Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; 8(4): 1188-1192

Received: 10-05-2020
Accepted: 12-06-2020

B Roopali
Department of Veterinary Medicine, College of Veterinary Science and AH, Durg, Chhattisgarh, India

S Roy
Department of Physiology and Biochemistry, College of Veterinary Science and AH, Durg, Chhattisgarh, India

M Roy
Department of Physiology and Biochemistry, College of Veterinary Science and AH, Durg, Chhattisgarh, India

Corresponding Author:
B Roopali
Department of Veterinary Medicine, College of Veterinary Science and AH, Durg, Chhattisgarh, India

Oxidative and anti-oxidant status in hypothyroid dogs

B Roopali, S Roy and M Roy

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-Bhillai, 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 glutathone

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 subjected 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 antioxidative 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-Bhillai, 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 Futhermore, 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 fromcephalic or saphenous veins aspetically. 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 bycentrifuging 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 cyano-methaemoglobin method Van-Kampen and Ziglstra (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 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) [8, 2].

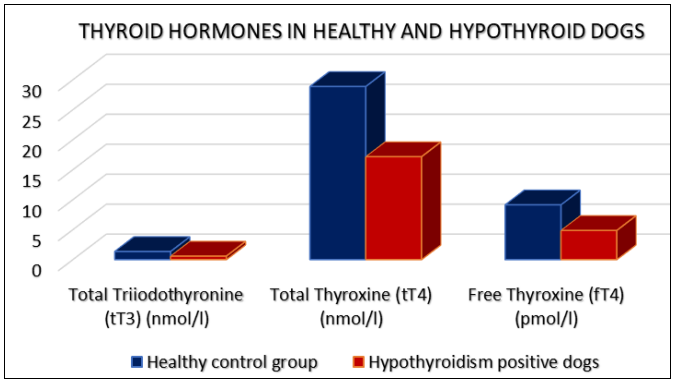

Fig 1: Thyroid hormones in healthy group and hypothyroid dogs

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, 19]who observed the mean malondialdehyde/ TBARS level to be significantly higher in hypothyroid group as compared to the control group. On the other hand, Mano *et al.* (1995) and Joshi *et al.* (2018) [17, 11] found the concentration of lipid peroxidases did not change in hypothyroid rats whereas, Brzezinska-Slebodzinska (2003) [3] reported that hypothyroidism lead to a significant decrease in lipid peroxidation end-product malondialdehyde. TBARS is a sensitive marker of lipid peroxidation, a breakdown product of the major chain reactions leading to definite oxidation of polyunsaturated fatty acids and thus serves as a reliable marker of lipid peroxidation (Nanda *et al.*, 2008 and Masullo *et al.*, 2018) [21, 19].

Hypothyroidism leads to decreased animal activity which contributes to hypercholesterolemia. Thus, in hypothyroidism higher cholesterol along with prolonged circulation time of ageing lipoproteins make them more susceptible to oxidation due to repeated exposure to a variety of oxidising species that allows formation and accumulation of lipid peroxidation products (Kanchan *et al.*, 2016) [13]. Therefore, oxidative stress is likely to be potentially related to the hypercholesterolemia due to thyroid dysfunction. The results are consistent with previous studies showing that hypercholesterolemia has a stronger influence on the development of oxidative stress in hypothyroidism (Santi *et al.*, 2010) [29].

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 catalse 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 H_2O_2 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) [32] didnot 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 dogs. 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].

