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# Studies on role of Thyroperoxidase (TPO) Enzyme in Primary Hypothyroidism Affected Dogs

**Raguvaran Raja[1]*, Debabrata Mondal[1], Jithin Mullakkalparambil Velayudhan[2], Ravi Shankar Kumar Mandal[3], Sonam Bhatt[4], Vivek Joshi[1] and Umesh Dimri[1]**

*[1]Division of Medicine, ICAR-Indian Veterinary Research Institute, Bareilly, U.P., INDIA*
*[2]Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, U.P., INDIA*
*[3]BASU, Patna, Bihar, INDIA*
*[4]National Research Center on Mithun, Nagaland, INDIA*

**Corresponding author: R Raja; E-mail: raguivri@gmail.com*

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**ABSTRACT**

The study was aimed to explore the role of auto antibodies against thyroperoxidase (TPO) as a causative factor of primary hypothyroidism in dogs. Dogs presented at Referral Veterinary Polyclinic, Indian Veterinary Research Institute during 2016-17, were screened for hypothyroidism on the basis of clinical signs like lethargy, thickening of skin, bilateral symmetrical alopecia and obesity. Serum and blood samples were collected for biochemical parameters, thyroid profile, TPO concentration and complete blood count. Twenty (20) dogs found positive for hypothyroidism during the study period. Adult dogs aged more than 5 years were commonly affected. Male and female dogs were equally susceptible to hypothyroidism and there was no sexual susceptibility. Clinical pathology revealed significant reduction in $FT_4$ level, altered protein, cholesterol and lipid metabolism in affected dogs. Thyroperoxidase ELISA study showed negligible role of auto-antibodies against TPO as a causative factor of primary hypothyroidism in dogs. All the affected dogs were treated with L-thyroxine (Eltroxin)® @ 20 – 40 mcg/kg bid for life long period along with other symptomatic therapy.

**HIGHLIGHTS**

- Primary hypothyroidism is often immune mediated disorder in canine.
- Autoantibodies against thyroperoxidase and thyroglobulin play pivotal role in the pathogenesis.

**Keywords:** Autoimmune, Hypothyroidism, Hypercholesterolemia, Thyroperoxidase

Thyroid gland, bilobed structure located lateral to trachea. Triiodothyronine ($T_3$) and L-thyroxine ($T_4$) are the important hormones secreted from the gland. Secretion is regulated via negative feedback mechanism through hypothalamic pituitary thyroid axis. Most of the secreted $T_4$ is deiodinated in liver and kidney to form the most potent $T_3$ and $rT_3$. L-thyroxine and triiodothyronine have high affinity towards plasma protein and all the metabolic action is mediated by $T_3$ only. Thyroid hormones interact with nuclear receptor and responsible for various metabolic process in body. Primary hypothyroidism is a common endocrine disorder in dogs (Chastain and Panciera, 1998). Lymphocytic thyroiditis and idiopathic atrophy are the two most common cause of adult onset of primary hypothyroidism. Lymphocytic thyroiditis occurs because of autoantibodies production against thyroglobulin (TG) (Haines *et al.*, 1984; Mooney, 2011). Histologically, it is characterized by infiltration of gland by lymphocyte, macrophage and plasma cells and end up with follicle destruction and secondary fibrosis. It has similar clinical

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and histologic attributes as that of Hashimoto's thyroiditis in man (Day and Shaw, 2008; Mooney, 2011). Idiopathic atrophy is marked histopathologically by replacement of thyroid parenchyma with adipose tissue. Affected thyroid glands are infiltrated by plasma cells and lymphocytes and there is follicular loss in addition to the presence of autoantibodies specific for thyroglobulin (Tg) and/or triiodothyronine (T3) and thyroxine (T4) in the serum (Nachreiner *et al*., 2002; Patzl and Mostl, 2003).

Thyroperoxidase, a membrane bound enzyme involved in oxidation of inorganic iodide and transfer of iodide to tyrosine residue of thyroglobulin resulting in formation of monoiodothyrosine (MIT) and diiodothyrosine (DIT). Coupling these molecules result in formation of $T_3$ and $T_4$. Hypothyroidism is associated with decreased concentrations of triiodothyronine ($T_3$), thyroxine ($T_4$), and increased thyroid stimulating hormone (TSH). These changes lead to increased body weight with altered lipid and glucose metabolism (Diekman *et al.*, 2000; Pucci *et al*., 2000; Hofer-Inteeworn *et al*., 2012). Despite of the etiological agents, primary hypothyroidism respond well with supplementation of L-thyroxine @ 0.02-0.04 mg/kg bid preferably in empty stomach. The course of treatment is always lifelong and one can anticipate reappearance of clinical signs after withdrawal of L-thyroxine supplementation. This study deals with role of TPO in auto-immune mechanism of primary hypothyroidism and clinico-pathological changes in affected dogs.

