Oxidative and anti-oxidant status in hypothyroid dogs

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Abstract
Oxidative stress is defined as an imbalance between the antioxidant defense systems and the rate of production of reactive oxygen species (ROS). While ROS production depends largely on the mitochondria, Thyroid hormones affect the cellular antioxidant status. The dogs presented to the Teaching Veterinary Clinical Complex, College of Veterinary Science & AH, Durg, private clinics and government Veterinary hospitals of Durg-Bhilai, Raipur and Rajnandgaon districts of Chhattisgarh for a period of two years from March 2018 to February 2020 were included for the present study. Thyroid hormones (tT3, tT4 and fT4), oxidative marker (TBARS) and antioxidant parameters (SOD, catalase and reduced glutathione) were estimated in hypothyroid and healthy dogs. In the present study, there was a significant decrease in tT3, tT4 and fT4 levels in hypothyroidism dogs as compared to healthy ones. The TBARS values in hypothyroidism positive dogs were significantly increased as compared to healthy control group. Hypothyroid dogs showed significant decrease in SOD, catalase and reduced glutathione values as compared to the healthy control group dogs.

Keywords: Canine, hypothyroidism, TBARS, SOD, catalase, reduced glutathione

Introduction
Hypothyroidism is the most frequently observed endocrine disease in dogs characterised by structural and / or functional abnormality in the thyroid gland, which leads to deficient production of thyroid hormones (Costa et al., 2016) [4]. The thyroid hormones (THs), namely, tetraiodothyronine (thyroxin, T4) and a much smaller proportion of triiodothyronine, exert actions at the cellular level by binding to a set of specialized receptors that couple to both genomic and non-genomic signaling pathways. Thyroid hormones are subject to transformations in the peripheral tissues, mainly in the form of deiodination. The general metabolic effect of THs is a relative acceleration of the basal metabolism that includes an increase of the rate of both catabolic and anabolic reactions (Nanda et al., 2008b) [22]. Hypothyroidism leads to the dysfunction of the respiratory chain in the mitochondria with accelerated production of free radicals (i.e., superoxide anion, hydrogen peroxide, and hydroxyl radicals as well as lipid peroxides), which consequently leads to oxidative stress (OS). ROS not only leads to lipid peroxidation and oxidative DNA damage but also interferes with physiologic adaptation and intracellular signal transduction, which ultimately causes activation of protein kinases and the mitogen activated protein kinase cascade leading to altered cellular functions (Yoshikawa and Naito, 2002) [36]. Hypothyroidism-associated ROS is the consequence of both increased production of free radicals and reduced capacity of the antioxidant defense. (Resch et al., 2002) [28]. Lipid peroxidation is reported to be high in hyperlipidemia, which is a consistent biochemical feature in hypothyroidism (Nanda et al., 2008 and Masullo et al., 2018) [21, 19]. The available data concerning oxidative stress and antioxidant parameters in canine hypothyroidism is scanty. Hence the present study was carried out to know the effect of hypothyroidism on oxidative markers in dogs.

Materials and Methods
The dogs presented to the Teaching Veterinary Clinical Complex, College of Veterinary Science & AH, Durg, private clinics and government Veterinary hospitals of Durg-Bhilai, Raipur and Rajnandgaon districts of Chhattisgarh for a period of two years from March 2018 to February 2020 were included for the present study. Dogs suspected for hypothyroidism having clinical signs either of bilateral symmetrical alopecia, rat tailed appearance, hyper pigmentation, pruritus, pyoderma, seborrhea, erythema, thinning of hair coat, lethargy, weight
gain, exercise and cold intolerance were considered for the estimation of thyroid hormones. Furthermore, oxidative markers were estimated in dogs positive for hypothyroidism.

**Healthy dogs**

Eight healthy dogs well vaccinated and dewormed without any clinical signs suggestive of canine hypothyroidism were kept as healthy control group for comparing the oxidative markers and thyroid profiles with hypothyroidism positive dogs.

**Collection of blood samples**

**Estimation of thyroid hormones**

Blood samples (2ml) from hypothyroidism suspected dogs (n=44) were collected either from cephalic or saphenous veins aspiratively. After collection, blood was allowed to clot at room temperature, and then centrifuged at 1,500 rpm for 10 minutes. Serum was collected and stored at -20 °C until further assay. Total triiodothyronine (tT3) (nmol/l), total Thyroxine (tT4) (nmol/l) and free Thyroxine (fT4) (pmol/l) were estimated by Radio immuno assay kits (RIA) (Gnanasekar et al., 2010) [9].

**Estimation of oxidative markers**

Around 2 ml of blood was collected in Acid citrate dextrose (ACD) for assessment of the following oxidative stress and antioxidant parameters. 10% RBC hemolysate was prepared by centrifuging the blood samples at 2000 rpm for 10 min and supernatant plasma were separated out. The sedimented cells were washed with sterile 0.85% NaCl solution three times. Washed erythrocytes were haemolysed with ninefold volume of distilled water to prepare 10% RBC hemolysate. Hemoglobin in the hemolysate was estimated by the cyanomethaemoglobin method Van-Kampen and Zigelstra (1961) [12].

**Estimation of TBARS**

TBARS/ Lipid peroxide level in 10% RBC hemolysate was determined as per Placer et al. (1966) [25] and was expressed as nmol/mg of hemoglobin (Hb).

