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# Evaluation of Vitamin $D_3$ - Calcium Therapy in Rachitic Dogs

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**ABSTRACT**

This study was designed to evaluate the influence of treatment on clinical signs, cortical indices (CI) of radius and ulna (measured radiographically), and serum concentrations of the Calcium (Ca), Phosphorus (P), alkaline phosphatase (ALP), 25-Hydroxyvitamin D and osteocalcin (OC, bone biomarker) in 12 rachitic dogs. The dogs were treated with vitamin $D_3$ @ 20, 000 IU/kg body weight (BW) intramuscularly at a weekly interval along with daily oral Ca supplementation @ 25 mg/kg BW for two months. Dogs were re-evaluated at 30$^{th}$ and/or 60$^{th}$ day post-treatment. Nine apparently healthy dogs were kept as control for comparison of CI and biochemical parameters. Data were analysed by paired and two-sample *t*-tests. Results showed appreciable improvement in most of the clinical signs within the 1$^{st}$ month of treatment. When compared to control group, at day 0, serum P concentration did not vary significantly ($p<0.05$), CI and serum concentrations of Ca, 25-Hydroxyvitamin D and OC were lower ($p<0.05$), while ALP was higher ($p<0.05$) in rachitic dogs. Moreover, during the treatment period, serum P concentration did not change significantly ($p>0.05$) while serum Ca increased ($p<0.05$) and ALP decreased ($p<0.05$) from the baseline values. There were significant differences between the pre- and post-treatment values of CI and Ca to P ratio ($p<0.05$). Serum concentrations of 25-Hydroxyvitamin D and OC increased post-treatment at day 60 ($p<0.05$). In this study, treatment with vitamin $D_3$ and Ca stimulated bone remodelling, as demonstrated clinically and by significant increase in the serum concentrations Ca, 25-Hydroxyvitamin D and OC.

**Keywords**: Rachitic dogs, cortical index, calcium, 25-Hydrpxyvitamin D, osteocalcin

Rickets is an important metabolic disease commonly seen in young growing dogs due to their rapid skeletal growth (Dittmer and Thompson, 2011). In pups, there is low availability of minerals such as calcium (Ca) or phosphorus (P) from the diet and this may be due to inadequate concentration, impaired absorption of minerals, and hypovitaminosis $D_3$ due to dietary deficiency (McMillan *et al*., 2006) or hereditary error in metabolism of vitamin $D_3$ (Dittmer and Thompson, 2011). Also, dogs cannot synthesis vitamin $D_3$ in their skin under the influence of ultraviolet light (How *et al*., 1994), so they are fully dependent on their dietary intake of vitamin D

Vitamin D metabolites play a vital role in active Ca absorption and skeletal mineralization, especially when dietary mineral content is low (Dittmer and Thompson, 2011). Faulty mineralization in bones causes wide-ranging deformities such as bowing of the radius and ulna, reduced bone density and thickening of distal metaphyses. Loss of bone is more pronounced near and at the end of the cortex leading to cortical wall thinning and medullary cavity expansion. Cortical wall thinning is of primary concern in osteoporosis due to the strong deterioration of bone mechanical properties. These bone deformities could be easily observed on clinical and radiographical examination (Kushwaha *et al*., 2009).

Mostly, diagnosis of rickets is based on the clinical and radiographic signs of limb and bone deformities, and serum biochemical alterations in Ca, P, alkaline phosphatase (ALP) activity, and 25-hydroxyvitamin D. Osteocalcin (OC), a bone biomarker, was also found to be lower in vitamin $D_3$ deficient dogs (Dittmer and Thompson, 2011).

Different treatment regimens have been followed for the management of rickets with variable results (Parthi *et al*., 2008; Kushwaha *et al*., 2009; Arora *et al.,* 2012). Treatment is successful in those cases where the bone is still in growing phase. Most cases respond to dietary supplementation of Ca, P and vitamin D within 2-3 months, but major bone deformities usually persist (McMillan *et al*., 2006). Hence present study was undertaken to evaluate the therapeutic efficacy of a combination of injectable vitamin $D_3$ and oral Ca in rectification of clinical, radiologic and biochemical alterations in rachitic dogs.

