CLINICAL, DIAGNOSTIC AND THERAPEUTIC STUDIES OF HYPOTHYROIDISM IN DOGS

By

D. SRIKALA,
B.V.Sc. & A.H.

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CERTIFICATE

D. SRIKALA has satisfactorily prosecuted the course of research and the thesis entitled **"**CLINICAL, DIAGNOSTIC AND THERAPEUTIC STUDIES OF HYPOTHYROIDISM IN DOGS " submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by her for a degree of any university.

Date:

(K. SATISH KUMAR)

Place: Major Advisor

CERTIFICATE

This is to certify that the thesis entitled "**CLINICAL, DIAGNOSTIC AND THERAPEUTIC STUDIES OF HYPOTHYROIDISM IN DOGS"** submitted in partial fulfillment of the requirements for the degree of **MASTER OF VETERINARY SCIENCE (Veterinary Clinical Medicine)** of **Sri Venkateswara Veterinary University**, is a record of the bonafide research work carried out by **D. Srikala** under our guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee

No part of the thesis has been submitted by the student for any other degree or diploma. The published part has been fully acknowledged. All the assistance and help received during the course of investigation have been duly acknowledged by the author of the thesis.

(K. SATISH KUMAR)
Chairman of Advisory Committee

Thesis approved by the student advisory committee.

Chairman: Dr. K. Satish Kumar,
Assistant Professor,
Dept. of Veterinary Clinical Medicine,
College of Veterinary Science,
R.nagar, Hyd-500030.

Member: Dr. D.S.Tirumala Rao,
Professor & University Head,
Dept. of Veterinary Clinical Medicine,
College of Veterinary Science,
R.nagar, Hyd-500030.

Member: Dr. K. Sadasiva Rao,
Professor and Head,
Dept. of Animal Reproduction, Gynaecology and Obstetrics
College of Veterinary Science,
R.nagar, Hyd-500030.

DECLARATION

I, **D. SRIKALA** here by declare that the thesis entitled, "**CLINICAL, DIAGNOSTIC AND THERAPEUTIC STUDIES OF HYPOTHYROIDISM IN DOGS**" submitted to **Sri Venkateswara Veterinary University** for the degree of **Master of Veterinary Science** in **Veterinary Clinical Medicine**, is a result of original research work done by me. I also declare that the thesis or part there of has not been published earlier elsewhere in any manner.

Date: **(D. SRIKALA)**

Place: Hyderabad

Name of the Author	:	**D. SRIKALA**
Title of the thesis	:	**CLINICAL, DIAGNOSTIC AND THERAPEUTIC STUDIES OF HYPOTHYROIDISM IN DOGS**
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Major Advisor	:	**Dr. K. SATISH KUMAR**
University	:	**SRI VENKATESWARA VETERINARY UNIVERSITY**
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"Dream as if you'll live forever, live as if you'll die today."

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ABSTRACT

Out of a total 10,172 dogs presented to the small animal medical ward of Veterinary Hospital, Bhoiguda, Teaching Veterinary Clinical Complex, College of Veterinary Science, Rajendranagar, Hyderabad, 47 were found hypothyroid. The overall prevalence of hypothyroidism in dogs was 0.46 per cent and it was 30.92 per cent among dogs exhibiting clinical manifestations suggestive of impaired thyroid function. The highest prevalence was recorded in Labrador Retriever (25.53 per cent) aged between 5-10 yrs (61.70 per cent) and spayed females (40.43 per cent) and lowest in Daschund and Pug (2.13 per cent), less than 5 yrs (6.83 per cent) and uncastrated males (12.77 per cent).

The common clinical manifestations were bilateral alopecia (82.98 per cent), rat tail appearance (72.34 per cent), puppy like coat (17.02 per cent), dry and lustreless coat (68.08 per cent), secondary skin disorders (76.60 per cent), exercise intolerance (78.72 per cent), dyspnoea at rest (25.53 per cent), obesity (68.08 per cent), lethargy in (74.47 per cent), corneal lipidosis (10.64 per cent), goitre and

lameness (10.64 per cent), pale mucosa (19.25 per cent), anaemia (27.6 per cent), nervous signs (25.53 per cent) and cyanosis and myxedema (8.51 per cent).

The primary disorders associated with hypothyroid dogs were related to skin and coat in 76.60 per cent, and were mainly of *Malassezia pachydermatis* or *Demodex canis* or pyoderma. Further, certain systemic disorders such as cardiovascular, nervous system, diabetes mellitus, renal and musculoskeletal disorders were also recorded. Severely affected hypothyroid dogs were presenting the severe signs related to both skin and coat and reduced metabolic rate. Whereas, dogs presented with only skin and coat abnormalities were considered mild and those presented with predominant skin and coat and mild to moderate reduced metabolic rate associated signs were diagnosed as moderately affected ones.

Out of 23 dogs in group I, alleviation of clinical signs occurred in 21 following 30-35 days of treatment with levothyroxine. The remaining two dogs presenting goitre, cyanosis and myxedema, severe nervous signs, pale mucosae and corneal lipidosis died after 10 and 15 days of initiation of therapy. Whereas, group II dogs treated with levothyroxine and carnitine showed clinical improvement from day 15 and complete alleviation of signs were recorded by day 30. No death was recorded in this group of dogs.

All the hypothyroid dogs revealed normocytic, normochromic and non-regenerative anaemia with non-significantly different parameters except PCV. Similarly, there was hypercholesterolemia, hypertriglyceridemia with elevated levels of CKMB, LDH, ALP and TSH with significantly low levels of TT4, fT4 and T3.

The thyroid lobes of the affected dogs were heterogenous, had an irregular thick capsule, ill-delineated abnormal shape, smaller size and were hyper echoic compared with sternothyroid muscles on day 0. Whereas, the mean length, width,

height and volume of the hypothyroid lobes were significantly decreased when compared to apparently healthy dogs. No significant difference was noticed with these findings following therapy.

The mean pre treatment values of LVEdD and LVEsD of both the groups were significantly increased ($p<0.01$) when compared with the mean values of healthy dogs. These dimensions after therapy were found to be significantly ($p<0.05$) different from the findings recorded prior to the treatment. The other dimensions viz., LVPWd, LVPWs, IVSd, IVSs, and EPSS of group I and II, were also different significantly ($p<0.05$) from that of healthy dogs. Following therapy (on day 45) there was a non-significant difference in these values. With respect to ejection fraction and fractional shortening, a significantly low ($p<0.01$) levels were noticed in all the hypothyroid dogs. However, a significant ($P<0.05$) increase was recorded in these values on day 45 post therapy. Whereas, no specific difference in severity and intensity of turbulence and mosaic pattern of colour flow was noticed on pulse wave and colour flow Doppler of mitral valve in all the dogs before and after treatment.

There was no significant difference in various haematological parameters such as TEC, Hb, PCV, TLC and DLC between the groups I and II. A significant difference was noticed in the values of CKMB and LDH ($P<0.01$) and cholesterol, triglycerides, total thyroxine, free thyroxine, triodothyronine and thyroid stimulating hormone ($P<0.05$) of group II dogs on day 45 after treatment as compared with group I. Whereas, no significant difference was noticed with the values of ALT and ALP in between the groups on day 45. The different abnormalities associated with thyroid gland morphology and texture that revealed during thyroid lobe ultrasonography such as irregular thick capsule, ill delineated, abnormal shape, smaller size, echogenic difference and thyroid lobe volume were not significantly different between the

groups. The post therapy levels of left ventricle dimensions such as LVEdD, LVEsD, LVPWd, LVPWs, IVSd, IVSs, EPSS, EF and FS were significantly different ($P<0.01$) among group II dogs in comparison with group I.

Hence, from the present investigation it may be concluded that, the prevalence of hypothyroidism in dogs was 0.46 per cent, with a highest prevalence in Labrador retriever, between 5-10 yrs and spayed females are at highest risk. Normocytic, normochromic and non-regenerative anemia was the basic hemotological finding. Hypothyroid dogs were also associated with cardiovascular (Dilated Cardiomyopathy) disorder. Hence, the role of echocardiography is not ignored in hypothyroid patients, particularly those representing with decreased metabolic rate signs. The common electrocardiographic abnormalities include bradyarrhythmias, low voltage R and P waves. A part from routine laboratory analysis of thyroid profile, ultrasonography of thyroid lobes forms an added advantage in diagnosing impaired thyroid function. Osteoarthritis is also associated with hypothyroid obese dogs. Levothyroxine along with L-carnitine was found to be more effective in hypothyroid dogs specifically those presented with signs of low metabolic rate.

INTRODUCTION

Believed to have been the first animals domesticated by humankind, dogs have been domesticated for about 12000 years. Dogs are considered as the best companions forever, human–canine bond has been respected and cherished from time immemorial. They teach us loyalty, friendship, love and care. In the present scenario the pets such as sniffer squads are shouldered with more responsibilities involving risk. Dogs can detect and differentiate a wide variety of odours, as their olfactory sense has no comparision with any living being. Unfortunately, certain endocrine disorders like *hypothyroidism* affect the dog's sense of smell and if undiagnosed can be ineffective in discharging its duty and then inviting risk to its own and of course to the mankind.

Hypothyroidism results in decreased production of thyroid hormones, thyroxine (T_4) and 3, 5, 3 triiodothyronine (T_3) from the thyroid gland. Naturally occurring hypothyroidism is a common disease in dogs but rare in cats (Rand et al., 1993). Thyroid hormones are iodine containing aminoacids synthesized in the thyroid gland. In blood, more than 99 per cent of T_4 and T_3 (T4 more than T3) are bound to plasma proteins. Dogs have lower avidity of thyroid hormone binding to serum proteins than do humans, which may result in lower total serum concentrations of T_4 and T_3, higher free hormone concentrations and more rapid clearance rates. Thyroid hormone synthesis and secretion are regulated primarily by changes in the circulating concentration of pituitary thyrotropin (TSH). Thyroid hormones have a wide variety of physiologic effects viz., increase the metabolic rate and oxygen consumption of almost all tissues, with the exception of adult brain, testes, uterus, lymphnodes, spleen and anterior pituitary. Thyroid hormones have positive inotropic and chronotropic effects on the heart and have

catabolic effects on muscle and adipose tissue, stimulate erythropoiesis and regulate both cholesterol synthesis and degradation. Thyroid hormones are also essential for the normal growth and development of the neurologic and skeletal systems (Ferguson and Peterson, 1992).

More than 95 per cent of canine primary hypothyroidism is believed to be acquired that results from destruction of thyroid gland associated with lymphocytic thyroiditis, idiopathic thyroid atrophy or unusually, thyroid neoplasia. Secondary hypothyroidism that results from deficiency of Thyroid Stimulating Hormone is uncommon in dogs. Tertiary hypothyroidism has not been documented in dogs. Congenital hypothyroidism (cretinism) can occur in dogs, but rarely diagnosed as it usually results in early puppy death.

