RESEARCH ARTICLE

Studies on Biochemical Changes as an Indicator for Fracture Healing in Goats

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Abstract

Goats having a long bone fracture (n=6) brought to Teaching Veterinary Clinical Complex, Jabalpur (MP, India) were included in the present study. Biochemical attributes were recorded preoperatively and on day 7th, 15th, 30th, 45th, 60th and 90th post-operative after immobilizing the fracture segment with dynamic compression plate. An initial increase in values of alkaline phosphatase followed by its decrease till 60th day and again an increase up to 90th day was observed during fracture healing. Level of creatinine kinase showed a decreasing trend. Gradual decrease in the values of serum calcium was observed till 45th post-operative day after which an elevation was observed. Significant decrease in the value of serum phosphorus was observed on 7th post-operative day and this decrease continued till 90th post-operative day. The values of total protein increased gradually from 15th day onwards. Blood glucose level fluctuated within normal range. From the above results it could be concluded that biochemical parameters play a pivotal role to ascertain the phase of fracture healing.

Keywords: Alkaline phosphatase, Blood glucose, Creatinine kinase, Fracture, Goat, Serum minerals, Total protein.

Ind J Vet Sci and Biotech (2024): 10.48165/ijvsbt.20.1.06

Introduction

Traumatic injuries are the major source of bone fracture. These injuries can occur in several situations and impair the patients ability to perform their normal day to day activities. Fracture healing is a complex physiological process, caused by the interaction of cellular elements that are activated and controlled by an array of cytokines and signaling proteins (Giannoudis *et al.,* 2007). This process is both temporal and spatial in nature and usually results in the formation of new bone, which is structurally and mechanically similar to the pre-fracture state (Gerstenfeld, 2003).

Fracture healing can be evaluated clinically and on the basis of radiograph. The correct evaluation of clinical parameters as well as radiographic interpretation is a challenge for the surgeon and hence to enhance his accuracy biochemical attributes often play a pivotal role. Moreover, early detection of fracture healing is neither possible clinically or radiographically and only biochemical parameters are able to forecast whether the healing will be rapid, delayed or will be a case of non-union, thus it provides prognostic value for the early detection of fracture healing complications. Estimation of these biochemical parameters during fracture healing process could enhance the accuracy to pin point exact stage of fracture healing and find out even the meagre risk of fracture healing complications (Cox *et al*., 2010). Early detection of fracture healing complications enables us to prevent them and avoid undesirable suffering by the patient (Coulibaly *et al*. 2011). The present study was therefore aimed to study the biochemical changes as an indicator for fracture healing in goats.

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How to cite this article: Kumar, D., Bhargava, M. K., Aparajita, J., Shahi, A., & Singh, R. (2024). Studies on Biochemical Changes as an Indicator for Fracture Healing in Goats. Ind J Vet Sci and Biotech. 20(1), 27-30.

Source of support: Nil

Conflict of interest: None

Submitted 26/08/2023 **Accepted** 25/10/2023 **Published** 10/01/2024

Materials and Methods

The work was approved by institutional ethical committee (121/AEC/Vety/2015 Dated: 22/05/2015). Six clinical cases of goats having long bone fracture presented at Teaching Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, Jabalpur, during the period May 2015 to June 2016 were included in the study. These goats were of either sex, aged between one to six years having long bone fractures, *viz.,* humerus, radius, ulna, femur and tibia fibula. Prior to surgery, the animals were kept off feed for 12h and off water for 6h. The surgical procedure was performed under general anaesthesia using Diazepam hydrochloride @ 0.5 mg/kg body weight intravenously, followed by Ketamine hydrochloride @ 5 mg/kg body weight

till effect. Maintenance of anaesthesia was done by repeated intravenous administration of Ketamine hydrochloride as per the requirement. The animals were administered amoxicillin + sulbactam @ 10 mg/kg b. wt., I/M, twice a day and continued up to 7th post-operative day. Similarly, Meloxicam @ 0.3 mg/kg b. wt., I/M, was administered for four post-operative days in all the six goats each having one long bone fracture.

Five milliliter of blood was collected from jugular vein of each animal pre-operatively and on 7th, 15th, 30th, 45^{th,} 60th and 90th post-operative day for analysis of biochemical attributes. Serum biochemical parameters such as blood glucose, creatinine kinase, alkaline phosphatase, serum calcium, serum phosphorus and total protein were determined by using semi-automatic analyzer and standard kits. The data was analyzed statistically using one-way ANOVA and Duncan's multiple range test (Snedecor and Cochran, 1994).