## MATERIALS AND METHODS

The study was conducted at small animal clinics, University Teaching Veterinary Hospital, Ludhiana, India from March 2014 to February 2015. A total of 375 young growing dogs (aged <1 year) presented for primary complaint of skeletal and gait deformities were examined for severity (normal, mild, moderate and severe) of clinical signs of lameness, pain on palpation of the limbs, bending/bowing of long bones, broadening of the distal metaphyses, and hind quarter weakness. Those showing moderate to severe clinical signs were subjected to radiography of the most affected limb (s) in an anterio-posterior plane and rickets was suspected on the basis of presence of bone deformities such as widening of physeal growth plate, thinning of cortex and poor mineralization of skeleton.

For further confirmation, approximately 5 ml of blood was collected from either the jugular or cephalic vein of the dogs showing above mentioned bone deformities. Serum was extracted and stored at -20°C for later analysis of Ca, P and ALP using dry chemistry method (MicroSlide, VITROS 52600 integrated system; Ortho clinical diagnostics, Rochester, USA).

Intact OC was measured using a commercially available solid phase hOST Enzyme Amplified Sensitivity Immunoassay (DIAsource® Immunoassays, Belgium). The human assays had been shown to have a good cross-reactivity with canine OC (Belic´ *et al*., 2012; Tharwat *et al*., 2014). The intra- and inter-assay coefficients of variations (CVs) were 3-5.5%. Serum 25-Hydroxyvitamin D was determined by the 25-Hydroxyvitamin D Total ELISA (DIAsource® Immunoassays, Belgium) which can determine 25-Hydroxyvitamin D Total ($D_2$+$D_3$). The intra- and inter-assay CVs were <10%.

Computerized radiographs of most affected forelimbs were used to measure the thicknesses of bone cortices and diameters using measurement tools on Master View analysis software (4.5.1), and CI [(Thickness of anterior cortex in mm - Thickness of posterior cortex in mm)/Total thickness of mid diaphysis in mm] of radius and ulna was calculated (Nazem *et al*., 2015) (Fig. 1).

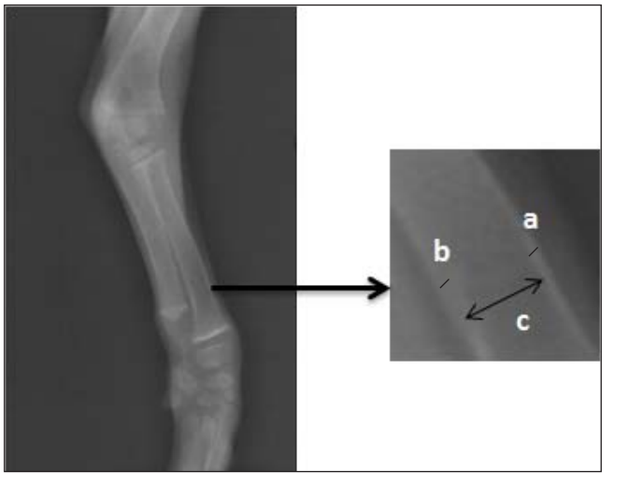


**Fig. 1:** Radiograph showing landmark for calculation of cortical index where 'a' is the thickness of the anterior cortex; 'b' is the thickness of the posterior cortex; and 'c' is the total thickness of the mid diaphysis

The dogs were responsive and had normal vital signs. All dogs had been vaccinated, and no other medical complications were reported by the owners. The animal use protocol was reviewed and approved by our institution's animal ethics committee.

Twelve dogs suffering from rickets were enrolled in the therapeutic study on the basis of owner's compliance. The affected dogs were administrated combination therapy with oral Ca [(Suspension Calcimax+; Meyer vitabiotics, Meyer Organics Private Limited] @ 25mg/kg, twice daily for 2 months and vitamin $D_3$ injection (Arachitol-6L

containing 6, 00, 000 IU or 15 mg cholecalciferol per 1 ml; Abbott India Limited) @ 20,000 IU/kg deep IM, at weekly interval for 6 occasions. Nine apparently healthy dogs of same age group were kept as healthy control.

To monitor the efficacy of treatment, rachitic dogs were evaluated on the basis of % improvement of clinical signs during the treatment period. For comparison of results between treatment and control groups, CI and biochemical parameters were measured only once in a control group. The mean values of CI and biochemical parameters on day 0 (pre-treatment) and 30 and/or 60 post-treatment were compared with that of the healthy dogs, whereas within treatment group, the mean values obtained on $30^{th}$ and/or $60^{th}$ days were compared with the previous values obtained on day 0. Owners were advised to apply the daily Ca dose to food and were instructed not to alter diet, environment and exercise schedule during the treatment trial.

