Physiological and haemato-biochemical changes during repair of femur fracture in dogs

Manjunath Patil, Dilipkumar D, Shivaprakash BV, Vivek R Kasaralikar, Vinay P Tikare and Ramesh BK

Abstract
The research was conducted in clinical cases of twenty four dogs with femur fracture and they were randomly divided into three groups viz., group I, II and III with eight animals in each group. Group I, II and III animals were treated with Dynamic compression plating, Locking compression plating and Intramedullary interlocking nailing techniques respectively. Healing of fractures was studied on before operation, immediately after operation, 30th, 60th and 90th post-operative days in 3 groups based on physiological and haemato-biochemical findings. In the present study bradypnoea, bradycardia and hypothermia were found on post-operative days. Progressive increase of packed cell volume, haemoglobin and total erythrocyte count on different post-operative days was seen. Physiological leucocytopenia, neutropenia and relative lymphocytosis was observed on post-operative days. Monocyte and eosinophil count were within physiological range in three groups of animals. Slight hypercalcemia, slight hyperphosphatemia and progressive decrease of serum alkaline phosphatase were found on different post-operative days. However, all physiological and haemato-biochemical findings during repair of femur fracture in dogs were within the normal physiological range in three groups of animals.

Keywords: Physiological, haemato-biochemical, dogs, femur, fracture

Introduction
A fracture is a break in continuity of hard tissues like bone, cartilage. Trauma is the most common cause of fractures in small animals, and can occur due to bending, torsional, shearing and compression forces, eventually resulting in oblique, wedge fragment, spiral or comminuted fractures. Fracture of long bone, particularly femur in dogs is a very painful condition. If timely care is not taken, it may result in complications, i.e., non-union, malunion and damage to muscles, haematoma, seroma, suppuration and gangrene of limb. To avoid such types of complications, cases should be treated at the earliest. Femoral fractures are generally not amenable to conservative repair, and some kind of internal fixation is generally required (Beale, 2004) [10]. The primary goal of fracture treatment is to achieve a healed fracture with normal bone alignment and promote immediate function of the affected limb. To know the healing of fracture presently radiography is routine practice. However, it’s difficult in field conditions to take the x-ray every time after orthopedic operation. Also, getting exposure to x-rays every time is harmful both to the patient and radiographer. So, in this context, other parameters viz., physiological and haemato-biochemical findings has any significant role on healthiness of patient and healing of fractures. More ever, there is a paucity of reports available on role of these parameters with respect to healing of fractures. Therefore, the present paper reports the physiological and haemato-biochemical changes during repair of femur fracture in dogs their by determines the healing of fractures.

Material and methods
Twenty four dogs of 2 months to 3 years of age with femoral fractures were presented to Department of Surgery and Radiology, Veterinary College, KVAFSU, Bidar constituted the present study material. Clinical symptoms before operation were non-weight bearing, swelling on thigh region, evinces crepitating sound and pain on palpation and limping. The similar clinical symptoms were observed by Bennet and May (1995) [11] and Johnson et al. (1998) [10]. These cases were divided into groups I, II and III consisting of eight dogs in each group and were treated with dynamic compression plating, locking compression plating and Intramedullary interlocking nailing techniques respectively based on the type of fracture. Food and water were withdrawn for 12 hours and 6 hours respectively before surgery in all the puppies. The affected limb hairs were clipped and shaved from rump region up to hock region.
and scrubbed with chlorhexidine solution \cite{1} followed by application of surgical spirit. The puppies were given pre-operative antibiotic amoxycillin sodium and cloxacillin sodium \cite{2} injection @ 20 mg per kg body weight intramuscularly and anti-inflammatory meloxicon \cite{3} injection @ 0.5 mg per kg body weight intramuscularly. The puppies were premedicated with atropine sulphate \cite{4} injection @ 0.045 mg per kg body weight intramuscularly, followed by triflupromazine \cite{5} injection @ 1 mg per kg body weight intravenously. After 15 minutes, anaesthesia was induced with 2.5 % thiopental sodium \cite{6} injection intravenously at dose rate of 12.5 mg/ kg body weight. Anaesthesia was maintained using the thiopental sodium given in incremental doses ‘to effect’. The animals were positioned in lateral recumbency with the affected limb above and were adequately draped. Approach of the femur fracture was performed by cranio-lateral incision \cite{Piermatti and Greeley, 1993}. Fracture reduction made mechanically by traction and manipulation. Post-operatively, antibiotic amoxycillin sodium and cloxacillin sodium injection @ 20 mg/kg body weight and meloxicon injection @ 0.5 mg/kg body weight intramuscularly for 8-10 days given. Daily surgical wound dressed aseptically and antiseptic cream (Lorexane \cite{7}) applied. The skin sutures were removed 10- 14 days after operation.