## MATERIALS AND METHODS

Dogs presented at RVP, IVRI during 2016-17, were screened for hypothyroidism on the basis of clinical signs such as lethargy, heat seeking, thickening of skin, bilateral symmetrical alopecia, rat tail appearance and obesity. Serum and blood samples were collected for biochemical parameters, thyroid profile, TPO concentration and complete blood count. Data was analysed by Independent T test (Two tailed) SPS 16 trail version.

## RESULTS AND DISCUSSION

Twenty (20) dogs found positive for hypothyroidism during the study period. Adult dogs aged more than 5 years was commonly affected (Fig. 1 & Table 1). Male (n-10) and female (n-10) dogs were equally susceptible to hypothyroidism and there was no sexual susceptibility in this study (Fig. 2). This was in accordance with study of other researchers (Khan CM, 2010). Labrador retriever dogs were highly (n-15) diagnosed with hypothyroidism which may be because of increased adoption of such breed by owners (Fig. 3) in the study region. Clinical examination revealed rat tail appearance with dermatitis (Fig. 4a & 4b) was the predominant finding in affected dogs. Obesity stands second to rat tail appearance. Some of the presented female dogs had been suffered with infertility problem. Calorigenesis is the earliest recognized effect of thyroid hormone and dogs with hypothyroidism face difficulty in maintaining body temperature (heat seeker). Few dogs in this study reported to have problem in maintaining body temperature. Thickening and puffiness of skin are result of glycosaminoglycans and hyaluronic acid accumulation in dermis (Doliger *et al*., 1995). Puffiness of skin was evident in only one dog. These clinical signs were in accordance with study of Kour *et al*. (2021). Hypothyroidism is often associated with secondary skin infection, such as malassezia dermatitis (Maya, 2007; Scott-Moncrieff, 2007). Four (24%) dogs were presented with severe dermatitis.

**Table 1:** Hospital based incidence of hypothyroidism

| Age wise incidence | Sex wise incidence | Breed wise incidence |
|---|---|---|
| < 5 yrs – 17 dogs | Male - 10 | Labrador retriever - 15 |
| > 5 yrs – 03 dogs | Female – 10 | German shepherd - 3 |
| | | Non descript - 2 |