Data were analysed using computer statistical software SPSS 16.0. Dogs showing moderate to severe clinical signs were combined together and their percentage distribution before and during the treatment trail were determined. Means and standard error (S.E.) means were calculated for CI and biochemical parameters included in the study. The significance of differences in variable observations within and between treatment and control groups were determined with paired and two-sample *t* tests, respectively. Statistical significance was set at $p < 0.05$.

## RESULTS

Out of the 375 cases presented with various musculoskeletal disorders, 40 cases (10.7%) were diagnosed for rickets. Among them, higher occurrence was recorded in the age group of 3-6 months (65%) followed by 0-3 months (20%), 6-9 months (10%) and 9-12 months (5%). Male dogs (77.5%) were found to be more affected with rickets than females. On clinical examination, most prominent signs (mild to severe) were broadening of distal metaphyses (100%), lameness (87.5%), bending/bowing of limbs (82.5%), pain on palpation of the limbs (70%) and hind quarter weakness (42.5%).

For the evaluation of treatment trail, 12 rachitic dogs were enrolled. Table 1 depicts the percentage distribution of dogs showing moderate to severe degree of clinical signs associated with rickets during the treatment period.

**Table 1:** Distribution (%) of dogs (n=12) showing moderate to severe clinical signs of rickets at day 0, 30 and 60 of the treatment trail

| Clinical/radiographic signs | Days of experiment | Distribution (%) of dogs showing moderate to severe clinical signs |
|---|---|---|
| Pain | 0 | 75 |
| | 30 | 16.67 |
| | 60 | 0 |
| Lameness | 0 | 41.67 |
| | 30 | 8.33 |
| | 60 | 0 |
| Bowing of limbs | 0 | 66.67 |
| | 30 | 66.67 |
| | 60 | 58.33 |
| Broadening of distal metaphyses | 0 | 83.33 |
| | 30 | 75 |
| | 60 | 75 |
| Weakness of hind quarters | 0 | 16.67 |
| | 30 | 8.33 |
| | 60 | 0 |

As the treatment progressed, clinical signs related to pain, lameness and hind quarter weakness were totally disappeared, whereas broadening of distal metaphyses and bowing of limbs showed mild improvement.

Results of biochemical parameters estimated at day 0, 30 and 60 are shown in Table 2. At the start of study, rickets affected dogs had significantly lower ($p<0.05$) Ca concentration and Ca to P ratio as compared to healthy dogs. Although numerically higher, but serum P levels in rachitic dogs did not vary significantly from that of healthy dogs. An ALP activity was significantly higher in rachitic dogs as compared with healthy dogs at the time of 1st admission (day 0). Calcium level and Ca to P ratio improved significantly ($p<0.05$) over the treatment period in treatment group and became parallel to that of healthy dogs at 30th day onwards. A non-significant decrease in serum P levels was observed towards the end of the treatment period in rachitic dogs. A significant decrease ($p<0.05$) in ALP activity was observed in rachitic dogs after one month of treatment but the values became comparable with that of apparently healthy dogs only on 60th day post-treatment

At day 0, CI of radius and ulna, and concentrations of 25-Hydroxyvitamin D and OC were significantly lower ($p<0.05$) in the affected dogs when compared with their healthy mates (Table 3). At day 60, CI of both radius (0.31±0.02) and ulna (0.33±0.01) of rachitic dogs increased significantly ($p<0.05$) and became comparable with that of healthy dogs. Similarly levels of 25-Hydroxyvitamin D and OC also increased significantly ($p<0.05$) in rachitic dogs on day 60 post-treatment and became parallel with that of healthy dogs.

## DISCUSSION

In the present study, rickets was reported in 10.7% of the total cases presented for various musculo-skeleton problems. Most of the dogs were male and between 3-6 months of age. Previous studies conducted in growing dogs also reported similar findings as observed in the present study (Kushwaha *et al*., 2009; Singh and Gopinathan, 2013).

Broadening of distal metaphyses, lameness and bowing/ bending of limbs were the most commonly encountered signs on clinical and radiographic examination which corroborates with the earlier studies involving rachitic dogs (Kushwaha *et al*., 2009; Dittmer and Thompson, 2011). The common clinical signs of rickets could be attributed to the fact that the deficiency of Ca and vitamin

**Table 2:** Results of biochemical parameters in ricketic dogs at day 0, 30 and 60 of the treatment trail compared with healthy dogs (Mean ± S. E.)