Follow up of the animals was done up to 3 months. The physiological and haemato-biochemical observations were recorded on before operation, immediately after operation, 30\textsuperscript{th} day, 60\textsuperscript{th} day and 90\textsuperscript{th} post-operative days in all groups of animals. The respiratory rate was monitored by observing the movement of rib cage. The heart rate was monitored by auscultation using stethoscope. The rectal temperature was recorded by placing clinical thermometer in the rectum of the dog.

For haematological observations, two ml blood was collected in EDTA coated vials using disposable syringes through cephalic or recurrent tarsal vein in all the cases. The estimations of packed cell volume (%), haemoglobin (g/dL), total erythrocyte count (x 10\textsuperscript{6}/µL) and total leukocyte count (x 10\textsuperscript{9}/µL) were carried out on fully automated haematology cell counter \cite{7}. Blood smear for differential leukocyte count were stained with Giemsa stain and cells were counted using Battlement method as described by Jain \cite{18} and individual cells were expressed in percentage.

For biochemical observations, blood samples were collected in clot activator vials to separate the serum. The parameters \textit{viz.}, serum calcium (mg/dL), serum inorganic phosphorus (mg/dL) and enzyme serum alkaline phosphatase (IU/L) were estimated by using ARTOS \cite{8} biochemical analyzer using respective diagnostic kit. Statistical analysis of data obtained was carried out by employing Student ‘t’ test as per the standard procedure outlined by Snedecor and Cochran \cite{19}. \par

\textbf{Results and discussion} \\
The details of all the physiological and haematological findings in the present study were given in table 1.

\textbf{Physiological Parameters} \\
The physiological parameters like respiratory rate, heart rate and rectal temperature remained elevated on pre-operative day. The respiratory rate and heart rate fluctuated within the normal physiological limits on post-operative days. However, rectal temperature was fluctuated within normal limits from 30\textsuperscript{th} to 90\textsuperscript{th} post-operative days. The elevated physiological parameters on pre-operative days could be attributed to the stress on the animal due to fracture \cite{Kelly, 1974}. \par

\textbf{Haematological Parameters} \\
The Packed cell volume and haemoglobin level on the 60\textsuperscript{th} and 90\textsuperscript{th} post-operative days were statistically higher when compared to pre-operative intervals in all the groups of animals. However, packed cell volume and haemoglobin were fluctuated within the normal physiological limits. The total erythrocyte count was significantly elevated on 60\textsuperscript{th} post-operative day when compared to pre-operative day in all the groups of animals. However, the total erythrocyte count was within the normal physiological limits. This showed that progressive increase of packed cell volume, haemoglobin and total erythrocyte count on post-operative days indicating erythropoiesis, however, within the normal physiological limits. This finding was in agreement with the findings of Singh \textit{et al.} \cite{20} they observed non-significant variation of packed cell volume, haemoglobin and total protein from the base values.

The total leukocyte count was higher on pre-operative day when compared to post-operative days in all the groups of animals. Physiological leucocytopenia was seen in all the groups of animals suggestive of gradual decrease in inflammatory reaction. This finding was in accordance with Maiti \textit{et al.} \cite{21} reported the total leukocyte count was increased significantly from the 1\textsuperscript{st} post-operative day and remained elevated up to the 30\textsuperscript{th} post-operative day in all animals and then gradually returned towards normal level at the 60\textsuperscript{th} post-operative day indicating resolution of inflammation and surgical stress. Leucocytosis occurred in condition where there was corticosteroid release in state of stress, pain, anaesthesia, trauma and surgical manipulation. The differential leucocyte count like neutrophil was significantly decreased on 60\textsuperscript{th} and 90\textsuperscript{th} post-operative day when compared to before operation in all the groups of animals. Contralaterally, the lymphocyte count was statistically increased on 30\textsuperscript{th}, 60\textsuperscript{th} and 90\textsuperscript{th} post-operative days when compared to pre-operative day in all the groups of animals. This showed that, neutropenia with relative lymphocytosis indicating gradual decrease of inflammatory reaction. However, they were within normal physiological limits on different post-operative days in all the groups of animals.

The monocyte count was higher on pre-operative day when compared to post-operative days in all the groups of animals. However, it was fluctuated within normal physiological limits in all the groups of animals. Also, eosinophil count between different post-operative days fluctuated within normal physiological limits.

These findings were in agreement with the findings of Tembharne \textit{et al.} \cite{22} observed that packed cell volume, haemoglobin, total erythrocyte count, total leucocyte count, monocytes, eosinophils and basophils count were within normal physiological range. However, lymphocytopenia was observed on 10\textsuperscript{th} post-operative day on treatment of the femur fracture in canine with the horn-peg prepared from the bovine horn. Also, Hansda \textit{et al.} \cite{23} observed non-significant changes of haemoglobin, total erythrocyte count, total leucocyte count and differential leucocyte count on 0 day and 20\textsuperscript{th} day.