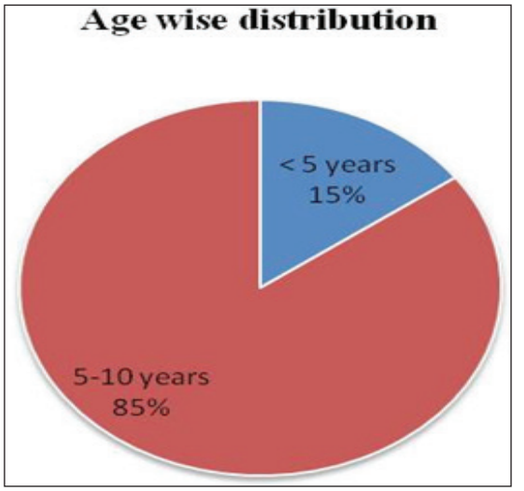


**Fig. 1:** Age wise distribution of hypothyroidism

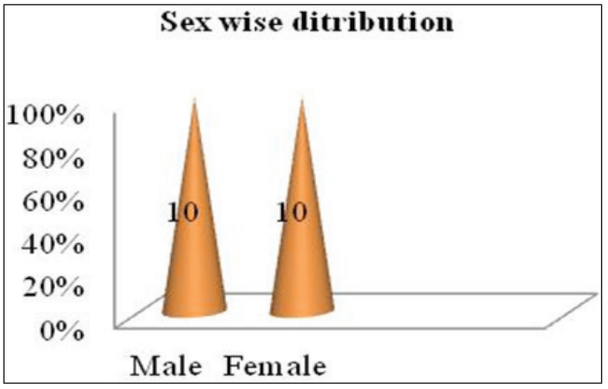


**Fig. 2:** Sex wise distribution of hypothyroidism

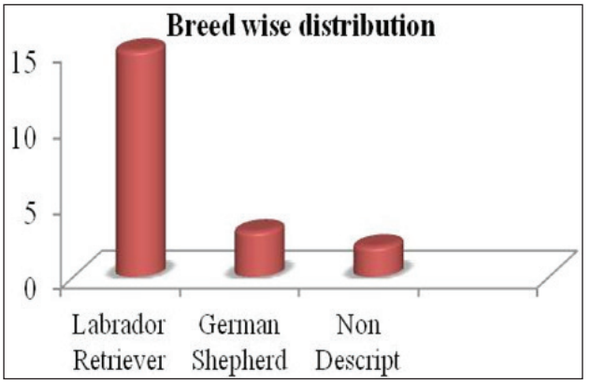


**Fig. 3:** Breed wise distribution of hypothyroidism

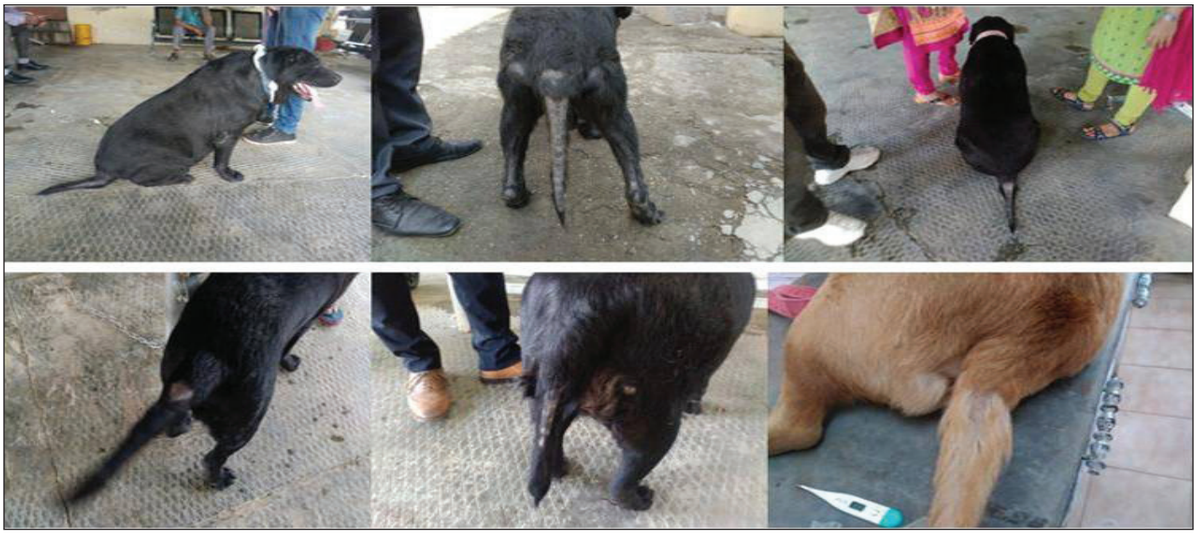

**Fig. 4a:** Dogs with characteristic rat tail appearance

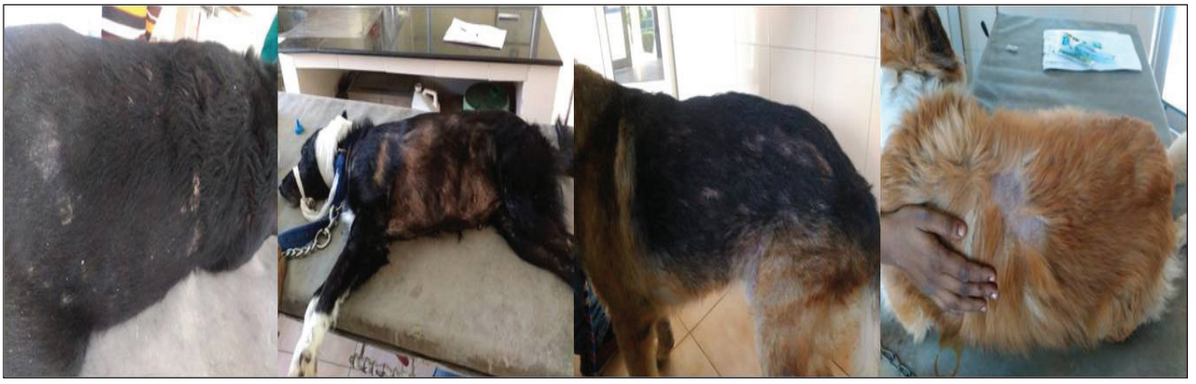

**Fig. 4b:** Dogs with varying degree of dermatitis

With respect to thyroid hormone concentration, there was significant ($p<0.05$) difference between diseased and control group in $FT_4$ concentration. In lymphocytic thyroiditis, thyroglobulin (Tg) in the circulation evokes immune response. It also presents thyroid hormones ($T_3$, $T_4$) to the immune system (Gaschen *et al*., 1993). Hence, dogs with lymphocytic thyroiditis have auto-antibodies against Tg and $T_4$ or $T_3$ (Thacker *et al*., 1992). Dogs with lymphocytic thyroiditis (91%) were positive for TGAB. Remarkably 5% and 6% dogs with non-thyroidal diseases and healthy dogs were positive for TGAB respectively (Iversen, 1998). Retrospective study on 45,131 canine thyroid hormone results showed 7.9% prevalence of thyroglobulin antibody (TgAA) in the overall dog population (Nachreiner *et al.*, 2000). TgAA-positive results occurred in 10 of 19 hypothyroid, 1 of 28 obese and 1 of 52 clinically healthy dogs. The clinically healthy TgAA-positive dog had additional evidence of hypothyroidism supported by low total T4, low free T and high canine TSH (Lee *et al*., 2004)