Deficiency of thyroid hormones affect all organs in the systems leading to decrease in basal metabolic rate (BMR). As the clinical signs are vague, diffuse and insidious in onset and relatively low accuracy of most biochemical tests and the fact that many factors like non thyroidal diseases, drugs and normal physiological fluctuations can lower circulating thyroid hormone concentrations, many times the condition is misdiagnosed clinically (Ferguson 1994). Further more hypothyroidism in canines can be associated with many systemic disorders. Even though, the human species are celeberating the world thyroid day (25^{th} May) the awareness regarding "Hypothyroidism" among the canine species is lagging much behind. Inspite of the sufficient literature available abroad, studies on clinically occurring primary hypothyroidism in dogs is scant and scarce in India. Keeping in view of the morbidity associated with hypothyroidism and its

associated complications in view an investigation is planned with the following objectives.

1. To study the prevalence of hypothyroidism in dogs.
2. To study the association of Dilated Cardiomyopathy (DCM) with hypothyroidism in dogs.
3. To study the efficacy of different therapeutic regimens of canine hypothyroidism.

Review of Literature

The most common endocrine disorder of dogs which till date possesses diagnostic and therapeutic challenges in field practice is Hypothyroidism. Hypothyroidism is the natural deficiency of thyroid hormone. The deficiency is produced by immune mediated destruction of thyroid gland, by natural atrophy of gland, dietary iodine deficiency or as a congenital problem (Dixon, 2004).

2.1 HISTORY

Vesalius first recognized and described the thyroid gland in 1543. As early as 1606, Paracelsus recognized a relationship between cretinism and the thyroid, and the first report on hyperthyroidism has appeared in 1786. Milestones of the 19th century were the description of hyperthyroidism or Graves Disease by Graves in 1835, the description of the triad of hyperthyroidism (Exopthalmus, goitre and tachycardia) by Von Basedow in 1840, the description of myxedema by Gull in 1873, the recognition of the relation of the later condition with thyroid atrophy by Ord in 1878, and the treatment of myxedema with thyroid extracts by Murray in 1891. Major in-sights into thyroid physiology were obtained when Magnus-Levy recognized that patients affected by hypothyroidism had a higher than normal oxygen consumption and that patients affected by myxedema had a lower than normal oxygen consumption. In

addition, Magnus-Levy also showed that administration of dessicated thyroid extract had profound influence on the basal metabolic rate and able to prevent spontaneous goiter in the dog by administering iodide (Marine and Lenhart, 1909). Thyroxine (T4) was isolated by Kendall in 1914 and synthesized by Harrington and Barger in 1927. Triiodothyronine (T3) was recognized as major biologically active thyroid hormone by Gross and Pitt in 1952. Sterling introduced an assay for measurement of T3 in 1969 and provided evidence for the peripheral conversion of T4 to T3 in 1970 (Eigenmann and Lubberink, 1985).

2.2 CANINE HYPOTHYROIDISM

Canine hypothyroidism can be primary, secondary, tertiary or congenital.

2.2.1 PRIMARY HYPOTHYROIDISM

Dixon et al., (2002) reported that primary hypothyroidism is a common endocrine disorder in canines and can be treated successfully using thyroid replacement therapy. Lethargy and mental demeanour were typically the first clinical signs to improve with significant body weight reduction occurring within 2 weeks of commencement of therapy.

Pikula et al., (2007) reported a clinical case of development of polyglandular failure syndrome (Schmidts syndrome) associated with hypothyroidism and hyperadrenocorticism in a 6 year old female Russian Terrier. The dog was presented with hypothyroidism in first occasion followed by hyperadrenocorticism.

Primary hypothyroidism is the most common that occurring in dogs when compared to others. Blois et al., (2008) reported a case of primary hypothyroidism in a two year old castrated male Australian Shepherd presented with nervous signs

Fors (2008) reported that primary hypothyroidism is the most common form of hypothyroidism in dogs and are manifested with a variety of neuromuscular signs.

Primary hypothyroidism is many times associated with Dilated Cardiomyopathy (DCM). The condition is manifested by the abnormalities of skin and coat along with bradycardia, arrythmias and first degree AV block. Levothyroxine supplementation helped in improving the primary hypothyroidism and its associated DCM by increasing contractility and increased left ventricle wall thickness (Gaalova et al., 2008).

Graham (2009) reported that hypothyroidism is a common endocrine disorder of dogs that may be both under and diagnosed. The common manifestations associated with primary hypothyroidism such as exercise intolerance, respiratory distress, alopecia and skin lesions are nonspecific.

2.2.2 SECONDARY HYPOTHYROIDISM

Secondary hypothyroidism caused by deficiency of Thyroid Stimulating Hormone (TSH) is rarely described in dogs, probably became a validated assay for canine TSH and has been unavailable until recently. The condition is caused by pituitary malformations and pituitary neoplasia (Manning, 1979).

Varshney et al., (2007) reported a case of secondary hypothyroidism in a Labrador bitch that was presented with lethargy, mental dullness, exercise intolerance, exertion, severe obesity, bilateral symmetrical alopecia. The condition was diagnosed based on TSH assay that revealed significantly low levels. The affected dog also revealed normal blood/serum glucose, proteins with increased cholesterol and triglyceride levels.

2.2.3 TERTIARY HYPOTHYROIDISM

Sheil et al., (2007) reported tertiary hypothyroidism in a 9 year old male Labrador retriever. The dog was diagnosed with pituitary dependent hyperadrenocoticism and administered mitotane therapy for 7 months. Gradually the

dog developed weight gain seborrhea and alopecia. Biochemical estimation confirmed hypothyroidism with low levels of T4 and T3 and elevated serum cholesterol and triglycerides.

2.2.4 CONGENITAL HYPOTHYROIDISM

Fyfe et al., (2003) reported congenital hypothyroidism with goiter in a fox terrier and opined that congenital hypothyroidism with goiter is associated with simple autosomal recessive traits. Affected pups exhibited inactivity, abnormal hair coat, stenotic ear canal and delayed eye opening with palpable ventrolateral cervical swelling. Thyroid profile revealed low T4 and T3 and high TSH concentrations.

Graham et al., (2007) documented that congenital anomaly of thyroid gland or pituitary that causes cretinism (congenital hypothyroidism) occurs in small proportion of canine population compared to those cases that result from irreversible acquired hypothyroidism.

Kolevska et al., (2007) presented neonatal transient hypothyroidism in two whippet puppies of 10 weeks age. The authors documented that puppies develop goiter associated with dietary iodine deficiency as the pups fed giblets and poultry meat. Laboratory examination revealed non-regenerative anaemia, hypercholesterolemia, significantly increased total thyroxine and increased TSH levels. Ultrasonography revealed enlarged thyroid gland.

2.2.5 EPIDEMIOLOGY

Primary hypothyroidism is common endocrine disorder in adult dogs beyond 2 years of age and is associated with thyroid atrophy (Mooney, 2003).

Hoh Woopil and Oh Taeho (2006) reported circadian variations of serum TT4, fT4 and 3,5,3 triiodothyronine on healthy dogs. The authors opined that measurement

of serum thyroid profile concentration from 11am to 14 hrs might be more significant to diagnose canine hypothyroidism.

Hypothyroidism is most commonly encountered endocrine disorder in dogs whose incidence is 1:156 to 1:500 (Borku and Aktas, 2007).

Seavers et al., (2008) studied thyroid status of Basenji dogs in Australia and concluded that dogs between 1to 14 years and spayed females were at increased risk for primary hypothyroidism.

2.3 PRIMARY HYPOTHYROIDISM

2.3.1 ETIOLOGY

2.3.1.1 THYROIDITIS

Hypothyroidism, primarily associated with lymphocytic thyroiditis which is characterized by diffuse infiltration of thyroid gland by lymphocytes, plasma cells and macrophages with formation of some lymphoid nodules and destruction of follicles, progressing to replacement of most of the thyroid by fibrosis connective tissue (Gosselin et al., 1981).

Lymphocytic thyroiditis and follicular atrophy are the most common lesions associated with clinical hypothyroidism in pet dogs. Lymphocyte thyroiditis resembles Haschimoto's thyroiditis in humans. The morphology of thyroid lesion and frequent occurrence of circulating thyroglobulin autoantibodies suggests that lymphocytic thyroiditis is immune mediated in pet dogs (Czuminska, 2001).

Primary hypothyroidism is a common endocrine disorder in adult dogs beyond two years of age and occur as a consequence of lymphocytic thyroiditis or thyroid atrophy (Mooney, 2003).

Castillo et al., (2006) reported that subclinical thyroiditis can be caused by infectious agents. The suspected dogs were clinically presented with signs of

hypothyroid whose T4 and T3 levels were significantly lower. The authors concluded that toxoplasmosis affects thyroid morphology, being able to alter its function with the development of autoimmune thyroiditis in susceptible individuals.

Porto et al., (2008) reported primary hypothyroidism in a Bull terrier dog that was diagnosed as canine lymphocytic thyroiditis as the cause for hypothyroidism.

2.3.1.2 DRUG INDUCED

Several drugs can affect thyroid function test results that lead to an erroneous evaluation of thyroid function. Drugs like glucocorticoids, propranol, sulfonamides, phenobarbital and NSAIDS can alter the synthesis, secretion, transport and metabolism (Daminet and Ferguson, 2003)

Singh et al., (2007) reported a case of hypothyroidism associated with a long term medication of phenytoin sodium and phenobarbital sodium combination for the treatment of epilepsy. Haematology revealed increased serum cholesterol and triglycerides with marked depression of thyroid profile.

Glucocorticoids, Sulphonamides and Phenobarbital are few common drugs that affect hypothalamic-pitutary-thyroid axis in many species. Usage of these drugs as therapeutic agents for respective disorders can have an affect on thyroid gland, hormone production and can lead to hypothyroidism. However, discontinuation of therapy with these drugs and subsequent replacement therapy with levothyroxine can help in improving the condition (Cardoso et al., 2007).

Klopmann et al., (2007) reported that a variety of anticonvulsant drugs used for epilepsy in dogs can lead to subnormal plasma thyroid hormone concentration.

Seelig et al., (2008) reported the usage of trimethoprim sulphamethaxazole and chloramphenicol in a 16 week old female boxer for 5 weeks has resulted in goitrous hypothyroidism.

2.3.1.3 DIETARY

Castillo et al., (2001) reported that excess iodine content of commercial diet causes alterations in thyroid function and morphology and its hormones have direction on bone formation. A significant decrease in the styloid epiphyseal surface was noticed on radiography among the dogs fed iodine excess commercial diet. The authors opined that commercial diets with high iodine content may cause hypothyroidism and changes in bone metabolism. Abnormally low iodine uptake was noticed in dogs fed commercial diet at the university animal clinic, Argentina. The results demonstrated that excess amount of iodine present in commercial diet caused a significant impairment of thyroid function and hypothyroidism.

Castillo et al., (2003) opined that increased dietary iodine alters thyroid morphology and function in puppies younger than 3 months old. Iodine excess causes alteration in thyroid activity, blocking both its characteristic function and cell proliferation. Depending on the dose of iodine and on the previous conditions of the gland, Iodine excess can have a goitrogenic effect and induce the blockage of hormone biosynthesis and secretion provoking hypothyroidism.

