 2005 showed that between male and female buffaloes, mature and immature, as well as left and right carpal joint, there was no significant difference in total protein content. Ameri and Gharib in 2005, in a study, detected that age and gender had no significant effect on the amount of total protein and albumin and globulin in synovial fluid of sheep. One of the main objectives of this study was to identify the synovial fluid protein structure of the carpal joint and comparing it with the blood serum. Based on physiological processes and according to the joint structure characteristics expected that indices in the synovial fluid, especially proteins that is has a unique molecular structure, to be different in synovial fluid and serum.

The results of this study and previous studies found that in buffalo, like most animals, sex and age had no significant effects on the total protein of synovial fluid. Statistical analysis showed that all parameters such as total protein, albumin, alpha globulins, beta globulins and gamma globulins in serum and synovial fluid of carpal joint are different significantly ($P<0.05$). Mean of total protein in synovial fluid was 0.99± 0.67 gr/dl and in serum 6.94± 0.16 gr/dl. A similar survey was also conducted in other animals.

Total protein of synovial fluid, in metacarpophalangeal joint of healthy horses reported

,
1.26 ± 0.15 gr/dl, which was significantly lower than in serum. (Nazifi *et al.,* 1998).

Van Pelt (1974) showed that the protein of synovial fluid is 1.081± 0.26 gr/ dl in the horse. Nazifi et al. (1998) in a research on physical indicators, biochemical and cytology of synovial fluid and blood serum of healthy adult camels, measured the total protein of elbow to be 2.54 ± 0.16 gr/dl. Sharifikhatire et al. (2009) in a study that compared blood biochemical parameters and synovial fluid founded that synovial fluid has significantly lower protein than serum; the researchers believe that this biomarker can be used for dilution method.

Nasim *et al.* (2010) reported that total protein of metacarpophalangeal joint in healthy Iranian fat-tailed sheep was 1.66±0.07gr/dl. Khazrainia and colleagues in 2009 also reported the amount of total protein in cow 1.18 ± 0.25 gr/dl. The total protein of synovial fluid increases significantly in bacterial, mycoplasma and yeast infection of joints (Khazrainia *et al.,* 2009), sometimes this amount is as much as serum proteins (Jacquse *et al.,* 2002; Vishal, 2011); this increase is not only in synovial fluid protein but is also seen in serum total protein (Decker *et al.,* 1959), which depends on the size of protein molecules, plasma concentration and the amount of peripheral vascular permeability (Jacquse *et al.,* 2002; Vishal, 2011).

In disease condition of the synovial fluid, protein increasing focuses more on alpha-2 (Decker *et al.,* 1959; Tetta *et al.,* 1990), that helps in diagnosis and in determining its intensity (Najizadeh et al., 2014). In this study, total protein was tested in both left and right carpal joint, which showed no significant difference. Pacchiani *et al.* (2004) in the assessment of stifle and shoulder joints in cat reported that there is no significant difference between the synovial fluid of right and left joints and between to different joint. Similar results on camel and cat were obtained (Bani Ismail and Al-Rukibat, 2005). In studies of different animals such as dog (Sawyer, 1963, Jacques 2002), alpaca and lama (Richard *et al.,* 2002), camel (Nazifi *et al.,* 1998), cow (Mojabi *et al.,* 1991) and buffalo (Baniadam and Razi Jalali, 1998) did not observe significant difference in total protein between left and right limb. The amount of protein increases in inflammatory condition and trauma to the joint and tendon which can be used in diagnosis. In non-infective acute inflammation, albumin decreases, but in non-infective chronic inflammation albumin and β-globulin increases. Proteins other than mucin become twice in trauma to the joint and degeneration in joints (Chauhan, 1995). The composition of plasma proteins, except some of them, directly derives from plasma. It has been found by electrophoresis that composition of proteins of blood plasma and synovial fluid are completely similar. Kushner (1991), by radioimmune diffusion, demonstrated that α-1-glycoprotein, transferrin, ceruloplasmin, and α-2-macroglobulin are similar in two fluids and have equal molecular weight. They also found in rheumatoid arthritis, the concentration of α-1-glycoprotein, transferrin, ceruloplasmin increases three times and α-2-macroglobulin increases 10 times than normal. This researcher claimed that the transition of plasma protein to synovial fluid depends to size, molecular shape and the degree of inflammation. The transition of proteins with molecular weight less than 100 Kilodalton do not relate to molecular weight, but the transition of materials with greater molecular weight depends to the degree of the inflammation in such condition like rheumatoid arthritis (Mojabi, *et al.,* 1991). Synovial fluid, like other biological fluids, is similar to serum but selective transportation of material from joint capsule and unique function of synovial fluid for protection of joint cause the quantity of materials in serum and synovial fluid be

,
different. In joint disorders, the quantity of some proteins by transportation from plasma and production in joint in conditions of inflammation, degeneration and infection change. Disease process, the prognosis of development of disease and assessment of treatment joint disorders can be known by analysis of the synovial fluid.

References

Ameri, M. & Gharib, Z. (2005) Analysis of synovial fluid from clinically healthy Iranian fat-tailed sheep. Comp. Clin. Path. 13, 4, 186-189.

Baniadam, A. & Razi Jalali, M. (2005) Studies on the normal synovial fluid from the carpal joints of Iranian water buffaloes: physical and biochemical parameters. Iran. J. Vet. Res. 6, 17-20.