Autoantibodies against thyroid hormone in hypothyroid dogs tend to interfere with radioimmunoassay determination of $TT_4$ and $TT_3$ (Nachreiner *et al.*, 2002). Estimation of $FT_4$ by equilibrium dialysis method stands accurate for the diagnosis of hypothyroidism as because $FT_4$ is unaffected by thyroid hormone antibodies (Peterson *et al*., 1997). In addition to that, concurrent treatment with glucocorticoids and acetylsalicylic acid tends to lower $TT_4$ concentration without affecting $FT_4$ (Daminet and Ferguson, 2003; Daminet *et al.*, 2003) TSH-stimulation test using bovine TSH (b-TSH) has been employed often in diagnosis of primary hypothyroidism (Kemppainen *et al*., 2001). But, gold standard status of TSH-stimulation test is questionable, because TSH values remain within reference range of 20 to 40 % in dogs with confirmed hypothyroidism (Khan, 2010). Falsely high TSH concentration is also observed in euthyroid dogs with nonthyroidal illness (Diaz Espineria *et al*., 2007). Studies like radionuclide scan, high resolution ultrasonography and thyroid biopsy are most reliable indicator of primary hypothyroidism but, it needs sophisticated instruments and skilled personnel (Resse *et al*., 2005).

TPO Elisa kit (Ca TPO ELISA, E08T0529, Blue Gene) was procured to study the role of TPO enzyme in autoimmune mechanism. Standard curve was obtained after plotting the OD and concentration of given six standards (0, 5, 10, 25, 50, 100). Samples were subjected to standard protocol as described in the instruction manual. From the obtained regression equation ($y$ = -67.74x + 79.87; $R^2$ = 0.860) concentrations of TPO in diseased and control group were estimated by incorporating OD values and revealed not much significant difference in comparison to healthy group. Autoantibody against TPO may be associated with primary hypothyroidism in adult animals. Antibodies against TPO play little role in thyroiditis of dogs in contrast to human thyroiditis (Skopek *et al*., 2006; Graham *et al.*, 2007). Blood and serum biochemical changes in the affected animal have been presented in Table 2. Clinical pathology revealed significant ($p<0.05$) reduction in $FT_4$ level, altered cholesterol and lipid metabolism in affected dogs. It was in accord with study of other researchers (Rinjenberk and Kooistra, 2010). Dog with primary hypothyroidism also exhibited multiple metabolic disorders (Raguvaran *et al*., 2017; Alenka Hrovat *et al*., 2019). Leptin and insulin concentrations were significantly higher in the hypothyroid compared to normal dogs (Tovi *et al*., 2010). Increased serum concentrations of adiponectin were recorded in hypothyroid dogs (Tovi *et al*., 2015). Hyperglobulinemia and hypoalbuminemia were the constant finding in affected dogs. With respect to serum protein concentration, there was significant hypoalbuminemia and hyperglobulinemia in diseased groups. Normocytic, normochromic, nonregenerative anaemia has been the classical finding in hypothyroid dogs. But, there was no significant difference between diseased and control group in complete blood count.

All the affected dogs were treated with L-thyroxine (Eltroxin)® @ 20 – 40 mcg/kg bid for life long period. Hypothyroidism is always accompanied by hypercholesterolemia and there is always risk of atherosclerosis. Possible complication of hyperlipidemia is neurological disturbance due to atherosclerosis and thromboembolisms (Hess *et al.*, 2003; Vitale *et al*., 2007). To avoid such complication, dogs with hypercholesterolaemia were treated with *L. carnitine* (mg/kg b.wt) @150 mg kg b.wt for 30 days along with either atorvastatin @1-2 mg/kg b.wt (dose tapered later) or ezetimibe @0.1-0.2 mg/kg b.wt 30 days. Dogs with secondary bacterial and fungal dermatitis were treated with Tab. Cefadroxil/Cephalexin @10-20 mg/kg b.wt for 3 weeks along with topical antifungal and antibacterial preparation. Advised high protein diet (Chicken, egg

albumin) along with regular exercise to facilitate fat loss. We also advised the owners to check thyroid profile of their pets annually.

## CONCLUSION

From our study, it was concluded that autoantibody against thyroperoxidase (TPO) plays little or neglible role in primary hypothyroidism of dogs. Bilateral symmetrical alopecia along rat tail appearance was noticed in most of the affected dogs and which may be considered as specific symptoms of hypothyroidism in dog.

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