Results and Discussion

The changes in the bio-chemical parameters at different time intervals of goats under fracture repair are presented in Table 1.

The pre-operative mean value of alkaline phosphatase which was towards the higher normal physiological limit further increased non-significantly up to 15th post-operative day with maximum value of 423.17±36.87 unit/L. Thereafter it declined reaching minimum value of 194.42±42 unit/L on 60th post-operative day. Thereafter it again increased on 90th post-operative day to 238.42±45.33 unit/L (Table 1). These observations were in close agreement with the findings of Rani and Ganesh (2003) in goats and Phaneedra *et al*. (2016) and Bhavani *et al*. (2022) in dogs. In present study, increase in the level of alkaline phosphatase up to 15th post-operative day might be due to increased osteoblastic activity. Osteoblast secretes large quantities of alkaline phosphatase, which is involved in the process of bone matrix formation and its mineralization. Alkaline phosphatase is believed to either increase the concentration of local inorganic phosphate or neutralizes inorganic pyrophosphate, which is an inhibitor of hydroxyapatite crystals that are necessary for fracture healing (Volpin *et al*., 1998). The increase in serum alkaline phosphatase level is also attributed to increased chondroblastic proliferation, to cause bone formation during healing of fracture with maximum contribution from the periosteum of destructed bone, which is a rich source of serum alkaline phosphatase (Rani and Ganesh, 2003; Phaneedra *et al*., 2016). Decrease in the value of alkaline phosphatase might be indicative of cessation of osteoblastic activity and receding of the values towards its base value may be due to ossification and consolidation of fractured bone. After 60th day interval, there was a gradual increase in the values of alkaline phosphatase till 90th day, which might be due to the fact that remodeling phase might have started after this time interval.

The pre-operative mean value (unit/L) of creatinine kinase (223.15±4.55) decreased significantly by 15th post-operative day (115.84±6.84), and thereafter further gradually decreased without significant differences to the least value of 65.33±7.79 on 90th post-operative day. The present findings corroborated with the report of Laurence (2000), who observed increased activity of creatinine kinase after surgery, which receded back to its normal value after healing. He attributed it to the extent of muscle damage due to surgery, and concluded that serum creatine kinase estimation could be a valid indicator of the extent of muscle damage. Higher value of creatinine kinase after the fracture and before the surgical intervention could be attributed to extensive muscle damage due to fracture. These increased values gradually returned to its normal reference range as the healing of injured tissue progressed at later intervals.

The pre-operative mean value of serum calcium decreased gradually and non-significantly reaching its minimum value

Table 1: Mean (± SE) values of biochemical parameters of goats (n= 6) at different time intervals

Days	Alkaline phosphatase (unit/ L)	Creatinine kinase (unit/L)	Serum calcium (mg/dL)	Serum phosphorus (mg/dL)	Total protein (g/dL)	Blood glucose (mg/dL)
0th	374.10^{a} ±24.92	223.15^{a} ±4.55	10.31 ±0.86	9.65^{a} ±0.20	6.09^{a} ±0.08	59.71 ±1.79
7th	401.36^{a} ±33.27	134.58^{b} ±14.24	10.09 ±0.80	8.23^{b} ±0.44	5.60^{a} ±0.21	69.91 ±3.14
15th	423.17^{a} ±36.87	115.84^{b} ±6.84	9.40 ±0.30	7.29^{b} ±0.37	6.73^{a} ±0.32	76.83 ±12.03
30th	302.93^{a} ±30.25	108.67^{b} ±7.66	9.22 ±0.29	7.23^{b} ±0.52	7.49^{b} ±0.62	75.19 ±6.23
45th	200.10^{b} ±36.18	85.98^{b} ±5.46	8.47 ±0.35	6.96^{b} ±0.41	7.17^{b} ±0.34	77.33 ±6.83
60th	194.42^{b} ±62.81	77.33^{b} ±8.74	9.42 ±0.53	6.54^{b} ±0.52	7.15^{b} ±0.40	76.75 ±4.49
90th	238.42^{b} ±45.33	65.33^{b} ±7.79	9.44 ±0.57	6.07^{b} ±0.20	7.29^{b} ±0.27	75.79 ±4.03

Values having different superscripts a,b show significant differences at 5% level between intervals.