**Estimation of Superoxide dismutase**

Superoxide dismutase activity in 10% supernatant tissues and RBC hemolysate was estimated as per Marklund and Marklund (1974) [18] with certain modifications suggested by Menami and Yoshikawa (1979) [20]. Each unit of the SOD activity is defined as the quantity of enzyme that inhibits auto-oxidation of pyrogallol by 50% under suitable experimental conditions and expressed as U/mg of Hb.

**Estimation of Catalase**

Catalase activity was estimated in erythrocyte haemolysate by appropriate dilution of the stock (1:10 dilution) haemolysate by the spectrophotometric method as given by Aebi (1983) [1] and expressed as U/mg of Hb.

**Estimation of Reduced Glutathione (GSH)**

Reduced glutathione activity, was determined by spectrophotometer as per standard protocol given by Prins and Loos, (1969) [26]. The GSH content was expressed as mmol/mg of Hb.

**Statistical analysis**

Statistical analysis of the data was done using ANOVA technique according to the method described by Snedecor and Cochran (1994) [34]. Statistically significant difference was considered at 5 percent level.

**Results and Discussion**

**Thyroid hormones**

There was a significant decrease in tT3, tT4 and fT4 levels in hypothyroidism positive dogs as compared to healthy ones (Fig.1). Similar findings of reduced thyroid hormones in hypothyroid dogs were reported by Durga (2017) and Anand (2019) [6, 7].

**Fig 1:** Thyroid hormones in healthy group and hypothyroid dogs

**Oxidative markers**

**TBARS**

The TBARS values in hypothyroidism positive dogs were significantly increased as compared to healthy control group. The data presented in this study show that hypothyroid dogs are susceptible to oxidative stress than healthy dogs (Fig.2). The results are in corroboration with the findings of Dumitriu et al., (1988); Sawant et al., (2011); Reddy et al., (2013); Varikasuvu et al., (2013) and Masullo et al., (2018) [7, 33, 27, 35].

**Enzymatic antioxidant parameters**

**Superoxide dismutase (SOD)**

In the present study, hypothyroid dogsshowed significant
decrease in SOD levels as compared to the healthy dogs (Fig. 3). Similar findings of decreased SOD activity were reported by Pasupathi and Latha (2008); Sahoo et al. (2008) and Varikasuvu et al. (2013) [24, 31 & 35]. The present finding are inconsistent with Dave et al. (2009) and Saeed et al. (2014) [6 & 30] who observed an elevation of SOD activity in hypothyroidism.

SOD is the first line of enzymatic defence against intracellular free radicals. It is reported that consequent accumulation of hydrogen peroxide causes inactivation of SOD activity. Decreased SOD activity would expose the cell membrane and other components to oxidative damage (Kono and Fridovich, 1982) [15].

Catalase
The catalase activity in hypothyroid dogs was significantly reduced as compared to healthy group (Fig. 4). The findings are in line with the results of Pasupathi and Latha (2008); Sahoo et al. (2008) and Masullo et al. (2018) [24, 31 & 19]. Whereas, Saeed et al. (2014) reported elevation of catalase levels in hypothyroidism. Catalase shares the function of catalyzing the decomposition of hydrogen peroxide to water. A low level of catalase activity, could primarily damage the endoplasmic reticulum in the cells. This finding suggests that the clinical condition of hypothyroidism decreases catalase activity and reduces antioxidant defence. When catalase activity is reduced in hypothyroidism, a possible excessive H₂O₂ in an organism could react with NO, producing peroxynitrite radicals or other hydroxyl radicals. These radicals will in turn react with cellular structures to cause damage, in a process known as lipid peroxidation (Halliwell and Chirico, 1993) [10].

Non-enzymatic antioxidant parameters
Reduced glutathione (GSH)
There was a significant decrease in reduced glutathione levels in hypothyroid dogs as compared to healthy ones (Fig. 5). Similar findings was reported by Varikasuvu et al. (2013) [35]. In contrast to our results, Das and Chainy (2001) [5] reported an elevation of GSH levels in hypothyroid rat whereas, Sarandol et al. (2005) [22] did not observe any significant changes in GSH levels. GSH is endogenously synthesized in the liver and is the first line of defence against prooxidant stress (Nicotera and Orrenius, 1986) [23]. This antioxidant molecule is one of the main parts of the cellular endogenous antioxidant systems.

The GSH-dependent defence system plays an important role against lipid peroxidation in cells. Insufficiency of GSH is one of the primary factors that permits lipid peroxidation. It has been reported that GSH plays and important role in the detoxification of hydroperoxides and prevents the effect of lipid peroxidation (Maddaiah, 1990) [16]. Thus the decreased production of GSH could be due to the overproduction of free radicals and increased lipid peroxidation in hypothyroidism. Thus, it is likely that cells are damaged by prolonged oxidative stress that far exceeds the capacity of the organs to synthesize antioxidant molecules (Komosinska-Vasser et al., 2000) [14].
Fig 5: Reduced Glutathione levels in healthy group and hypothyroid dogs

Conclusion
In conclusion, our present study suggests that hypothyroid dogs accompanied with reduced tT3, tT4 and fT4 levels showed a very high production of ROS and oxidative stress, characterised by enhanced lipid peroxidation activity (TBARS) and concomitant decline in antioxidant defence mechanisms (SOD, catalase and reduced glutathione).

References


