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Serum minerals and bone specific enzymes status in chronic bone disorders in canines

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Abstract
Several studies have been conducted in establishing the relationship between bone development and serum minerals such as calcium, phosphorus and the enzymes like alkaline phosphatase (ALP) as well as tartare -resistant acid phosphatase (TRAP). The role of magnesium in contributing to skeletal disorders is less known. In this study, the influence of serum ALP and TRAP in contributing to development of chronic skeletal disorders such as osteoporosis, hypertrophic osteodystrophy along with the serum concentration of calcium, phosphorus and magnesium will be discussed. Thirty healthy large breed dogs under 18 months of age were used for this study. Serum enzymes and phosphorus were estimated using fully automated biochemical analyzer. The levels of serum calcium and magnesium were detected with atomic absorption spectrophotometry (AAS). A significant increase in the serum ALP and TRAP were observed due to the intensive bone re-modelling in the course of bone disorders. The levels of calcium, phosphorus and magnesium were significantly lower in the diseased pups. Overall, this study helps to analyze the development of chronic skeletal disorders in growing pups and aids in early diagnosis and treatment.

Keywords: Alkaline phosphatase, tartare -resistant acid phosphatase, calcium, serum phosphorus, serum magnesium, chronic skeletal disorders

Introduction
The young growing animals are more prone to skeletal disorders due to many factors such as improper diet and excessive exercise. The large breed pups require more nutrients, especially minerals like calcium and phosphorus for their skeletal development. The initial twelve months are crucial for pups as their skeletal system is highly susceptible to physical and mechanical injury due to increased metabolic activity during growth. The most frequently occurring chronic skeletal disorders are hypertrophic osteodystrophy, osteochondrosis, hip dysplasia and rickets.
The role of calcium and phosphorus in contributing to bone development and maintained has been well established. These elements constitute the hydroxyapatite crystals of bones and thereby determining the bone strength. Magnesium is an intracellular cation that activates and stimulates the proliferation of osteoblasts. The acute reduction in level of magnesium concentration affects the parathyroid hormone function and eventually disturbs the calcium homeostasis. Enzymes like ALP and TRAP are vital for bone mineralization and their levels are high during active bone-remodeling. The ALP helps in increasing the local concentration of inorganic phosphate ions and help in mineralization along with other functions such as promoting the hydroxyapatite crystal formation. The elevated serum ALP is observed during pathological or physiological skeletal re-modelling. Tartarated resistant acid phosphatase is an enzyme secreted by osteoclast and is used as an indicator for the activity of the bone resorption cells. The serum TRAP will be elevated especially during intensive bone resorption. The current study was undertaken to study the status as well as the roles of these serum minerals and enzymes in chronic skeletal disorders in canine for early diagnosis.

Material and Methods
Large breed dogs below 18 months of age with symptoms suggestive of chronic skeletal disorders such as rickets, osteodystrophy, bending of bones were used for the study. Ten apparently healthy dogs brought to the hospital for routine health-check up without any symptoms of skeletal deformities served as control. Five mL of blood sample was collected in serum separation vials from each animal for the estimation of enzymes and minerals.
The serum samples were analyzed for calcium and magnesium using Atomic Absorption

Spectrophotometer (AAS) with Graphite Furnace and Flow Injection Analysis System (FIAS) (M/s Perkin Elmer, USA). Serum phosphorus (mg/dL) was analyzed using Fully Automated Biochemical Analyzer, (Selecta Pro S Lite, Netherland) utilizing kits purchased from Coral Clinical Systems, Tulip Diagnostics Pvt. Ltd, Mumbai. Activity of serum Acid phosphatase (TRAP: IU/L) and Alkaline phosphatase (ALP: IU/L) were quantified by fully automated biochemical analyzer (Selecta Pro S Lite, Netherland).

Results and Discussion

Serum Minerals

Calcium

In the present study significantly lower levels of serum calcium were noticed in diseased dogs compared to control dogs (Table.1), which was in accordance with the findings of Campbell and Doughlas (1965) [3] in dogs, where they observed that hypocalcemia in pups caused poor growth along with skeletal disorders and pathological fractures. Calcium formed the structural basis for hydroxyapatite and was required during bone formation and maintenance (Flynn, 2003) [7]. McMillian *et al*. (2006) [11] noted that lower levels of serum calcium led to reduced bone mineralization. Hutchinson *et al*. (2012) [9] observed osteopenia in dogs having hypocalcemia. Ferguson and Hartles (1963) [6] observed large resorptive cavities in large bones in hypocalcemia rats. Significantly lower levels of serum calcium in the diseased dogs in this study might be due to decreased absorption of calcium. Decreased levels of serum calcium might have contributed to the development of chronic skeletal disorder by reducing bone mineralization.