| Parameter | Days of experiment | Ricketic dogs (n=12) | Apparently healthy dogs (n=9)[1] |
|---|---|---|---|
| Calcium (Ca) (mmol/L) | 0 | $2.17\pm0.10^{aA}$ | $2.86\pm0.13^{b}$ |
| | 30 | $2.82\pm0.07^{aB}$ | $2.86\pm0.13^{a}$ |
| | 60 | $2.96\pm0.06^{aB}$ | $2.86\pm0.13^{a}$ |
| Phosphorus (P) (mmol/L) | 0 | $2.24\pm0.13^{aA}$ | $1.92\pm0.11^{a}$ |
| | 30 | $2.00\pm0.14^{aA}$ | $1.92\pm0.11^{a}$ |
| | 60 | $1.86\pm0.14^{aA}$ | $1.92\pm0.11^{a}$ |
| Ca : P | 0 | $1.01\pm0.08^{aA}$ | $1.50\pm0.06^{b}$ |
| | 30 | $1.50\pm0.13^{aB}$ | $1.50\pm0.06^{a}$ |
| | 60 | $1.70\pm0.13^{aB}$ | $1.50\pm0.06^{a}$ |
| Alkaline phosphatase (IU/L) | 0 | $645.67\pm52.49^{aC}$ | $118.11\pm2.47^{b}$ |
| | 30 | $306.67\pm32.95^{aB}$ | $118.11\pm2.47^{b}$ |
| | 60 | $181.33\pm25.59^{aA}$ | $118.11\pm2.47^{a}$ |

[1]Parameters measured only once; within rows, means with different superscripts (a, b) differ significantly ($p<0.05$); within columns, for each parameter, means with different superscripts (A, B, C) differ significantly ($p<0.05$)

D causes failure of mineralization at the metaphyses of long bones resulting in its thickening by a zone of proliferating cartilage cells (Rosol and Capen, 1997) leading to enlargement of metaphyseal region. Further, due to the inherent weakness, the physes deforms and bows as it cannot support the body weight leading to bending and rotation of limbs (Malik *et al*., 1997).

We found reduced levels of Ca and 25-Hydroxyvitamin D in blood and disturbed Ca: P ratio in rachitic dogs that might have caused defects in bone mineralization resulting in development of rickets. Measurement of circulating levels of 25-Hydroxyvitamin D represents the most reliable assessment of vitamin D status. In young animals, vitamin D is required for systematic growth and mineralization of cartilage in growth plate. In the deficiency of vitamin D, mineralization of cartilaginous matrix fails to occur and hence bone formation is blocked (Hazewinkel and Tryfonidou, 2002).

Increased ALP activity reported in this study is probably due to increased osteoblastic activity, which is a sign of incomplete mineralization of organic matrix (Johnson *et al*., 1988; Parthi *et al*., 2008).

The CI of radius and ulna was lower in rachitic dogs as compared to their healthy mates. Present study identified morphometrical variation between forelimb bones of healthy and rachitic dogs by measuring CI. Cortical index is very important and practical index that can be used to assess bone health (Hiney *et al*., 2004). Several studies have explained the use of radiographs to study morphometry of human and animal bones (Dogan *et al*., 2007; Kushwaha *et al*., 2012; Nazem *et al*., 2015).

We also estimated concentration of serum OC (a biomarker of bone formation); value of which was significantly lower in rachitic dogs as compared with apparently healthy dogs, and hence could be used as an additional supportive parameter for diagnosis of rickets in dogs. To our knowledge, no literature defining levels of OC status in the young growing dog affected with rickets is available.

The main purpose of current study was to assess the influence of vitamin $D_3$ and Ca supplementation on correction of clinical signs, biochemical alterations and CI in rachitic dogs. We observed substantial reduction in severity of clinical signs related to pain, lameness and hind quarter weakness in rachitic dogs; although the broadening of distal metaphyses and bowing of limbs could show only mild improvement. One month after the first visit, the owners informed that all dogs had recovered to some extent and that no further worsening of condition had been observed. Similar findings were also reported by Kushwaha *et al*. (2009) who found satisfactory to good clinical response with oral Ca and injectable vitamin $D_3$ in terms of improvement in weight bearing, reduction in lameness and pain, and decrease in the enlargement of metaphyses and straightening of forelimbs in ricketic dogs. Johnson *et al.* (1988) while treating a 3 month old ricketic pup with oral synthetic vitamin D analog and intravenous Ca glubionate every other day for 32 weeks had found narrowing of physes by at least 50% after 2

**Table 3:** Results of cortical index (CI), 25-Hydroxyvitamin D and osteocalcin in ricketic dogs at day 0 and 60 of the treatment trail compared with healthy dogs (Mean ± S.E.)