2.3.1.4 MISCELLANEOUS

Levine et al., (2005) reported that myasthenia gravis associated with hypothyroidism in canines. The authors reported this complaint in a 12 year old female Poodle that was presented with the history of paraperisis, dyspnoea and tenesmus.

Schenck (2007) documented that certain endocrine disorders like hyperthyroidism (cats) and hypothyroidism (dogs) is also associated with disorders of calcium metabolism in respective species.

Bansal et al., (2007) reported idiopathic hypothyroidism in a GSD bitch that was presented with the clinical signs of hair loss, change in skin colour and confirmed by the low levels of T3 and T4.

Thyroid gland plays a key role in metabolism and consequently hypothyroidism has been a common diagnosis for decreased performance in sledge dogs. The authors opined that a variety of environmental factors influence thyroid hormone production such as light exposure, climate, lassitude, exercise and season. Results of the study indicated that thyroid hormone plays an integral role in thermoregulation and is greatly affected by environmental cues (Dunlap et al., 2008).

2.3.2 PATHOPHYSIOLOLGY

Production of T4 is regulated by the pituitary gland located at the base of ones brain. This gland is called the master gland as it regulates hormone production in the adrenal system, the thyroid system, the reproductive system and more. The pituitary produces a substance called Thyroid Stimulating Hormone (TSH). When T4 levels are dropping, the pituitary gland stimulates the thyroid gland to make and release more T4. Active thyroid hormone serves as a sort of a volume dial for metabolism. Since, virtually every cell in the body can be affected by reduced levels of thyroid hormone it is not surprising that reduced level of thyroid hormone lead to symptoms in multiple body systems. (Rand et al., 1993).

Thyroid hormones have a wide variety of physiologic effects viz., increasing the metabolic rates and oxygen consumption of almost all tissues, with the exception of adult brain, testes, uterus, lymphnodes, spleen and anterior pituitary. Thyroid hormones have positive inotropic and chronotropic effects on the heart. These have catabolic effects on muscle and adipose tissue, stimulate erythropoiesis and regulate both cholesterol synthesis and degradation. Thyroid hormones are also essential for

the normal growth and development of the neurologic and skeletal systems (Ferguson and Peterson, 1992).

2.3.3 DIAGNOSIS

2.3.3.1 HISTORY

Credille et al., (2001) opined that exercise intolerance, lethargy, and putting up weight inspite of normal appetite are the important history findings of hypothyroidism in dogs.

Or et al., (2005) reported discolored hair, alopecia with lethargy as the basic information that can be collected as history at the time of presentation of the dog.

Hypothyroidism is the natural deficiency of thyroid hormones produced by immune mediated destruction of thyroid gland is presented with several dermatological abnormalities, lethargy and obesity with cold intolerance (Satish Kumar et al., 2007).

2.3.3.2 CLINICAL MANIFESTATIONS

2.3.3.2.1 SKIN AND HAIR

Alopecia and non-pruritic dermatitis are the significant hair and coat abnormalities among 54 dogs of primary hypothyroidism. T3 and T4 levels of these patients were significantly lower than in control dogs (Chakrabarthy et al., 2001).

The effects of hypothyroidism on canine skin were determined by comparing morphologic, morphometric hair cycle differences in skin biopsy samples. Hypothyroid dogs had a greater number of follicles in telogen and fewer hair shafts than healthy dogs and whereas, following levothyroxine replacement therapy the hypothyroid dogs had an increased number of follicles in the growing stage of hair cycle (Credille et al., 2001).

Or et al., (2005) reported discolored hair Rottweiler with hypothyroidism. Serum AST, ALT, ALP and cholesterol were with in normal range with low levels of thyroid profile. The dog was successfully treated with levothyroxine @ 20 mcg/ kg b. wt daily.

Mayr (2007) reported that generalized *Malassezia* dermatis is one of the common secondary skin disorder noticed in hypothyroid dogs. Further, authors also reported concurrent bacterial skin infections. The lesions are localized over the ventral neck, paws, axilla, inguinal areas, skin folds and perineal region. Alopecia X is the term concurrently used to describe a group of skin diseases without systemic clinical signs such as absence of pruritus and hyperpigmentation. Many times it is associated with hypothyroidism.

Hypothyroidism is the natural deficiency of thyroid hormones produced by immune mediated destruction of thyroid gland is presented with several dermatological abnormalities. Satish Kumar et al., (2007) reported generalized hair loss (88%), rat tail appearance (83%), pigmentation and pruritis (27%), brittle, dry and lustrous hair coat (83%), puppy like coat (22%).

Bansal et al., (2007) reported that dermatological disorders associated with primary hypothyroidism in dogs are dry, thick, and hyper pigmented skin with alopecia on ventrum, dorsum, neck and tail.

Fialkovikova et al., (2008) reported that primary hypothyroidism in canines is manifested by nonpruritic alopetic lesions on the ventral thighs, rat tail appearance with changed colour of hair coat, brittle and dry coat and scaling of skin.

2.3.3.2.2 PHYSICAL CONDITION

Land et al., (2006) studied the prevalence and risk factors for obesity in adult dogs and concluded that hypothyroidism, hyperadrenocorticism, ruptured cruciate

ligament are the common causes. Obesity is most likely to occur in older dogs of certain breeds like Labrador, Dalmatian and Daschund that are associated with hypothyroidism.

Scott (2007) suggested that canine hypothyroidism is presented with a wide range of clinical signs. The most common decreased BMR characterized by lethargy and obesity coupled with dermatological manifestations. However, other manifestations such as, reproductive dysfunctions, clinical heart disease and behavioural abnormalities are less compelling.

Ajit Kumar et al., (2008) assessed the body condition and body fat of dogs suffering with hypothyroidism and concluded that obesity was the most common physical abnormality noticed with hypothyroid dogs which further affects fertility.

2.3.3.2.3 CARDIOVASCULAR

Fernandez et al., (2005) stated that cardiomegaly and heart dilatation is the common associated abnormalities recorded in nutritional hypothyroidism effected African wild dogs.

Stepanovic and Stefanovic (2005) reported that hypertension is defined as secondary when it occurs as a consequence of certain chronic disorders like hypothyroidism, hyperadrenocorticism and Diabetes mellitus.

Thyroid hormones have direct and indirect effects on heart that results in depression of left ventricular functioning in canine hypothyroidism. The condition was manifested by exercise intolerance, dyspnoea at rest, generalized weakness, lethargy which are associated with decreased contractility and low heart rate (Gaalova et al., 2008).

Lethargy, exercise intolerance and dyspnoea at rest were the common cardiac related manifestations in dogs. (Rossmeisl et al., 2009)

Ischemic and haemorrhagic stroke are characterized by its abrupt onset is the 3rd leading cause of death in humans but rare in dogs. Many times the underlying conditions that may be associated with canine stroke include hypothyroidism, hypertension and coagulopathy (Wessmann et al., 2009)

2.3.3.2.4 NEUROMUSCULAR

Fors (2006) stated that primary hypothyroidism in dogs is associated with a variety of neurological signs including lower motor neuron disease, peripheral vestibular syndrome, facial, laryngeal paralysis, megaoesophagus and myasthenia gravis. The diagnosis is confirmed based on neurological signs coupled with low T3, T4 and TSH levels.

Klopmann et al., (2006) suggested that hypothyroidism is a common endocrinological disorder in older and large breed dogs and has various clinical symptoms. Many times these patients also manifest nervous signs like ataxia, hyperflexia, vestibular signs and facial nerve paralysis.

Higgins et al., (2006) presented hypothyroid associated central vestibular disease in 10 dogs. Authors studied from 1999 to 2005 and concluded that dogs between 5-10 years referred for progressive neurological disease were diagnosed for hypothyroidism based on increased serum cholesterol, triglycerides with significant low levels of TT4, fT4 but increased TSH concentrations.

In a retrospective study conducted by, Georgescu and Codranu (2007) reported that 15-29 percent of dogs were showing neurological abnormalities. The most common neurological manifestation being related to peripheral nervous system. Neurological examination of hypothyroid dogs revealed tetraparesis with inability to walk, decreased muscular tone and myotatic reflexes. All the dogs were significantly responded to levothyroxine therapy for a month. Primary hypothyroidism in dogs has

been associated with a variety of neuromuscular signs including generalize peripheral neuropathy, vestibular syndrome, facial, laryngeal paralysis, megaoesophagus and myasthenia gravis.

Seydel (2007) reported aggressive behavioural pattern towards strangers and others dogs that is associated with revealed low levels of total thyroxine.

Hypothyroidism has been associated with a variety of neurological signs such as tetraparesis, central and peripheral vestibular signs, facial paralysis and paraperesis (Vitale and Olby, 2007)

Singh et al., (2007) documented epileptic seizures in a dog with hypothyroidism. The biochemical profile revealed increased levels of serum cholesterol and triglycerides with marked depression of thyroid profile.

Kang et al., (2007) reported that a male Labrador retriever presented with mental retardation, circling and head pressing with adypsia as typical manifestation. Biochemical findings revealed low levels of thyroid profile T3, T4 and TSH with the presence of antithyroid antibodies in serum and cerebrospinal fluid.

Pettigrew et al., (2007) documented arrested physical development and neurological abnormalities in 9 weeks terrier puppies. It was diagnosed as hypothyroidism with low serum T4, fT4 and elevated levels of TSH.

A dog presented with the signs of chronic mild to moderate ataxia, obesity and lethargy was treated with levothyroxine but of no response. Post mortem examination revealed marked thyroid gland atrophy and severe CNS atherosclerosis (Blois et al., 2009)

2.3.3.2.5 RESPIRATORY SIGNS

Georgescu and Codranu (2007) reported affected dogs presented with exercise intolerance, respiratory distress, laryngeal paralysis are associated with primary hypothyroidism in dogs.

2.3.3.2.6 MYXEDEMA

Finova and Greco (2007) documented that hypothyroidism is a common endocrinopathy in dogs that is caused by lymphocytic thyroiditis and idiopathic thyroid atrophy. Myxedema coma is a rare and potentially fatal manifestation of severe hypothyroidism.

Satish Kumar et al., (2007) opined that myxedema is the most unusual manifestation in hypothyroid dogs. The authors reported myxedema in 03 dogs out of 34 cases reported during a two year study.

2.3.3.2.7 OCCULAR

Williams et al., (2007) opined that hypothyroidism in canine patients can be predisposed to keratoconjuntivitis sicca.

Durieux et al., (2008) suggested that ocular changes are not common in hypothyroidism but few abnormalities such as, corneal dystrophy (represented as white spot on eye surface), ulceritis and lipidosis are rarely associated.

2.3.3.3 LABORATORY ANALYSIS

2.3.3.3.1 HAEMATOLOGY

Chakrabarthy et al., (2001) reported significantly low mean values of hemoglobin, PCV and TEC suggesting anemia in hypothyroid related alopetic dogs.

Mooney (2003) opined that hypothyroidism occurs in adult dogs of more than 2 years as a sequlae of lymphocytic thyroiditis or thyroid atrophy. Though haematology is nonsignificant but hypothyroid dogs presented normocytic, normochromic, nonregenerative anaemia.

In primary hypothyroidism Haematologically no significant abnormalities appeared, though normocytic, normochromic non regenerative anaemia was a common finding (Bansal et al., 2007).