on 45th post-operative day. Thereafter, a gradual increase in the value was observed on day 60th and 90th post-operatively (Table 1). These observations were in accordance with the findings of Jawre (2012) in bovines, and Gupta (2015) in goats. Steep increase of serum calcium level in the experimental animals during the entire period of treatment was also observed by Somasundaram (2021). Higher value of serum calcium in the initial stage can be attributed to increased osteoclastic activity, leading to resorption of dead bone. A gradual decrease in its level, till 45th post-operative day interval could be due to deposition of this excessive calcium at the fracture site. Increase in the values of serum calcium from 60th post-operative day onwards, might be due to initiation of remodeling phase. Newton and Nunamaker (1985) were of opinion that acid phosphatase released by osteoclast first cause demineralization, which may be responsible for increase level of calcium in serum and then removal of organic matrix.

The pre-operative mean value of serum phosphorus (9.65±0.20 mg/dL) decreased significantly by 7th post-operative day (8.23±0.44 mg/dL) and thereafter further decreased gradually and non-significantly till 90th day of surgery. These findings were in accordance with the report of Rani and Ganesh (2003) in goats. Higher values of serum phosphorus at initial intervals can be attributed to osteoclastic activity leading to resorption of dead bone, thereby resulting in increased level of serum phosphorus, as also observed in the present study.

The mean value of total protein fluctuated non-significantly between 0th and 15th day post-operative, with a slight decline on 7th day, and thereafter increased significantly on day 30th which then fluctuated non-significantly till 90th post-operative day (Table 1). These findings were similar to the observations of Gabriel *et al*. (2014) in goats and Singh (2015) in dogs, who observed protein level towards lower reference range during initial phase of fracture healing and suggested that it might be due to inflammatory condition, which would have decreased daily feed intake of animals, thereafter rise in total protein might be due to improved appetite of the animals, decreased inflammation and initiation of the healing process.

The pre-operatively mean value of blood glucose was the lowest (59.71±1.79 mg/dL), which increased gradually till day 30th day post-operative, and thereafter the values fluctuated within normal physiological limit non significantly. Similar findings were also reported by Kaneko (1997) in goats, De'Souza (2012) in dogs and Jawre (2012) in bovines. They were of opinion that, fluctuation in the values of glucose could be because of variation in regular dietary intake of animals. In the present study initially low level of glucose observed may be due to fasting of the animal before undertaking the surgery. Moreover, disruption of bone and surrounding tissue due to etiology of fracture and surgical procedure would have lead to inflammation in the initial stage, which might have reduced the appetite of the animal and thus decrease in feed intake and the value of blood glucose. Fluctuation of these values within normal physiological limit would be due to return of the animal on its normal diet after completion of inflammatory phase of fracture healing and also due to variation in daily feed intake.

Fracture healing is a continuous physiological process to achieve union of fracture segments. Till date clinically validated method to measure fracture healing is not available. Fracture evaluation clinically and radiographically often is a misleading and hence it should always be supplemented with bio-chemical changes for accurate assessment of fracture healing procedure. From the above findings, it could be concluded that alkaline phosphatase activity gives an accurate picture of stage of fracture healing. Its higher activity in the initial stage is an indication of increased osteoblastic activity which is suggestive of reparative phase, it is followed by its reduced activity an indication of completion of reparative phase and again an increase in its activity, an indicator for initiation of remodeling phase. Creatinine kinase is a sole factor to measure the extent of damage to tissue and its reparative phenomenon. Calcium and phosphorus is an indicator to know the resorption consolidation and remodeling phase of fracture healing. Protein and glucose focuses on daily dietary intake of the animal and its reduced value in initial stage is supportive of trauma, inflammation and pain, and reduced appetite. However at later stage the values of these two arbitraries fluctuated within normal range.

Conclusions

From the findings of present study, it can be concluded that alkaline phoshphatase, calcium and phosphorus can be an imperative indicators to find out the stage of fracture healing, and creatinine kinase can be an important marker to find out the extent of muscle damage. Total protein and glucose indicate the health status of the animal. All these parameters together can predict the outcome of fracture healing.

Acknowledgement

We wish to thank the Honorable Vice Chancellor, NDVSU, Jabalpur; as well as Dean, College of Veterinary Science, Jabalpur, for their constant support. First author further wish to express his gratitude to the Madhya Pradesh Biotechnology Council, Bhopal, for providing funds for the research project "Augmentation of fracture healing in goats using regenerative medicine and bone substitutes", under which this research was conducted.

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