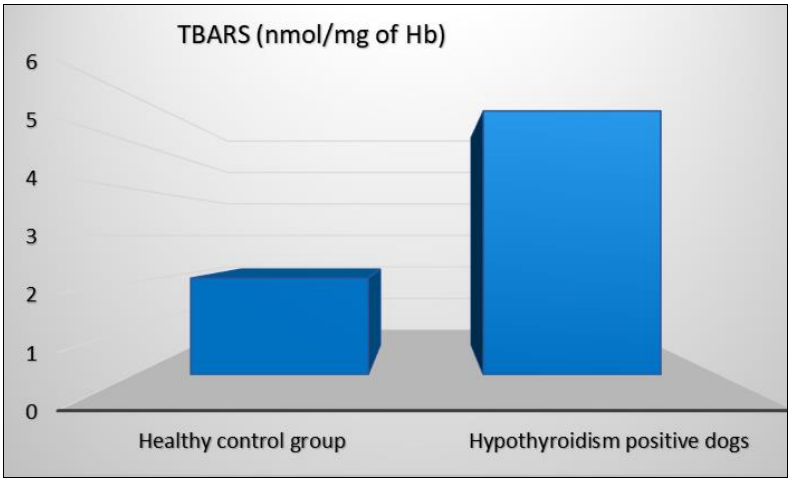

Fig 2: TBARS in healthy group and hypothyroid dogs

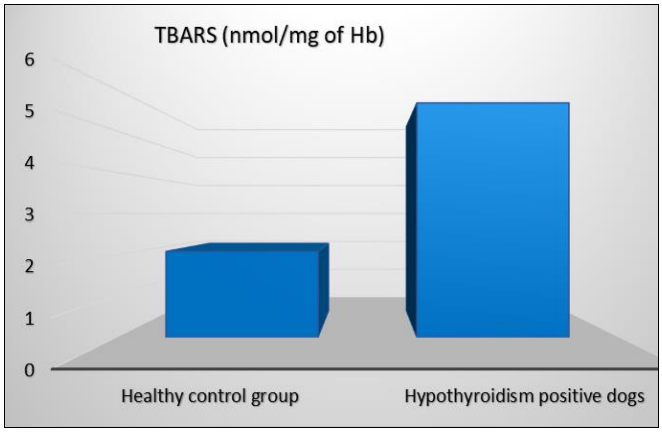

Fig 3: Superoxide dismutase levels in healthy group and hypothyroid dogs

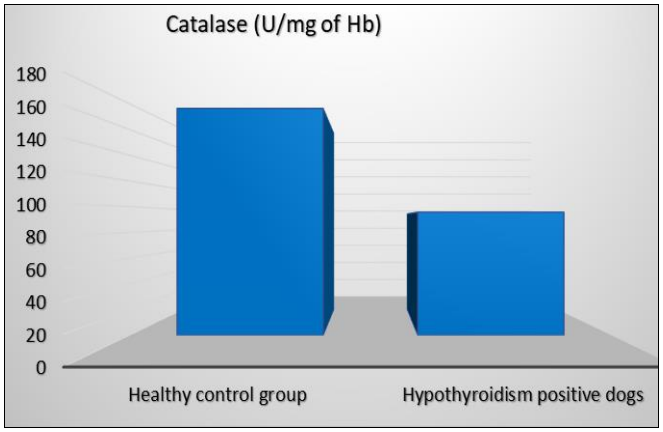

Fig 4: Catalase levels in healthy group and hypothyroid dogs

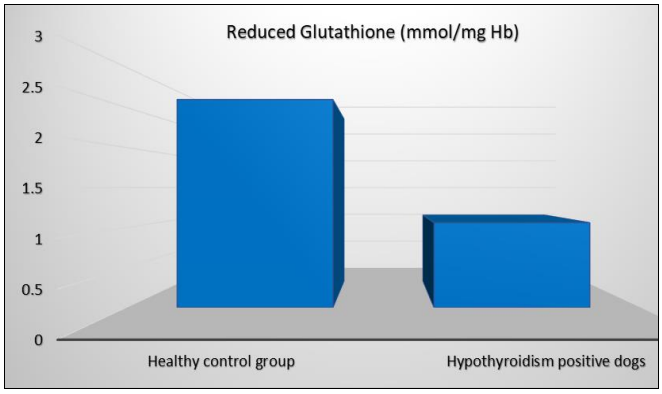

Fig 5: Reduced Glutathione levels in healthy group and hypothyroid dogs

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

1. Aebi HE. Catalase. In: Bergmeyer HU Ed., Methods of Enzymatic Analysis, Verlag Chemie, Weinhem, 1983, 273-286.
2. Anand. Studies on thyroid profile in dogs with special referance to radio immuno assay. M.V.Sc thesis submitted to Bombay veterinary college, Mumbai, 2019.
3. Brzezinska-Slebodzinska E. Influence of hypothyroidism on lipid peroxidation, erythrocyte resistance and antioxidant plasma properties in rabbits. Acta Veterinaria Hungarica. 2003; 51(3):343-351.
4. Costa GM, Steffi LA, Francisco AFXJ, Daniel de AV, Janaina SAME. Dermatological manifestations associated with canine hypothyroidism: A review. Revista Brasileira de Higiene e Sanidade Animal. 2016; 10(4):781-797.
5. Das K, Chainy GBN. Modulation of liver mitochondrial antioxidant defense system by thyroid hormone. Biochem. Biophys. Acta. 2001; 1573:1-13.
6. Dave BN, Paradkar NM. Total superoxide dismutase, Cu/Zn superoxide dismutse and glutathione peroxidasein untreated hyperthyroidism and hypothyroidism. J KSci. 2009; 11(1):6-10.
7. Dumitriu L, Rodica B, Ursu H, Mariana P, Ionescu V. Significance of high levels of serum malondialdehyde (MDA) and ceruloplasmin (CP) in hyper- and hypothyroidism. Endocrinologie. 1988; 26(1):35-8.
8. Durga. Clinico-diagnostic and therapeutic studies on hypothyroidism in dogs. M.V.Sc thesis submitted to Sri Venkateswara Veterinary University, Tirupathi, 2017.
9. Gnanasekar R, Nagvekar UH, Sivaprasad N. Development and validation of a two-step free thyroxine radioimmunoassay based on antibody coated tubes. Journal of Liquid Chromatography & Related Technologies. 2010; 33:1576-1586.
10. Halliwell B, Chirico S. Lipid peroxidation: its mechanism, measurement, and significance. Am J Clin Nutr. 1993; 57:715.
11. Joshi B, Sangeeta S, Aman S, Seema G, Vanishree BJ. A Study of Lipid Peroxidation and Total Antioxidant Capacity in Hyperthyroid & Hypothyroid Female Subjects. Galore International Journal of Health Sciences and Research, 2018, 3(4).
12. Van-Kampen EJ, Ziglstra WG. Colorimetric determination of hemoglobin. Clin Chem Acta. 1961; 6:53-88.
13. Kanchan A, Shashi S, Atreyee B, Aggarwal HK. Effect of thyroxine replacement therapy on the redox status of primary hypothyroid patients. Indian Journal of Basic and Applied Medical Research. 2016; 6(1):52-59.
14. Komosinska-Vassev K, Olczyk K, Kucharz EJ, Marciz C, Kotulska A. Free radical activity and anti oxidants defense mechanisms in patients with hyperthyroidim due to Grave's disease during therapy. Clinical chimica Acta. 2000; 300:107-117.
15. Kono IF. Superoxide radical inhibits catalase. J Biol. Chem. 1982; 257:5751-5754.
16. Maddaiah VT. Glutathione correlates with lipid peroxidaiton in liver mitochondria of triiodothyronine-injected hypophysectomised rats. FASEB J. 1990; 4:1513-18.