Bani Ismail, Z. & Al-Rukibat, R. (2006) Synovial fluid cell counts and total protein concentration in clinically normal fetlock joints of young dromedarian camels. J. Vet. Med. A. 53, 5, 263-265.

Chauhan, RS. (1995) Veterinary Clinical and Laboratory Diagnosis. Jaypee Brothers Medical, New Dehli, Vol. 1, 1th ed. PP: 20-25.

Decker, B., McKenzie, B.F., McGuckin, WF. & Slocumb, CH. (1959) Comparative distribution of proteins and glycoproteins of serum and synovial fluid. Arthritis. Rheum. 2, 2, 162-177.

Khazraneian, P., gharaghozlou, M.J., Nazifi, S., Najafi, J., Hasani Tabatabaei, M. & Youssefy, P. (2009). Microbiological and clinicopathological studies on bovine arthritis in Iran. J. Vet. Res. 61, 1, 33-38.

Kumar, M. & Agarwal, K. (2001) Biochemical alterations of synovial fluid in induced traumatic arthritis in buffalo calves (*Bubalus bubalis*). Indian. J. Vet. Surg. 22, 2, 87-91.

Kumar, M. & Agrawal, K. (2001) Synovial fluid changes following intra-articular synovial fluid transfusion in induced traumatic arthritis in buffalo calves (*Bubalus bubalis*). Indian. J. Anim. Sci. 71, 12, 1153-1155.

Latimer, K., Mahaffey, E. & Prasse, K. (2003) Clinical Pathology: Veterinary Laboratory Medicine. Iowa State Press, Iowa, USA. PP: 27-89.

Madison, J. Sommer, M. & Spencer, P. (1991) Relations among synovial membrane histopathologic findings, synovial fluid cytologic findings, and bacterial culture results in horses with suspected infectious arthritis: 64 cases (1979-1987). J. Am. Vet. Med. Assoc. 198, 9, 1655-1661.

Mojabi, A., Norouzian, I. & Chavoushi, M.(1991) Evaluation of some non-electrolytes in normal synovial fluid of cow. J. Vet. Res. 46, 3-4, 9-28.

Najizadeh, MH., Pourjafar, M., Chalmeh, AA., Badiei, K., Nazifi, S. & Naghib, M. (2014) Synovial fluid inflammatory cytokines and proteins in clinically healthy and arthritic joint of dromedary camels (*Camelus dromedarius*). Iran. J. Vet. Med. 8, 1, 53-57.

Nasim, S., Nazifi, S. & Meimandi Parizi, A. (2010) Cellular and biochemical parameters of synovial fluid of fetlock joint clinically normal Iranian fat-tailed sheep. J. Vet. Lab. Res. 1, 2, 115-124.

,

Nazifi, S., Rezakhani, A. & Gheisari, H. (1998). Physical, biochemical and cytologic properties of blood and synovial fluid in clinically normal adult camel (*Camelus dromedarius*). J. Vet. Med. A. 45, 1-10, 155-160.

Pacchiana, PD., Gilley, RS., Wallace, LJ., Hayden, DW., Feeney, DA., Jessen, CR. & Aird, B. (2004) Absolute and relative cell counts for synovial fluid from clinically normal shoulder and stifle joints in cats. J. Am. Vet. Med. Assoc. 225, 12, 1866.

Rohde, C., Anderson, D.E., Desrochers, A., St-jean, G., Hull, BL. & Rings, M. (2000) Synovial fluid analysis in cattle: a review of 130 cases. Vet. Surg. 29, 341-346.

Sharifi Khatir, A., Qujeq, D. & Yousef Ghahhary, B. (2009) Determination of lactate level in serum and joint knee synovial fluid in patients with arthritis. Q. Horizon. Med. Sci. 15, 2, 40-45.

Smithies, O. (1959) Zone Electrophoresis in Starch Gels and its Application to Studies of Serum Proteins. Advances in Protein Chemistry. Vol. 14. PP: 65-113.

Stashak, T.S. (2002) Adams Lameness in Horses. Lippincott Williams and Wilkins, Philadelphia, 5th ed. PP: 349-357.

Swan, A., Amer, H. & Dieppe, P. (2002) The value of synovial fluid assays in the diagnosis of joint disease: a literature survey. Ann. Rheum. Dis. 61, 493-498.

Tetta, C., Camussi, G., Modena, V., Divittorio, C. & Baglioni, C. (1990) Tumor necrosis factor in serum and synovial fluid of patients with active and severe rheumatoid arthritis. Ann. Rheum. Dis, 49, 665-667.

Van Pelt, R. (1974) Interpretation of synovial fluid findings in the horse. J. Am. Vet. Med. Assoc. 165, 1, 91-95.

Vishal, A.A., Mishra, A. & Raychaudhuri, SR. (2011). A Double Blind, Randomized, Placebo Controlled Clinical Study Evaluates the Early Efficacy of Aflapin® in Subjects with Osteoarthritis of Knee. Int. J. Med. Sci. 8, 7, 615-622.

Watkins, G. H. (2000). Arthritis, In: Martin, W. B. and Aitken, I. D., Diseases of Sheep, Blackwell, Oxford, 3th ed. PP: 249–253.