Phosphorus

In the current study a significantly lower serum phosphorus concentration was observed in dogs affected by chronic skeletal disorders (Table.1), which is similar to the observations of Campbell and Doughlas (1965) [3] in mice, Dittnmer and Thompson (2011) [4] and Taylor *et al*. (2009) [17] in dogs. Campbell and Doughlas (1965) [3] observed that in mice fed with diet low in phosphorus developed rickets and osteoporosis. Dittnmer and Thompson (2011) [4] reported rickets in dogs with hyperphosphatemia. Taylor *et al* (2009) [17] suggested that low phosphorus concentration dogs caused reduced bone mineral density. Inorganic phosphate contributed to hydroxyapatite, the component that provided mechanical resistance to organic matrix of bone (Bonjour, 2011) [2]. Hypophosphatemia in the current study might have caused weakening of organic matrix of the bone due to improper mineralization leading to skeletal disorder.

Magnesium

In the current study hypomagnesemia was observed in the affected dogs (Table.1) which was in accordance with findings of Rondanelli *et al.* (2010) [14] and Sojka (1995) [15] in humans, where they suggested that lower magnesium levels contributed to cessation of bone growth due to reduced osteoblast activity and suppressed bone mineralization. However, contrary to this Velazguez *et al*. (1999) [19] observed that lower serum magnesium levels did not contribute to development of chronic skeletal disorders in humans. The skeletal changes observed in affected dogs of this study might be due to reduced osteoblast activity and suppressed bone mineralization caused by significantly lower levels of magnesium.

Serum enzymes

Alkaline Phosphatase

In the present study a significantly higher levels of serum alkaline phosphatase (ALP) were observed in affected dogs compared to control (Table.1). Nizet *et al.* (2020) [13] in humans, Taylor *et al*. (2009) [17] and Arora *et al*. (2012) [1] in dogs observed similar results. Alkaline phosphatase found on the osteoblast membrane boosted the local phosphate ion concentration, thus promoted bone calcification and during bone damage ALP was released to circulation (Hoshi *et al.*, 2001) [8]. Taylor *et al*. (2009) [17] and Arora *et al*. (2012) [1] observed that growing dogs suffering from rickets had elevated serum ALP. The increased levels of serum ALP in dogs under study might be due to improper bone mineralization and increased bone re-modelling during growth phase.

Acid Phosphatase

Dogs with chronic skeletal disorders had significantly elevated serum tartarated resistant acid phosphatase (TRAP) compared to control dogs (Table.1). This was similar to the findings of Terpos *et al*. (2005) [18] in dogs and Fagerlund (2009) [5] in humans, where they recorded that TRAP concentration in the serum was increased during chronic skeletal disorders like osteoporosis. Stepan *et al*. (1983) [16] observed an elevated TRAP in humans affected with osteodystrophy. Moonga *et al*. (1990) [12] found increased serum TRAP levels in osteoporosis in humans. Acid phosphatase, a robust and sensitive biomarker for osteoclast activity and resorption of bone elevated during increased osteoclast activity (Janckilla and Yam, 2009) [10]. The affected dogs in the study had a significantly elevated serum TRAP indicating elevated osteoclast activity that might have contributed in development of bone disorders.

Conclusion

In the current study, the serum calcium, phosphorus and magnesium levels were significantly lower in dogs having skeletal disorders compared to control dogs. While the serum ALP and TRAP levels were significantly higher in diseased dogs compared to control dogs. The determination of skeletal disorders is vital to provide a quality life for pets. Addition of diets containing optimum levels of minerals during the early stages of growth will help to prevent development of skeletal diseases in pups to a greater extent.

Table 1: Mean ± SE values of serum minerals, Homocysteine and enzymes in control dogs and animals with skeletal disorders

Sl. No.	Parameters	Control	Diseased	P value
		Mean ± SE (n = 10)	Mean ± SE (n = 30)	
1	Ca mg/dL	10.68 ± 0.38	7.73 ± 0.16	0.001**
2	P mg/dL	7.72 ± 0.60	6.23 ± 0.46	0.016*
3	Mg mg/dL	2.68 ± 2.6	2.01 ± 0.10	0.19*
4	ALP IU/L	199.19 ± 30.18	276.53 ± 16.63	0.007**
5	ACP IU/L	3.36 ± 0.45	5.61 ± 0.23	0.000**

** Highly significant (p<0.01), * Significant (p<0.05)

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