Andronic et al., (2008) suggested that haematological and biochemical tests be performed along with clinical examination of skin and hair of dogs suffering with hypothyroidism.

2.3.3.3.2 BIOCHEMICAL FINDINGS

Gomathy et al., (2004) suggested that relation between thyroxine and cholesterol concentration for the maintenance of glossy, healthy skin coat and hence the deficiency could result in various kinds of skin and coat abnormalities.

Elevated levels of plasma Creatine Kinase MB (CKMB), ALT, AST, LDH were the common biochemical alterations apart from significantly low levels of T3, T4 and TSH are the most common biochemical abnormalities noticed in affected dogs (Rossmeisl, 2009)

The common biochemical alterations in primary hypothyroid dogs are mild to moderately elevated ALT, ALP, cholesterol and triglycerides along with significantly decreased T4, T3 and TSH (Satish Kumar et al., 2007).

Andronic et al., (2008) reported hypercholesterolemia, hypertriglyceridemia with increase levels of plasma urea, ALT, ALP and beta- globulins in primary hypothyroidism canine cases.

Increased serum cholesterol levels, Creatine Kinase MB and alkaline phosphatase activities were the major biochemical alterations noticed in 6 Doberman pinscher dogs suspected for primary hypothyroidism (Suraniti et al., 2008).

2.3.3.3.3 THYROID PROFILE

Ramsey et al., (1997) opined that TSH measurements are a useful additional diagnostic test in cases of suspected hypothyroidism in dogs but analysis of fT4, TT4 and T3 are not to be ignored.

Peterson et al., (1997) indicated that measurement of serum fT4 and TSH concentrations is useful for diagnosis of hypothyroidism in dogs. However, dogs with confirmed hypothyroidism may sometimes also have serum TSH concentrations with in reference limits.

The diagnosis of hypothyroidism is achieved by demonstration of low circulating TT4 with elevated TSH concentrations. However, in some cases this combination doesn't occur and other diagnostic tests such as fT4 and thyroglobulin autoantibody analysis are required (Mooney, 2003).

Measurement of canine thyrotropin had an excellent specificity and suggests that the measurement of TSH is a valuable tool in confirming canine hypothyroidism. However, due to the low sensitivity of TSH assays it cannot be recommended to exclude the disease (Boretti and Reusch, 2004). The diagnostic value of assessment of TSH using current assays shows normal values in a high percentage of hypothyroid dogs. The authors concluded that in most of the hypothyroid dogs T4 is decreased but with the presence of autoantibodies to T4 the values can be normal or increased.

Breyer et al., (2004) opined that though hypothyroid profile estimations are commonly used to diagnose canine hypothyroidism, assessment of autoantibodies to thyroglobulin (TGAA) is more useful tool in the diagnosis of thyroid dysfunction and can be used as a marker as an auto immune thyroiditis.

Even though T4 and T3 levels were considered in assessing thyroid function in dogs these values varied according to the breeds such as smaller breeds like Spitz had

highest T4 (1.96±0.14mcg/dl) large breeds GSD will had highest T3 (102.83± 6.89ng/dl) Jagpreeth et al., (2006).

Although primary hypothyroidism can be diagnosed clinically by skin and other manifestations determination of fT4 and TSH levels are of more significant. Suraniti et al., (2008) reported that free T4 values with in the normal range but with highly elevated TSH in 6 Doberman pinscher dogs between 6-8 years of age suffering with hypothyroidism.

2.3.3.4 ELECTROCARDIOGRAPHY

Gonul et al., (2002) reported that in hypothyroidism can exhibit changes include changes in rhythm disturbances such as sinoatrial block, low amplitudes of P and R waves on ECG.

Sinus rhythm, bradycardia, low amplitude of P and R waves with normal P, R and QRS intervals are the common electrocardiographic abnormalities in secondary hypothyroidism (Varshney et al., 2007).

Gaalova et al., (2008) reported that bradycardia, low voltage QRS complexes, arrythmias and first degree AV block are the common electrocardiographic changes associated with primary hypothyroidism.

2.3.3.5 ULTRASONOGRAPHY

2.3.3.5.1 THYROID ULTRASONOGRAPHY

Reese et al., (2005) opined that ultrasonography of thyroid gland is an effective tool for the investigation of thyroid gland diseases to discriminate between

euthyroid sick and hypothyroid dogs. While assessing thyroid gland ultrasonography maximal cross sectional area, thyroid volume and echogenicity were measured.

Thyroid lobes of hypothyroid patients are ultrasonographically examined for size, shape and echogenicity and homogeneity. The thyroid lobe volume was estimated and found no difference between healthy and euthyroid dogs with nonthyroidal illness. The thyroid lobes were fusiform and triangular in shape in longitudinal and transverse planes, respectively. Bromel et al., (2005) opined that determination of thyroid size and volume by ultrasound may be a useful adjunctive test for differentiating hypothyroid and euthyroid dogs.

Castillo et al., (2006) suggested ultrasonography of thyroid lobes is the easiest and fastest way of diagnosing primary hypothyroidism in dogs. They further opined that this is the additional diagnostic tool to rule out false positive patients.

Marco and Larsson (2006) evaluated the use of cervical ultrasonography as a diagnostic method for hypoytyroidism. Dogs confirmed by thyroid profile were subjected for cervical ultrasonography that revealed significantly lower total thyroid gland volume when compared with normal dogs. Ultrasonography of thyroid lobes is an additional non-invasive diagnostic tool to confirm hypothyroidism in dogs. The affected thyroid lobes in hypothyroid dogs were sonographically smaller, hypoechoic, heterogenous, misshapen and ill delineated. However, thyroid hormone replacement therapy (THRT) improved ultrasonographic characters of thyroid gland in the affected patients.

Primary hypothyroidism is a frequent endocrine disorder in the adult dog. However, false positive diagnosis are common because of the relatively low accuracy of most commonly used biochemiocal tests. Taemens et al., (2007) opined that thyroid ultrasonography is an additional diagnostic tool of primary hypothyroidism

and concluded that thyroid ultrasonography is more sensitive, gives quick result as compared to biochemical tests and involves no sedation of the patient.

2.3.3.5.2 ECHOCARDIOGRAPHY

Hypothyroidism results in reversible cardiac changes consisting of increased period and left ventricle and systolic diameter and fractional shortening with low P and R wave amplitude. However THRT was found to improve the functional parameter of cardiovascular system (Panciera, 1994).

Right ventricular and intravenous septal hypokinesia, decreased left ventricular systolic-diastolic with left atrium diameter and left ventricular fractional shortening are the major echocardiographic abnormalities reported in dogs diagnosed for hypothyroidism (Gonul et al., 2002).

Gaalova et al., (2008) reported that depression of left ventricular functioning is associated with hypothyroidism. 2D echocardiography of hypothyroid dogs showed reduced contractility and reduced left ventricular wall thickness before treatment..

2.3.3.6 RADIOGRAPHY

Archibald (1974) opined that cervical and thorasic radiographs are of additional diagnostic tools to study hypothyroidism (goitre) and associated DCM. Goitrous swelling represents as soft tissue at the caudal portion of larynx

Stanin et al., (2005) suggested that radiological diagnosis of cardiac diseases was one of the non-invasive techniques that have promising results in diagnosis of cardiac diseases like dilated cardiomyopathy

2.3.4 ASSOCIATED DISORDERS OF HYPOTHYROIDISM

2.3.4.1 CARDIOVASCULAR DISORDERS

Hypothyroidism is associated with abnormalities in lipid metabolism. Anna Rita et al., (1999) opined that the risk of coronary heart disease assumed to be associated with hypothyroidism.

Bakrel et al., (2003) opined that changes in left ventricular dimensions and left ventricular pressures are common secondary complications that are associated with primary hypothyroidism in dogs. The authors reported that left ventricular systolic and diastolic pressures, left ventricular wall thickness and interventricular septum thickness were significantly low in hypothyroid dogs compared with healthy ones.

Vressler et al., (2003) reported portal vein and aortic thrombosis in a Siberian husky that had erlichiosis and hypothyroidism. The condition was diagnosed based on ultrasound examination in said dog that is under treatment with levothyroxine for primary hypothyroidism.

Stephen et al., (2003) stated that thyroid hormone have direct and indirect effect on heart. Hence, concluded that hypothyroid dog had a depression on left ventricular function i.e., associated with decreased contractility and left ventricular wall thickness which can be corrected by levothyroxine supplementation.

Philips and Harkane (2003) reported DCM as a concurrent cardiovascular complication in hypothyroid dogs. The dogs had demonstrated improvement in myocardial contractility, increased fractional shortening and reductions in left atrial size, left ventricle and systolic and diastolic diameters following supplementation with levothyroxine.

Mac Gregor et al., (2004) reported cholesterol based pericardial effusion and aortic thrombo-embolism in a 9 year old dog with hypothyroidism that was presented with exercise intolerance, general weakness, lethargy and nocturnal cough.

Fernandez et al., (2005) reported cardiomegaly and heart dilatation are the common associated cardiovascular abnormalities in hypothyroid African wild dogs.

Vitale and Olby (2007) documented that hypothyroidism is associated with hyperlipedemia that predisposes to atherosclerosis, increased blood viscosity and thromboembolic events. The authors also reported dogs suffering with hypothyroidism had an evidence of iliac thrombosis and atherosclerosis on ultrasound examination.

Fialkovikova et al., (2008) reported that primary hypothyroidism presented with non-pruritic alopetic lesions, change in colour of hair coat, obesity is associated with slow progression of DCM.

Gaalova et al., (2008) stated that cardiovascular system abnormalities particularly DCM is a common associated disorder in primary canine hypothyroidism. The condition can be diagnosed using 2D echocardiography that showed reduced contractility and reduced left ventricular wall thickness.

2.3.4.2 DIABETES MELLITUS

Hess et al., (2000) opined that hyperadrenocorticism, urinary tract infections, dermatitis and diabetes mellitus are concurrently associated with hypothyroidism. They concluded that treatment of these disorders may improve the prognosis of dogs with diabetes mellitus and may prevent complications like insulin resistance or diabetic ketoacidosis.

Hess et al., (2003) documented the association between diabetes mellitus, hypothyroidism and atherosclerosis in dogs and concluded that diabetes mellitus and hypothyroidism are more prevalent in dogs with atherosclerosis compared to dogs without atherosclerosis.

2.3.4.3 RENAL INSUFFICIENCY

Mansfield and Mooney (2006) reported lymphocytic, plasmocytic thyroiditis associated glomerulonephropathy in a boxer. The dog was diagnosed for primary hypothyroidism based on antithyroglobulin antibody titer suggesting immune mediated thyroiditis with a concurrent protein loosing glomerulonephropathy. Thyroid replacement therapy for 12 months improved the dog clinically

2.3.4.4 MUSCULOSKELETAL DISORDERS

Parades et al., (2003) reported about the effects of moderate to severe osteo-arthritis on canine hypothyroidism and concluded that osteoarthritis need not to be considered a factor influencing thyroid function evaluation in dogs.