Our present findings were differed with the findings of Nagaraja \cite{24} who observed that neutrophilia, lymphopenia, monocytes and eosinophilia during healing of femur fracture and haemoglobin was in the normal range.
Biochemical parameters
The serum calcium level on the 60th and 90th post-operative days was statistically higher when compared to pre-operative intervals in all groups of animals. However, the serum calcium level was within the higher normal physiological limits. These findings are in agreement with the earlier workers viz., Lauren and Kelly (1969) [23], Pandey and Udapa (1981) [27], Rao (1991) [30] and Vasantha (1991) [38].

The serum inorganic phosphorous level was significantly elevated on 30th, 60th and 90th post-operative days when compared to pre-operative day. However, the serum inorganic phosphorous value was within the normal physiological limits. The results are in agreement with the findings of Henderson and Nobel (1926) [15], Pandey and Udapa (1981) [27], and different from findings of Soliman and Hassan (1964), Vasantha (1991) [38] and Prachasilpchai et al. (2003) [29], who observed a non-significant change in serum phosphorous level during fracture healing.

The serum alkaline phosphatase level was significantly higher on pre-operative day when compared to the post-operative days in all groups of animals. These results are in agreement with the earlier workers Hunsberger and Ferguson (1932) [17], Brinker (1965) [12], Shirfin (1970) [32], Katerjian and Arsenia (1975) [20], Singh et al. (1976) [44], Hosking (1978) [16], Pandey and Udapa (1981) [27], Volpin et al. (1986) [39], Sahay et al. (1988) [31], Vasantha (1991) [38], Maiti et al. (1999) [24], Manjubala et al. (2001) [25], Hegade et al. (2007) [14] and Phaneendra et al. (2016) [26]. The elevated alkaline phosphatase level could be attributed to proliferation of osteogenic cells and maximum contribution from peristium of destructed bone, which is a rich source of alkaline phosphatase. In Group III animals higher level of serum alkaline phosphatase was present when compared to group I and II animals, respectively, this might be due to less stable fixation in group III. This finding differed from the findings of Volpin et al. (1986) [39] and Konmennou et al. (2005) [22].

Table 1: Mean ± SE, values of physiological and haemato-biochemical in different groups of femoral fracture repair in dogs