17. Mano T, Sinohara R, Sawai Y, Oda N, Nishida Y, Mokurno T. Changes in lipid peroxidation and free radical scavengers in the brain of hyper-and hypothyroid aged rats. J Endocrinol. 1995; 147:361-365.
18. Marklund S, Marklund G. Involvement of superoxide anion radical in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974; 47:469-74.
19. Masullo LF, Rejane AM, Romélia PGL, Tarcísio P, Marilena F, Pedro A. Levothyroxine Replacement Improves Oxidative Status in Primary Hypothyroidism. Frontiers in Endocrinology, 2018, 9.
20. Menami M, Yoshikawa H. A simplified assay method of superoxide dismutase activity of clinical use. Clin Chem Acta. 1979; 92:337-42.
21. Nanda N, Bobby Z, Hamide A. Oxidative stress and protein glycation in primary hypothyroidism. Male/female difference. Clin Exp Med. 2008; 8:101-8.
22. Nanda N, Bobby Z, Hamide A. Association of thyroid stimulating hormone and coronary lipid risk factors with lipid peroxidation in hypothyroidism. Clin Chem Lab Med. 2008b; 46:674-9.
23. Nicotera P, Orrenius S. Role of thiols in protection against biological reactive intermediates. Adv Exp Med Biol. 1986; 187:41-51.
24. Pasupathi P, Latha R. Free radical activity and antioxidant defence mechanisms in patients with hypothyroidism. Thyroid Sci. 2008; 3(12):1-6.22.
25. Placer ZA, Cushman LL, Johnson BC. Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical system.Anal Biochem. 1966; 16:359-64.
26. Prins HK, Loos JA. Biochemical Methods in Red Cell Genetics. United States: Academic Press, 1969, 127-9.
27. Reddy VS, Gouroju S, Suchitra MM, Suresh V, Sachan A, Srinivasa Rao PV, Bitla AR. Antioxidant defense in overt and subclinical hypothyroidism. Horm Metab Res. 2013; 45(10):754-8.
28. Resch U, Helsel G, Tatzber F, Sinzinger H. Antioxidant status in thyroid dysfunction. Clin Chem Lab Med. 2002; 40:1132-4.
29. Santi MMMF, Duarte RN, Moresco. Association between thyroid hormones, lipids and oxidative stress biomarkers in overt hypothyroidism. Clinical Chemistry and Laboratory Medicine. 2010; 48(11):1635-1639.

30. Saeed Naazeri, Mosayeb Rostamian, Mehdi Hedayati. Impact of Thyroid Dysfunction on Antioxidant Capacity, Superoxide Dismutase and Catalase Activity. Zahedan Journal of Research in Medical Sciences. 2014; 16(1):51-54.
31. Sahoo DK, Roy A, Bhanja S, Chainy GB. Hypothyroidism impairs antioxidant defence system and testicular physiology during development and maturation. Gen Comp Endocrinol. 2008; 156(1):63-70.
32. Sarandol E, Tas S, Dirican M, Serdar Z. Oxidative stress and serum paraoxonase activity in experimental hypothyroidism: effect of vitamin E supplementation. Cell Biochem Funct. 2005; 23:1-8.
33. Sawant SU, Chandran S, Alan FA, Rajan MGR. Correlation between Oxidative Stress and Thyroid Function in Patients with Nephrotic Syndrome. Int J Nephrol, 2011.
34. Snedecor GW, Cochran WG. Statistical methods. Edn 8th, Affiliated East-West Press, New Delhi, 1994, 68.
35. Varikasuvu S, Shivakrishna G, Suchitra MM, Aparna B. Antioxidant Defense in Overt and Subclinical Hypothyroidism. Hormone and Metabolic Research, 2013, 4510.
36. Yoshaikawa T, Naito Y. What is oxidative stress? JMAJ. 2002; 45:271-6.