Cho et al., (2007) carried out a retrospective study of 94 hyperkalemia dogs and concluded that thyroid hormone plays a role in calcium metabolism and during thyroid dysfunction hypercalcemic state exists remitting in depletion of bone calcium.

Schenk (2007) documented that calcium homeostasis is affected in hypothyroid dogs resulting in weakening of long bones and there by leading to increased risk for fracture and osteo arthritis in severly obese hypothyroid dogs.

Chronic canine hypothyroidism resulted in substantial but subclinical phenotypic myopathic changes indicative of altered muscle energy metabolism and depletion of skeletal muscle carnitine. These abnormalities may contribute to nonspecific clinical signs such as lethargy and exercise intolerance often reported in hypothyroid dogs (Rossmeisl et al., 2009)

2.3.4.5 SKIN DISORDERS

Pipan (2005) reported that pyoderma a common skin disorder can occur in concurrent with hypothyroidism among various breeds of dogs. Apart from antibiotic therapy supplementation of levothyroxine will help in alleviating the signs.

German shepherd dog pyoderma (GSP) is a unique recurrent deep pyoderma that begins over lumbosacral region and progress to affect multiple regions of the body. The underlying disease process involved in the development of GSP includes flea allergy, dermatitis, atopic dermatitis, food allergy and hypothyroidism (Rosser, 2006).

Canine demodicosis and malasseziosis are the two important skin associated complaints that were found aggravated in dogs with hypothyroidism. *Malassezia pachydermatis* is a common secondary infectious agent that causes canine malasseziosis in dogs which is a common associated disorder of primary hypothyroidism. The complaint is manifested by dry hair coat, scaling of skin alopecia and moist eczematous lesions on ventral abdomen and ear canal (Mayr, 2007).

Even though alopecia X is a common skin abnormality encountered in primary hypothyroid dogs malasseziosis and pyoderma are the frequent concurrent skin disorders that occurs as secondary invaders and is responsible for pruritis (Satish Kumar et al., 2008).

2.3.5 THERAPEUTIC MANAGEMENT OF CANINE HYPOTHYROIDISM

2.3.5.1 Thyroxine

Dixon et al., (2002) reported that clinical resolution of metabolic signs can be expected within 2weeks, whereas dermatological signs may take up to 3 months following thyroid replacement therapy in dogs diagnosed for primary hypothyroidism. Further, a decrease in circulating cholesterol and triglyceride concentration with an increase in RBC count can be used to indicate overall effect of thyroid hormone replacement therapy (THRT).

Bansal et al., (2007) reported the efficacy of oral thyroxine (Eltroxine 100mcg) when administered at one tablet BID for 4 months in a German shepherd bitch. Clinical improvement with respect to hair growth, presumption of normal reproductive cycle, normalization of body weight and gradual improvement in T3 and T4 levels occurred during four months of therapy.

Finova and Greco (2007) suggested that levothyroxine commonly used as oral supplement in treating primary canine hypothyroidism.

Satish kumar et al., (2008) studied the efficacy of levothyroxine @ 10 mcg/kg BID for first 15 days followed by SID for 30-90days depending on clinical recovery.

Gaalova et al., (2008) opined that cardiovascular abnormalities disappeared upon supplementation of levothyroxine at 20mcg/kg bwt for considerable period.

Traon et al., (2008) studied pharmacokinetic properties of levothyroxine in dogs of starting dose of 20mcg/kg b wt SID was more efficacious in treating canine primary hypothyroidism.

Traon et al., (2009) reported the efficacy of liquid solution of levothyroxine towards canine primary hypothyroidism. A total of 35 dogs with naturally occurring hypothyroidism were treated with liquid levothyroxine orally once daily with starting dose of 20mcg/kg bwt. The dose was adjusted for every 4weeks based on improvement in clinical signs and serum total T4 and TSH. The authors concluded that all the hypothyroid dogs had rapid clinical and hormonal responses to supplementation with liquid thyroxine solution at a starting dose of 20mcg/kg bwt once daily.

2.3.5.2 NEUTRICEUTICALS

Weinsier et al., (1984) documented that L-carnitine supplementation along with levothyroxine helps improve the thyroid dysfunction faster as low thyroid

function may indicate a need for carnitine to help overcome low energy levels and the tendency to gain weight.

III. MATERIALS AND METHODS

3.1 **Materials**

3.1.1 Patients

Dogs referred from various hospitals of Hyderabad and presented to Veterinary Hospital Bhoiguda, Teaching Veterinary Clinical Complex, College of Veterinary Science, Rajendranagar, Hyderabad during the period from March 2009 to March 2010 were considered for the study.

3.1.2 Clinical material

3.1.2.1 Blood

Whole blood samples were preserved with ethylene diamino tetra acetate (EDTA*) @ 1mg /ml of blood for complete blood picture.

3.1.2.2 Serum

Sera were separated and the samples were transferred into Appendorff tubes and stored in refrigerator at -20^{0}C for biochemical analysis.

3.1.2.3 Skin sampling

Deep skin scrapings were collected using blunt scalpel blade. Impression smears from moist skin lesions and sampling from dry scaly lesions using moistened sterile swab were collected to rule out *Demodicosis* and *Malasseziosis*.

3.1.3 Diagnostic equipment

3.1.3.1 Biochemical analyzer

Sera samples were screened using *star 21 plus*** semi automatic biochemical analyzer supplied by M/S Rapid Diagnostics, New Delhi.

** EDTA supplied by M/S SD Fine Chemicals, Ahmedabad*

*** Star 21 Plus, M/S Rapid diagnostics, New Delhi.*

3.1.3.2 ELISA Reader

Sera samples for thyroid profile was screened using *LISA elisa reader / washer** supplied by M/S Rapid Diagnostics, New Delhi.

3.1.3.3 Ultrasound scanner

Ultrasonography of thyroid gland and 2D-echocardiography were performed using *IXOS vet*** ultrasound/Doppler machine supplied by Esoate Pie Medicals, Netherlands. Linear L10-5 and micro convex array C5-2 R13 cardiac probes were used to perform thyroid scan and echocardiography, respectively.

3.1.3.4 Electrocardiogram

Six lead electrocardiograph *BPL Cardiart-108**** was used to record the electrocardiogram. Crocodile clamps used for connecting electrical circuits were modified to reduce tension and used to connect the electrocardiogram electrodes to the patient skin after applying commercially available conducting gel*****.

3. 1. 3. 5 X-Ray machine

Thoracic radiographic studies were done using *Heliophos-D****** *500* mA X-ray machine. Left lateral and/or dorsoventral exposures were taken at inspiration. They were analysed according to Fagin (1988).

3.2 Methods

3. 2. 1 Selection of cases

Dogs of various breed, age and sex presented with few/all of the following clinical signs suggestive of hypothyroidism such as bilateral symmetrical alopecia, exercise intolerance, obesity, dyspnoea at rest and generalized weakness were

** LISA Elisa reader / Washer, M/S Rapid diagnostics, New Delhi.*
*** Colour Doppler / Ultra Sound Scanner, M/S Esoate Piemedical, Netherlands.*
**** Cardiart – 108, M/S BPL India Limited.*
***** Cardiac Gel, M/S Care Medi systems, Secunderabad, Andhra Pradesh.*
******Heliphos – D 500mA X-ray Machine, Seimens, India.*

considered for the present study. All the clinical data pertaining to the selection of cases were recorded in a data sheet specially designed for the present investigation, the details of which are furnished in appendix-A.

3.2.2 Clinical cases

Out of the total patients diagnosed for hypothyroidism 47 dogs confirmed to be ailing with hypothyroidism based on haematobiochemistry, ECG, radiography, thyroid ultrasonography and echocardiography were selected and subjected to different therapeutic regimens. The selected 46 patients were randomly divided into 2 groups, Gr-I and II. Out of 23 dogs of each group care was taken to include 15 hypothyroid dogs associated Dilated Cardiomyopathy (DCM).

3.2.3 Collection of samples

Blood was collected on day 0 (before therapy), 15, 30 and 45 (after therapy) from the peripheral (cephalic/saphenous) veins of hypothyroid dogs using sterile disposable syringes into clean, dry glass vials having EDTA and also into sterile clean and dry glass tubes for serum separation. Serum was separated by centrifugation at 2000rpm for 5min and collected into serum collection vials. However, blood was also collected from apparently healthy dogs on day 0 to establish normal values. Further skin scrapings and impression smears from affected areas were also collected to rule out secondary skin disorders associated with hypothyroidism.

3.2.4 Haematology

The following haematological parameters were estimated immediately.

3.2.4.1 Total Erythrocyte Count (TEC)

The estimation of TEC was done by haemocytometer method as described by Schalm *et al.,* (1986). The results were expressed as number of erythrocytes x 10^6 / µl of blood.

3.2.4.2 Packed Cell Volume (PCV)

The PCV was estimated by using haemocytometer method as described by Schalm *et al.,* (1986). The results were expressed in percentage.

3.2.4.3 Haemoglobin (Hb)

The estimation of Hb was conducted using acid-haematin method as described by Schalm *et al.,* (1986). The results were expressed in grams per deciliter (g/dl) of blood.

3.2.4.4 Total Leucocyte Count (TLC)

The estimation of TLC was done by haemocytometer method as described by Schalm *et al.,* (1986). The results were expressed as number of leucocytes x 10^3 / µl of blood.

3.2.4.5 Differential Leucocyte Count (DLC)

Differential leucocyte count was calculated after staining the blood smear with Leishman's stain as per the procedure described by Schalm *et al.,* (1986) and the values were expressed as percentage of the cells.

3.2.5 Serum biochemistry

The following biochemical parameters were estimated using biochemical auto analyzer and commercially available diagnostic kits.

3.2.5.1 Serum cholesterol

The serum cholesterol was estimated by the standard method as detailed by Nader et al., (2001). The cholesterol level was expressed in mmol/dl. The detailed procedure is furnished in appendix - B

3.2.5.2 Triglycerides

The serum cholesterol was estimated by the standard method as detailed by Nader et al., (2001). The triglycerides level was expressed in mmol/dl. The detailed procedure is furnished in appendix - C

3.2.5.3 Alkaline Phosphatase (ALP)

The serum ALP was estimated by the Modified International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) method as detailed by Tietz, (1976). The enzymatic activity of serum ALP was expressed in U/L. Detailed procedure is furnished in appendix - D.

3.2.5.4 Alanine Amino Transferase (ALT)

The serum ALT was estimated by the Modified International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) method as detailed by Tietz, (1976). The enzymatic activity of serum ALT was expressed in U/L. Detailed procedure is furnished in appendix -E.

3.2.5.5 Aspartate Amino Transferase (AST)

The serum AST was estimated by the Modified International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) method as detailed by Tietz, (1976). The enzymatic activity of serum AST was expressed in U/L. Detailed procedure is furnished in appendix - F.

3.2.5.6 Creatine Kinase MB (CKMB)

The creatine Kinase MB activity was estimated by the standard method as detailed by Wu and Browers (1982). The CKMB activity was expressed in U/L. The detailed procedure is furnished in Appendix - G.

3.2.5.7 Lactate Dehydrogenase (LDH)

The enzymatic activity of lactate dehydrogenase was estimated by the standard method as detailed by Teitz, (1976). LDH activity is expressed in U/L. Detailed procedure is furnished in appendix – H.