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Parameter</th>
<th>Groups</th>
<th>Before operation</th>
<th>Immediately after operation</th>
<th>30th day</th>
<th>60th day</th>
<th>90th day</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Respiratory Rate (breaths/min)</td>
<td>I 36.8±2.1cts, II 37.2±2.9cts, III 38.5±1.3cts</td>
<td>24.25±3.78c</td>
<td>27.25±0.60c</td>
<td>26.63±0.83c</td>
<td>25.88±1.13cts</td>
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<tr>
<td>2</td>
<td>Heart Rate (beats/min)</td>
<td>I 101.5±4.5cts, II 106.1±3.2cts, III 111.7±1.6cts</td>
<td>70.25±2.38cts</td>
<td>31.75±0.90cts</td>
<td>30.38±1.55cts</td>
<td>29.13±3.15cts</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Rectal Temperature (°F)</td>
<td>I 102.9±0.23cts, II 103.2±0.24cts, III 104.9±0.16cts</td>
<td>99.38±0.69cts</td>
<td>100.40±0.10cts</td>
<td>101.58±2.23cts</td>
<td>100.25±0.14cts</td>
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<tr>
<td>4</td>
<td>Packed Cell Volume (%)</td>
<td>I 31.5±1.00cts, II 33.6±1.90cts, III 30.9±2.18cts</td>
<td>34.00±0.73cts</td>
<td>44.83±1.42cts</td>
<td>46.23±1.10cts</td>
<td>47.00±0.95cts</td>
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<tr>
<td>5</td>
<td>Haemoglobin (g/dL)</td>
<td>I 9.29±0.18cts, II 9.60±0.29cts, III 10.10±0.39cts</td>
<td>10.13±0.23cts</td>
<td>11.20±0.30cts</td>
<td>12.43±0.38cts</td>
<td>13.15±0.10cts</td>
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<tr>
<td>6</td>
<td>Total Erythrocyte Count (x10^6/µL)</td>
<td>I 5.05±0.18cts, II 5.63±0.25cts, III 5.07±0.23cts</td>
<td>5.51±0.76cts</td>
<td>6.31±0.22cts</td>
<td>7.04±0.12cts</td>
<td>8.05±0.12cts</td>
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<tr>
<td>7</td>
<td>Total Leukocyte Count (x10^3/µL)</td>
<td>I 13.1±0.32cts, II 15.0±0.34cts, III 14.1±0.66cts</td>
<td>12.74±0.72cts</td>
<td>12.63±0.71cts</td>
<td>10.40±0.59cts</td>
<td>8.84±0.27cts</td>
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<tr>
<td>8</td>
<td>Neutrophil (%)</td>
<td>I 78.7±2.16cts, II 80.1±1.54cts, III 75.3±1.37cts</td>
<td>71.88±1.38cts</td>
<td>64.63±1.37cts</td>
<td>65.01±0.86cts</td>
<td>60.38±1.57cts</td>
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<tr>
<td>9</td>
<td>Lymphocyte (%)</td>
<td>I 18.0±1.01cts, II 16.38±0.50cts, III 21.13±1.36cts</td>
<td>25.88±1.33cts</td>
<td>32.63±1.62cts</td>
<td>33.88±1.58cts</td>
<td>34.38±1.46cts</td>
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<td>10</td>
<td>Monocyte (%)</td>
<td>I 1.88±0.48cts, II 1.50±0.31cts, III 2.13±0.12cts</td>
<td>0.88±0.37cts</td>
<td>0.63±0.17cts</td>
<td>1.13±0.12cts</td>
<td>1.38±0.50cts</td>
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<td>11</td>
<td>Eosinophil (%)</td>
<td>I 2.13±0.47cts, II 2.00±0.35cts, III 1.50±0.15cts</td>
<td>1.25±0.15cts</td>
<td>1.80±0.45cts</td>
<td>2.63±0.30cts</td>
<td>2.38±0.25cts</td>
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<tr>
<td>12</td>
<td>Serum Calcium (mg/dL)</td>
<td>I 8.79±2.22cts, II 9.14±1.18cts, III 9.31±0.09cts</td>
<td>9.07±0.19cts</td>
<td>9.81±0.27cts</td>
<td>9.30±0.12cts</td>
<td>11.00±0.17cts</td>
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<tr>
<td>13</td>
<td>Serum Inorganic Phosphorus (mg/dL)</td>
<td>I 2.82±1.13cts, II 3.19±0.07cts, III 3.11±0.09cts</td>
<td>2.95±1.12cts</td>
<td>3.73±1.64cts</td>
<td>4.19±0.17cts</td>
<td>4.68±0.21cts</td>
<td></td>
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<tr>
<td>14</td>
<td>Serum Alkaline Phosphatase (IU/L)</td>
<td>I 120.1±1.88cts, II 118.4±1.29cts, III 122.2±1.79cts</td>
<td>110.35±1.51cts</td>
<td>88.30±1.40cts</td>
<td>78.20±1.13cts</td>
<td>73.19±1.18cts</td>
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</table>

Conclusion
In the present study, respiratory rate, heart rate and rectal temperature were elevated on pre-operative days and there after values declined to normal range suggesting reduction of fracture stress on the animals. Progressive increase of packed cell volume, haemoglobin and total erythrocyte count from immediately after operation to 90th day indicating erythropoiesis. Physiological leucocytopenia, neutropenia with...
relative lymphocytosis on different post-operative days indicating resolution of inflammation and surgical stress. Monocyte and eosinophil counts were within normal physiological limits in all the groups of animals indicating free from anaphylactic reactions. Slight hypercalcemia, slight hyperphosphatemia and progressive decrease of serum alkaline phosphatase on different post-operative days indicating osteosynthesis of bone. In conclusion, the above physiological and haematomo-biochemical findings on different post-operative days helped in knowing the healing of fracture.

References
1. Savlon-Chlorhexidine gluconate solution IP, 1.5% v/v, Johnson and Johnson Ltd., Baddi
2. Moxel-Amoxycillin sodium and Cloxacillin sodium IP, 300 mg/ml, 3g vial, Alembic pharmaceuticals Ltd., Vadodara.
3. Melonex-Meloxicam IP, 5 mg/ ml, Intas pharmaceuticals Ltd., Ahmedabad
4. Atropine sulphate-Atropine sulphate IP, 1mg/ml, 10ml vial, Domesto Ltd., Vijayawada.
5. Siquil-Triflupromazine HCI IP, 20 mg/ml, 5 ml vial, Sarabhai Chemicals Ltd., Vadodara.
6. Thiosol Sodium- Thiopentone sodium IP 500 mg vial, Neon Laboratories Ltd., Mumbai.
7. Lorexane-Gamma Benzene Hexachloride and Profavine Hemisulphate with Cetrimide cream IP 0.10 % w/w, Virbac Animal Health India Pvt Ltd., Mumbai.
9. ARTOS® Biochemical analyser and kits, M/s Swemed diagnostics, Bengaluru, India.