| Parameters | Days of experiment | Treatment group II (n=12) | Apparently healthy dogs (n=9)[1] |
|---|---|---|---|
| CI (Radius) | 0 day | $0.26\pm0.04^{aA}$ | $0.35\pm0.05^{b}$ |
| | 60 day | $0.31\pm0.02^{aB}$ | $0.35\pm0.05^{a}$ |
| CI (Ulna) | 0 day | $0.27\pm0.01^{aA}$ | $0.37\pm0.02^{b}$ |
| | 60 day | $0.33\pm0.01^{aB}$ | $0.37\pm0.02^{a}$ |
| 25-Hydroxyvitamin D (ng/mL) | 0 day | $10.34\pm3.79^{aA}$ | $102.59\pm4.44^{b}$ |
| | 60 day | $116.36\pm5.59^{aB}$ | $102.59\pm4.44^{a}$ |
| Osteocalcin (ng/mL) | 0 day | $16.91\pm2.42^{aA}$ | $28.89\pm0.92^{b}$ |
| | 60 day | $28.73\pm2.57^{aB}$ | $28.89\pm0.92^{a}$ |

[1]Parameters measured only once; within rows, means with different superscripts (a, b) differ significantly ($p<0.05$); within columns, for each parameter, means with different superscripts (A, B) differ significantly ($p<0.05$)

weeks of therapy; and by 6 weeks, the physes became normal in width and appearance. Many studies involving animal models supported the positive correlation between bone healing and vitamin $D_3$ supplement, in which the vitamin D metabolites stimulated the repair of fractured bone and increased the callus strength (Delgado-Martinez *et al*., 1998; Theyse *et al*., 2006).

Concentrations of Ca, 25-Hydroxyvitamin D and OC showed significant increase after the treatment trail. Kushwaha *et al*. (2009) and Ghoke *et al*. (2012) also reported rise in Ca and vitamin D levels after their administration in rachitic dogs. Increasing trend in the serum Ca might be a positive indication of correction of mineral imbalance. The main target organs for vitamin D are the bone, kidney, intestine, and parathyroid glands. In the intestine, vitamin $D_3$ promotes active uptake and transcellular transport of Ca and P. From the present results, it may be assumed that supplementation of vitamin $D_3$ helps in absorption of Ca from the intestine as well as its deposition into the bones. Over the study period, mean 25-Hydroxyvitamin D levels in rachitic dogs increased significantly. Either oral or parenteral vitamin $D_3$ supplementation can be used to improve the vitamin D status of the growing dogs (Chesney, 2012). In rachitic dogs, weekly injections of vitamin $D_3$ at dose rate of 20, 000 IU/kg BW is well within the safety margin and did not cause toxicity as no hypercalcemia was seen even after 6 injections at weekly intervals (Nakamura *et al*., 2004; Sakals *et al*., 2006).

Supplementation of rachitic dogs with vitamin $D_3$ and Ca significantly reduce the activity of ALP. In a study conducted by Parthi *et al*. (2008), ALP activity decreased by more than 50% in animals treated with Ca-vitamin $D_3$-Zinc, biophosphonate compound, anabolic steroid and transforming growth factor β. The decreased ALP activity indicates mineralization of the cartilage osteoid matrix.

In our study, significant rise in serum OC post-treatment compared as to baseline values was observed. The non-collagenous protein OC, a product of osteoblasts, is regarded as a sensitive indicator of bone formation (Pullig *et al*., 2000). Although widely used in human medicine, their use in veterinary medicine are mostly limited to preclinical and clinical studies involving assessment of bone response to medical and surgical interventions and for the detection of musculoskeletal injuries (Allen, 2003; Frisbie *et al*., 2010). In vitro studies have shown that vitamin D directly affects the ability of osteoblasts to regulate the expression of several genes, consequently, the proliferation of osteoblasts and the production of type I collagen, ALP and OC increased (Van Leeuwen *et al*., 2001).

Dogs undergoing treatment in present study showed increase in long bone CI indicating bone remodelling which corroborate with the results of the previous studies (Kushwaha *et al*., 2009; Ghoke *et al*., 2012).

Our study shows that treatment of rachitic dogs with vitamin $D_3$ and Ca for 60 days could result into correction of clinical signs, levels of serum biochemical parameters and CI. However, bending of limbs and broadening of distal metaphyses were the irreversible changes which could not be corrected even after 60 days of treatment. The results demonstrates that the prognosis in growing dogs with presumptive vitamin $D_3$ responsive rickets can be good and that application of a similar therapeutic approach is warranted.

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