3.2.6 Thyroid function tests

The following thyroid function tests were assessed using ELISA reader.

3.2.6.1 Thyroxine (T4)

The quantitative determination of total and free thyroxine from serum samples was done using microplate enzyme immunoassay, as suggested by Nelson and Wilcox (1996). The values are expressed in mcg/dl and ng/dl. Detailed procedure is furnished in appendix – I.

3.2.6.2 Triiodothyronine (T3)

The quantitative determination of Triiodothyronine from serum samples was done using microplate enzyme immunoassay, as suggested by Larsen, (1972). The values are expressed in ng/dl. Detailed procedure is furnished in appendix – J.

3.2.6.3 Thyroid Stimulating Hormone (TSH)

The quantitative determination of Thyroid Stimulating Hormone (TSH) from serum samples was done using microplate enzyme immunoassay, as suggested by Boelaert and Franklyn, (2005). The values are expressed in U/ml. Detailed procedure is furnished in appendix – K.

3.2.7 Skin sample analysis

Skin scrapings were examined under microscope using specified procedures described by Soulsby (2006). Smears made from moist swab and/or cellophane tape

impressions were also examined under oil immersion after staining with new methylene blue by DIFF quick method (Moris, 1999).

3.2.8 Electrocardiography

The ECG was recorded using the standard bipolar and augmented unipolar limb leads at 25 mm/s speed and interpreted as described by Tilley (1992). The ECG was recorded on dogs in right lateral recumbency on a non-conducting top table. The leads were connected proximal to the olecranon process on the caudal aspect of the appropriate forelimb and over the patellar ligament on the cranial aspect of the appropriate hind limb. However, in patients with severe respiratory distress, ECG was recorded in standing position.

3.2.9 Radiography

Based on the results of physical examination and ECG findings a few cases with DCM were further subjected to radiographic examination. Thoracic radiographs were taken in the left lateral and/or dorsoventral exposures and analysed (Fagin 1988). Further cervical and forelimb radiography was also taken in patients exhibiting goitre and lameness.

3.2.10 Ultrasonography

The following parameters were studied in hypothyroid dogs using Linear L10-5 probe.

3.2.10.1 Thyroid ultrasonography

Thyroid ultrasonography was performed in selected hypothyroid dogs both on day 0 (before therapy) and day 45 (after therapy). However, thyroid ultrasonography was also done in apparently healthy dogs for comparison. Hair was clipped from the ventral aspect of the neck, in a region from the larynx to 10-15 cm more caudally.

Dogs were in dorsal recumbency without the use of tranquilisers or anaesthesia and were restrained manually.

Each lobe was first observed in a transverse plane. Scanning was started in the midline, just caudal to the larynx, followed by a slow gliding motion of the probe caudally plate (1). Landmarks used for the localization of each lobe were laterally located common carotid arteries, the medially located trachea and the ventrally located sternothyroid muscles. An additional landmark for the left lobe was the dorsally located oesophagus. The maximum height and maximum width of each lobe, which were not necessarily located in the same plane, were measured on transverse images by use of electronic callipers with a precision of one-tenth of a millimetre. Following this, length was calculated on longitudinal image of each lobe was obtained either by slowly rotating the probe 90^0 or by using a longitudinal image of the carotid artery and the trachea as land marks, according to a previously described approach (Wisner et al., 1991). Any difference in echogenicity throughout the parenchyma resulted in the interpretation of a heterogenous lobe. The relative overall echogenicity of each lobe was compared with the overlying sternothyroid muscle. The delineation of each lobe was recorded as being smooth or irregular. The shape of the lobes was assessed only on transverse images, and this at the level of the maximum height of the lobe. Each lobe was recorded as normal in shape if it had a triangular to polygonal shape. Any variation to this shape, being rounded, ovoid or amorphous in shape was considered abnormal and recorded as such. The maximum width, the maximum height and the maximum length of thyroid lobe were used to calculate the volume according to the following formula:

Volume (cm^3) = length (cm) x width (cm) x height (cm) x Π/6

3.2.10.2 Echocardiography

Hypothyroid dogs associated with DCM were subjected to following procedures using microconvex array c5-2 R13 cardiac probe.

3.2.10.2.1 M-mode echocardiography

M-mode echocardiography, one-dimensional technique was employed, wherein which uses a very narrow ultrasound beams images a small portion of the heart and detects only the axial motion of structures. Transthoracic echocardiograms were obtained with the unsedated dogs in right lateral recumbancy. Access to the right side of the thorax was facilitated by use of a table with a special cut-out to allow the transducer to be directed upward towards the site of maximal cardiac pulsation (Allworth et al., 1995). Transducer is located parasternally between right third and sixth intercostal spaces between sternum and costochondral junction (Thomas et al., 1994).

3.2.10.2.2 M-mode measurements

M-mode recordings were taken at the high papillary level. Measurement of left ventricular dimension at end–diastole (LVEdD) and end–systole (LVEsD) was made intraluminally from the trailing edge of the septal wall image to the leading edge of the ventricular free wall. End–diastolic and end–systolic measurements of the thickness of the Inter Ventricular Septum (IVSd, IVSs) and left Ventricular Posterior Wall (LVPWd, LVPWs) were made using trailing edge (AllWorth et al., 1995). Further, Ejection fraction (EF) and Fractional Shortening (FS) were also calculated using the following the formula;

$$\text{Ejection Fraction (EF \%)} = \frac{\text{LVEdV - LVEsV}}{\text{LVEdV}} \text{ x } 100$$

- *LVEdV – Left Ventricular Volume at End – diastole*
- *LVEsV – Left Ventricular Volume at End – systole.*

$$\text{LVEdD - LVEsD}$$

$$\text{Fractional Shortening (FS \%)} = \frac{\text{LVEdD}}{\text{LVEdD}} \times 100$$

End point septal separation was measured from the point of maximal cranial motion of the cranial mitral valve leaflet (E point) to the interventricular septum during the rapid–filling phase of diastole (Calvert and John, 1986). All the measurements were made in millimetres. Further, pulsed wave Doppler and colour flow Doppler studies were also attempted in order to assess the mitral valve insufficiency and regurgitation as per the technique suggested by Dominique and Marc-Andre (2008).

3.2.11 Therapeutic trial

Out of the 47 total dogs diagnosed for hypothyroidism 46 patients (including 30 dogs with associated DCM) were selected for therapeutic trial. They were randomly allotted to one of the following treatment groups viz., group I and II with 23 (including 15 DCM dogs) in each. The therapy was carried out for 45 days. However, the remaining one dog diagnosed for hypothyroidism was also treated with respective drugs for stipulated period.

3.2.11.1 Group I

Dogs in group I were treated with Levothyroxine* at the initial dose rate of 20 mcg/kg bwt orally, SID on empty stomach for 45 days.

3.2.11.2 Group II

Dogs in group II were treated with Levothyroxine* at the initial dose rate of 20 mcg/kg bwt along with L-carnitine** @ 50mg/kg after meals orally, SID for 45 days. However, all the dogs of both the groups were maintained with levothyroxine @10mcg/kg bw SID on empty stomach.

3.2.11.3 General considerations in the treatment

The pet owners were advised to restrict physical activity and continue the medication for the prescribed duration. Arrhythmias were tackled by suitable treatment. Thyroid profile and serum cholesterol and triglycerides were monitored in the course of treatment.

** Tab. Eltroxin (Levothyroxine, 25, 50 and 100 mcg), M/S Glaxo Smithklime, Mumbai*
*** Tab. Carnitor (Levocarnitine, 500 mg), M/S Elder Pharmaceuticals Ltd., Uttaranchal.*

3.2.11.4 Monitoring of the patient

The hypothyroid dogs under treatment trial were monitored for a period of 45 days and the different treatment regimens were evaluated at fortnight intervals based on improvement of clinical signs, hematobiochemical findings, electrocardiography, radiography and ultrasonographic features.

3.3 Statistical analysis

The data collected were statistically analyzed by employing t-test as per the methods described by Snedecor and Cochran (1994).

IV. RESULTS

4. Hypothyroidism

4.1 Prevalence

A total 10,172 dogs of various breed, sex and age were presented with the signalment of different systemic diseases to the small animal medical ward of Veterinary Hospital, Bhoiguda, Teaching Veterinary Clinical Complex, College of

Veterinary Science, Rajendranagar, Hyderabad from April 2009 to March 2010. Out of them, 182 were showing the signs suggestive of hypothyroidism such as bilateral alopecia, obesity, exercise intolerance and dyspnoea at rest. Among them hypothyroidism was diagnosed in 47 dogs. The remaining 135 dogs were confirmed as hyperadrenocorticism (22), flea allergic dermatitis (13), demodicosis (65) and atopic dermatitis (35). The overall prevalence was 0.46 per cent and it was 30.92 per cent among dogs exhibiting clinical manifestations. The study revealed more acquired hypothyroidism (97.87 per cent) compared with a rare prevalence (2.13 per cent) of congenital.

4.1.1 Breed wise prevalence

The breed wise prevalence of hypothyroidism in the dogs is presented in table 1 and fig. 1. The highest prevalence was in Labrador retriever (25.53 per cent) followed by Spitz (23.40 per cent), German shepherd (19.15 per cent), Golden Retriever (12.77 per cent), Non-descript breed and Doberman pinscher (8.15 per cent) and in Daschund and Pug (2.13 per cent) the prevalence was lowest.

4.1.2 Age wise prevalence

The age wise prevalence of hypothyroidism in dogs is detailed in table 2 and fig. 2. The highest prevalence (61.70 per cent) was recorded in dogs between 5-10 years of age followed by 17.03 per cent in 10-14 years old dogs, 14.89 per cent and 6.83 per cent in dogs aged more than 14 years and less than 5 years, respectively.

4.1.3 Sexwise prevalence

The prevalence of hypothyroidism in male castrated and uncastrated was 31.91 per cent and 12.77 per cent, respectively. Whereas, the same in female spayed and unspayed dogs were 40.43 per cent and 14.89 per cent, respectively. The data is presented in table 3 and fig.3.

4.2 Clinical manifestations of hypothyroid patients

Almost all the hypothyroid dogs exhibited similar manifestations and presented in table 4 and fig.4. The common clinical manifestations were bilateral alopecia in 39 (82.98 per cent), rat tail appearance in 34 (72.34 per cent), puppy like coat in 08 (17.02 per cent), dry and lustreless coat in 32 (68.08 per cent), secondary skin disorders in 36 (76.60 per cent), exercise intolerance in 37 (78.72 per cent), dyspnoea at rest in 12 (25.53 per cent), obesity in 32 (68.08 per cent), lethargy in 35 (74.47 per cent), corneal lipidosis in 05 (10.64 per cent), goitre and lameness in 5 (10.64 per cent), pale mucosa in 9 (19.25 per cent), anaemia in 13 (27.6 per cent), nervous signs in 12 (25.53 per cent) and cyanosis and myxedema in 04 (8.51 per cent). Auscultation of heart revealed bradycardia and arrhythmias that were similar in all the dogs. Whereas, auscultation of thyroid region on either side of larynx of goitre dogs revealed thyroid thrill. These dogs also presented with the signs of lameness particularly of forelimbs. The classical clinical manifestations associated with hypothyroidism in dogs are depicted in plates 2 to 14.

4.3 Disorders associated with hypothyroidism in dogs

The primary disorder associated with hypothyroidism was related to skin and coat in 36 (76.60 per cent) dogs and was caused by infestation of *Malassezia pachydermitis* (20 dogs) followed by *Demodex canis* in 7 dogs, mixed infestation of both in 5 dogs and bacterial pyoderma among 4 dogs. This was followed by cardiovascular disorder in 30 (63.83 per cent) dogs, nervous system in 12 (25.53 per cent), diabetes mellitus in 5 (10.64 per cent), renal disorders such as hydronephrosis in 3 (6.38 per cent) and musculoskeletal disorders in 2 (4.26 per cent) dogs. The data is presented in table 5 and fig. 5.

4.4 Primary hypothyroidism

The results of investigations carried out in healthy dogs and those with hypothyroidism were classified into two groups, Group I and II are presented in the following headings.

4.4.1 Haematological findings

The mean ± SE of PCV, Hb, TEC and TLC before therapy in group I dogs were 37.12 per cent ± 1.12 per cent, 11.63 ± 0.24g/dl, 7.04 ± 0.48 X 10^6/µl and 7.66 ± 0.76 X 10^3/µl, respectively. These values were 38.02 ± 1.22 per cent, 11.29 ± 0.14g/dl, 7.28 ± 0.08 X 10^6/µl and 7.23 ± 0.36 X 10^3/µl in dogs of group II. Whereas, for apparently healthy dogs the results of above parameters were 39.16 ± 0.18 per cent, 12.92 ± 0.14g/dl, 7.48 ± 0.66 X 10^6/µl and 7.18 ± 0.22 X 10^3/µl, respectively. The differential leukocyte count in group I and II dogs were 69.12 ± 0.22 and 68.98 ± 0.24 per cent neutrophils, 25.62 ± 0.56 and 25.22 ± 0.32 per cent lymphocytes, 3.08 ± 0.72 and 2.98 ± 0.12 per cent eosinophils and 2.18 ± 0.36 and 2.82 ± 0.36 per cent monocytes, respectively. Similar parameters in healthy dogs were 67.92 ± 0.76 per cent, 26.66 ± 0.28 per cent, 2.88 ± 0.52 per cent and 2.54 ± 0.92 per cent, respectively (table 7 and fig. 7).

4.4.2 Biochemical findings

The mean ± SE values of CKMB (49.46 ± 2.34, 52.28 ± 2.15 and 24.18 ± 0.44 U/L), LDH (134.42 ± 3.25, 138.34 ± 4.44, 84.85 ± 0.26 U/L), ALT (32.64 ± 1.26, 31.94 ± 1.02 and 31.28 ± 0.84 U/L), ALP (92.24 ± 0.88, 90.22 ± 1.02 and 76.22 ± 0.24 U/L), cholesterol (11.88 ± 1.22, 12.54 ± 1.04 and 5.82 ± 0.58 mmol/L), triglycerides (4.98 ± 1.84, 4.02 ± 1.98 and 1.12 ± 0.32 mmol/L), TT4 (0.82 ± 1.06, 0.98 ± 0.92 and 3.22 ± 0.12 mcg/dl), fT4 (0.44 ± 1.26, 0.84 ± 1.02 and 2.82 ± 0.56 ng/dl), T3 (28.54 ± 1.34, 31.02 ± 1.02 and 78.68 ± 0.08 ng/dl) and TSH (7.08 ± 1.02,

7.92 ± 1.14 and 2.26 ± 0.42 ng/ml) were recorded in hypothyroid dogs of group I, II and apparently healthy dogs, respectively (table 8 and fig.8)

4.4.3 Electrocardiographic changes

Electrocardiographic abnormalities were detected in all the hypothyroid dogs of both the groups. The results are shown in table 9 and fig. 9. These observations were bradycardia (53.54 ± 3.12 and 51.22 ± 2.98 bpm), bradyarrythmias, low voltage R (0.82 ± 0.12 and 0.76 ± 0.38 mv) and P wave (0.14 ± 0.04 and 0.12 ± 0.22 mv), respectively (plate 15 and 16), in group I and II hypothyroid dogs. Whereas, these observations in healthy dogs were 102.28 ± 2.64 bpm, normal rhythm, 1.96 ± 0.98 mv and 0.36 ± 0.02 mv, respectively. However, R wave amplitude of DCM associated hypothyroid dogs (plate 17) of group I and II were greatly elevated (2.88 ± 0.62 and 2.92 ± 0.84 mv).

4.4.4 Radiographic observations

The radiographic observations are presented in table 10 and fig. 10. Out of 47 dogs subjected to radiography 24 (51.06 per cent) showed cardiomegaly (plate 18), 6 (12.77 per cent) auricular bulge and left atrial enlargement (plate 19), 5 (10.64 per cent) had goitre and arthritis and 12 (25.53 per cent) dogs did not show any abnormality. Further, x-ray of the neck region of goitre dog revealed area of soft tissue swelling at the caudal portion of the larynx which was hazy on skiagram (plate 20). Whereas, the x-ray of the hypothyroid obese dogs presented with the signs of lameness revealed inflamed joints, particularly of carpal joint (plate 21).

4.4.5 Ultrasonographic observations

4.4.5.1 Thyroid Ultrasonography

The thyroid lobes of all the hypothyroid dogs were visible and all measurements and results related to homogenicity of the lobes, relative echogenicity, capsule delineation and the shape of the lobes on transverse and longitudinal section were recorded. The hypothyroid lobes were heterogenous, had an irregular thick capsule, ill-delineated abnormal shape, smaller size (plate 22 and 23) compared with healthy dogs and were hyper echoic compared with sternothyroid muscles.

4.4.5.2 Echocardiography

Out of the total 46 dogs subjected for 2D echocardiography, 30 hypothyroid patients were diagnosed for dilated cardiomyopathy (DCM). The mean values of left ventricular dimensions such as, LVEdD, LVEsD, LVPWd, LVPWs, IVSd, IVSs, EPSS, FS and EF were 51.88 ± 0.26 and 52.36 ± 0.42 mm, 44.12 ± 0.24 and 43.88 ± 0.64 mm, 5.66 ± 0.54 and 5.24 ± 0.12 mm, 8.12 ± 0.58 and 8.82 ± 0.44mm , 4.96 ± 0.29 and 5.02 ± 0.88 mm, 5.88 ± 0.54 and 5.82 ± 0.82mm, 9.12 ± 0.80 and 9.44 ± 0.13mm, 14.16 ± 2.04 and 15.88 ± 1.98 per cent and 33.46 ± 1.22 and 31.64 ± 1.02 per cent in hypothyroid dogs of group I and II, respectively. Similar parameters of apparently healthy dogs were 37.98 ± 0.39 mm, 26.55 ± 0.49 mm, 6.76 ± 0.22 mm, 9.27 ± 0.18 mm, 5.82 ± 0.23mm, 6.97 ± 0.21 mm, 4.62 ± 0.16 mm, 30.12 ± 0.26 per cent and 65.88 ± 2.24 per cent (table11 and fig. 11).

The echocardiographic observations of DCM associated hypothyroid dogs are depicted in plates 24 to 28.

4.5 Classification of intensity of hypothyroidism in dogs:

Severity of intensity of hypothyroidism in dogs is presented in table 6 and fig.6. Out of 47 hypothyroid dogs 27 (57.45 per cent) were severe, 13 (27.66 per cent) and 07 (14.89 per cent) were moderate and mild, respectively (plates 29).

This classification was based on the values of thyroid profile. Further, these levels were correlated with different clinical signs and their intensity in each dog. Almost all the severely affected hypothyroid dogs were presenting more pronounced manifestations of both skin and coat abnormalities (bilateral symmetrical alopecia, rat tail, dry hyperpigmented and hyperkeratosis of skin) and decreased metabolic rate abnormalities (obesity, exercise intolerance, lethargy, dyspnoea at rest, anemia and cyanotic tongue). Some were also showing corneal lipidosis and goitre. The thyroid profiles of these severely affected hypothyroid dogs were very low (T4-1.14±0.86mcg/dl; fT4-0.98±1.02ng/dl; and T3-36.00±1.86ng/dl) and severely elevated TSH (6.02±0.94ng/ml) when compared with apparently healthy dogs (3.22±0.12mcg/dl, 2.82±0.56ng/dl, 78.68±0.08ng/dl, 2.26±0.42ng/ml). Similarly, CKMB (46.26±1.82U/L), LDH (120.28±2.20U/L), cholesterol (9.92±1.04mmol/L) and triglycerides (4.02±0.90mmol/L) were also extremely higher than apparently healthy dogs (24.18±0.44U/L, 84.85±0.26U/L, 5.82±0.58mmol/L and 1.12±0.32mmol/L). Echocardiographic M-mode measurements of left ventricle dimensions were greatly altered in all the 27 dogs showing severe manifestations. 13 dogs considered as moderately hypothyroid were presented with predominant skin and coat abnormalities but with mild to moderate signs related to reduced metabolic rate. The thyroid profile was (T4-2.02±1.18mcg/dl; fT4-1.24±1.06ng/dl; T3-48.72±1.86ng/dl and TSH-5.32±1.18ng/ml). Serum CKMB (36.26±1.28U/L), LDH (102.26±2.54U/L), cholesterol (7.36±1.62mmol/L), triglycerides (2.60±1.08mmol/L) were also moderately elevated compared to healthy dogs. Non-significantly different M-mode measurements of left ventricular dimensions were noticed in these dogs. Further, secondary skin disorders such as, malasseziosis, demodicosis and / pyoderma was also noticed in severe and moderately affected dogs. Whereas, the remaining 07

dogs showed only skin and coat abnormalities. No echocardiography abnormalities were noticed in these dogs. The thyroid profile was (T4-2.98±0.16mcg/dl; fT4-2.48±0.06ng/dl; T3-62.70±0.52ng/dl and TSH-2.78±0.82ng/ml). Serum CKMB (28.10±0.98U/L), LDH (92.74±0.32U/L), cholesterol (6.44±0.74mmol/L) and triglycerides (1.60±0.54mmol/L) were mildly elevated. Hence these dogs were considered as mildly affected.

4.6 Therapeutic trial

The findings recorded before initiation of therapy (day0) and during the course of therapy on day 15, 30 and 45 of treatment in the 2 groups of hypothyroid dogs subjected for therapeutic trial are presented in this chapter.

4.6.1 Group I

4.6.1.1 Clinical manifestations

All the 23 dogs in this group showed similar manifestations such as bilateral alopecia, rat tail appearance, puppy like coat, dry, lustreless coat, exercise intolerance, obesity and lethargy. Two dogs of this group also showed corneal lipidosis, paraplegia, facial paralysis, cyanosis, myxedema and goitre. Secondary skin disorders like malasseziosis and demodicosis were also recorded in 19 dogs.

When therapy was instituted bilateral alopecia disappeared after 25 days in 5 dogs and after 35 and 45 days in the remaining 10 and 5 dogs, respectively. Remaining skin and coat abnormalities like rat tail appearance, puppy like coat and dry and lustreless coat were subsided in 2, 1 and 7 dogs after 25 days and in remaining 13, 2 and 5 dogs following 35 days. Improvement in exercise intolerance, dyspnoea at rest were started after 25 days in 7 and 1 dog, respectively and disappeared after 35 days in all the dogs of this group. Similarly, obesity and secondary skin disorders improved after 25 days in 3 and 8 dogs and in remaining 12

and 11 dogs disappearance of these signs were recorded after 35 days during the course of therapy. Signs related to corneal lipidosis, pale mucosae, anaemia, nervous system and cyanosis and myxedema improved after 25 days in 1, 1, 5, 3 and 1 dogs and after 30 days in the remaining dogs. Whereas, goitre swelling was partially improved in one dog after 45 days following therapy (table 12a).

Two dogs presenting goitre, cyanosis and myxedema (1), severe nervous signs (seizures, syncope, paralysis), pale mucosae (1) and corneal lipidosis (1) died after 10 and 15 days of initiation of therapy and in the remaining 21 dogs alleviation of clinical signs took 30-35 days (table 12).

4.6.1.2 Haematological values

The values recorded before (day 0) and after therapy (day 45) are presented below and shown in table 13 and fig. 13.

Packed cell volume (PCV)

The mean PCV of group I dogs was 37.12 ± 1.12 per cent before therapy, that was significantly low ($P<0.05$) compared with the mean value of apparently healthy dogs (39.16 ± 0.18 per cent). A non-significant increase was seen after therapy, with the mean value reaching 38.02 ± 0.12 per cent on day 45

Haemoglobin (Hb)

The mean pretherapeutic Hb concentration (11.63 ± 0.24 g/dl) of group I patients was non-significantly low when compared with mean values of healthy dogs (12.92 ± 0.14 g/dl). The post therapeutic mean Hb concentration (12.42 ± 0.25 g/dl) was found to be significantly ($P<0.05$) increased on day 45.

Total erythrocyte count (TEC)

The dogs of this group showed mean TEC of 7.04 ± 0.48 x 10^6/µl on day 0, which was similar to apparently healthy dogs (7.48 ± 0.66 X 10^0/µl). This value showed a significant increase ($P<0.05$) on day 45 post therapy (7.52 ± 0.42 X 10^6/µl).

Total leucocyte count (TLC)

The mean TLC values of hypothyroid dogs of this group was 7.66 ± 0.76 X 10^3 /µl, that was similar to apparently healthy dogs (7.18 ± 0.22 X 10^3/µl) on day 0. These dogs showed a non significant difference (7.88 ± 0.88 X 10^3 /µl) after therapy on day 45.

Differential leucocyte count (DLC)

The mean neutrophil count on day 0 was 69.12 ± 0.22 per cent. The mean lymphocyte, eosinophil and monocyte counts of this group of dogs were 25.62 ± 0.56, 3.08 ± 0.72 and 2.18 ± 0.36 per cent, respectively before treatment. There was no significant difference between these values in hypothyroid dogs and the values of neutrophils (67.92 ± 0.76 per cent) lymphocytes (26.66 ± 0.28 per cent), eosinophils (2.88 ± 0.52 per cent) and monocytes (2.54 ± 0.92 per cent) recorded in healthy dogs. After therapy on day 45 the values were (68.12 ± 0.42 per cent) neutrophils, (26.06 ± 0.45 per cent) lymphocytes, (3.02 ± 0.34 per cent) eosinophils and 2.80 ± 0.28 per cent monocytes that were not significantly different from pretreatment levels.

4.6.1.3 Biochemical values

The results of estimations of various biochemical parameters group I dogs before (day 0) and after therapy (day 45) are presented below and shown in table 14 and fig. 14.

Creatine Kinase MB (CKMB)

A significant increase ($P<0.01$) was recorded in the mean CKMB levels (49.46 ± 2.34 U/L) of hypothyroid dogs of this group on day 0 when compared to healthy dogs whose mean values were 24.18 ± 0.44 U/L. These values significantly ($P<0.01$) decreased to 36.25 ± 1.12 U/L following therapy on day 45.

Lactate dehydrogenase (LDH)

In the hypothyroid dogs, the mean pre therapeutic LDH concentration was 134.42 ± 3.25 U/L that was significantly ($P<0.01$) high compared to mean concentration of healthy dogs (84.85 ± 0.26 U/L). After therapy the LDH levels were significantly decreased ($P<0.01$) with a mean value of 98.28 ± 2.02 U/L by day 45 when compared to pre therapeutic value.

Alanine aminotransferase (ALT)

In dogs of this group the mean pretherapeutic enzymatic activity of ALT was 32.24 ± 1.06 U/L, which was similar to that of healthy dogs whose mean value was 31.28 ± 0.84 U/L. On day 45 following therapy the mean value (30.28 ±0 .54 U/L) was not significantly altered when compared to pre-treatment values.

Alkaline phosphatise (ALP)

In hypothyroid dogs of group I, the ALP level had a mean pretherapeutic value of 92.24 ± 0.88 U/L, that was significantly higher ($P<0.05$) when compared to that of healthy dogs whose mean concentration was 76.22 ± 0.24 U/L. There was a significant decrease ($P<0.05$) in the ALP levels (78.94 ± 0.16 U/L) of these dogs on day 45 following therapy.

Cholesterol

In hypothyroid dogs, the mean pre therapeutic cholesterol level was 11.88 ± 1.22 mmol/L which was significantly (P<0.01) high compared to mean concentration of healthy dogs (5.82 ± 0.58 mmol/L). After therapy the same were significantly decreased (P<0.01) to a mean value of 7.98 ± 1.07mmol/L by day 45 when compared to pre therapeutic value.

Triglycerides

In dogs of this group the mean pretherapeutic levels of triglycerides was 4.98 ± 1.84 mmol/L, that was significantly (P<0.01) high compared to mean concentration of healthy dogs (1.12 ± 0.32 mmol/L). On day 45 following therapy the mean triglycerides value (2.64 ± 0.22 mmol/L) was significantly (P<0.01) altered when compared to pre-treatment values.

Total Thyroxine (TT4)

In the hypothyroid dogs, the mean pre therapeutic total thyroxine levels was 0.82 ± 1.06 mcg/dl that was significantly (P<0.05) low compared to mean concentration of healthy dogs (3.22 ± 0.12mcg/dl). After therapy the thyroxine levels were significantly increased (P<0.05) to a mean value of 2.28 ± 0.50mcg/dl.

Free thyroxine (fT4)

In the hypothyroid dogs of group I the mean pre therapeutic free thyroxine levels on day 0 was 0.64 ± 1.26 ng/dl that was significantly (P<0.05) low compared to mean concentration of healthy dogs (2.82 ± 0.56ng/dl). After therapy the free thyroxine levels were significantly increased (P<0.05) to a mean value of 1.90 ± 0.06ng/dl by day 45.

Triodothyronine (T3)

In group I dogs, the mean pre therapeutic triiodothyronine levels was 28.54 ± 1.34 ng/dl that was significantly (P<0.05) low compared to mean concentration of

healthy dogs (78.68 ± 0.08ng/dl). After therapy the triiodothyronine levels were significantly increased (P<0.05) to a mean value of 64.00 ± 1.53ng/dl by day 45 when compared to pre therapeutic value.

Thyroid stimulating hormone (TSH)

In the hypothyroid dogs, the mean thyroid stimulating hormone (TSH) levels was 7.08 ± 1.02 ng/ml on day 0, that was significantly (P<0.01) high compared to mean concentration of healthy dogs (2.26 ± 0.42ng/ml). After therapy the TSH levels were significantly decreased (P<0.05) to a mean value of 3.02 ± 1.16ng/ml.

4.6.1.4 Thyroid ultrasonographic findings

The hypothyroid lobes of the affected dogs were heterogenous (06 dogs), had an irregular thick capsule (04 dogs), ill-delineated abnormal shape (07 dogs), smaller size (05 dogs) and were hyper echoic (07 dogs) compared with sternothyroid muscles on day 0. Where as, the mean ± SE of length, width and height of the hypothyroid lobes were 1.12 ± 0.05, 0.68 ± 0.32 and 0.52 ± 0.02 cm, respectively that were significantly decreased (P<0.05) when compared to apparently healthy dogs (1.82 ± 0.52, 0.94 ± 0.32 and 0.72 ± 0.02 cm), respectively. These values (1.28 ± 0.07, 0.72 ± 0.48 and 0.60 ± 0.10 cm) on day 45 after therapy had no significant improvement when compared to pretherapy values. The mean relative volume of thyroid gland of the hypothyroid dogs of group I was 0.21 ± 0.02cm^3 that was significantly decreased (P<0.05) when compared to the thyroid volume of apparently healthy dogs (0.64 ± 0.06cm^3). Whereas, the mean volume of thyroid gland on day 45 following therapy was 0.28 ± 0.02cm^3 that was non-significantly different when compared to day 0. However, all these parameters did not showed much improvement and did not became

normal any time during the follow-up period and all thyroid glands were considered abnormal after day 45 (table 15 and fig. 15).

4.6.1.5 Echocardiographic findings

In the dogs of this group the mean pre-treatment values of LVEdD and LVEsD were 51.88 ± 0.26 and 44.12 ± 0.24mm, which were significantly increased ($P<0.01$) when compared to the mean values of healthy dogs (37.98 ± 0.39 and 26.55 ± 0.49 mm). These dimensions after therapy (by day 45) were found to be 49.54 ± 0.52 and 41.90 ± 0.24mm, which were significantly ($p<0.05$) different from the findings recorded prior to the treatment. The other dimensions viz., LVPWd (5.66 ± 0.54mm), LVPWs (8.12 ± 0.58mm), IVSd (4.96 ± 0.29mm), IVSs (5.88 ± 0.54mm) and EPSS (9.12 ± 0.86mm) noted on day zero were also significantly different ($p<0.05$) from that of healthy dogs (6.76 ± 0.22mm, 9.27 ± 0.18mm, 5.82 ± 0.23mm, 6.97 ± 0.21mm and 4.62 ± 0.16mm). Following therapy (on day 45) there was a non-significant increase in the dimensions of LVPWd (6.05 ± 0.12mm), LVPWs (8.95 ± 0.02mm), IVSd (5.07 ± 0.38mm), IVSs (6.05 ± 0.74mm), with a non-significant decrease in EPSS (7.88 ± 0.44mm) when compared with the pre treatment values (Table 16 and fig. 16). With respect to ejection fraction and fractional shortening, the values on day 0 were 33.46 ± 1.22 per cent and 14.16 ± 2.04 per cent which were significantly low ($P<0.01$) when compared to healthy dogs (65.88 ± 2.24 and 30.12 ± 0.26 per cent, respectively). However, a significant ($P<0.05$) increase to 42.74 ± 1.08 and 20.32 ± 0.76 per cent were recorded in this group dogs on day 45 post therapy. However, no specific difference in severity and intensity of turbulence and mosaic pattern of colour flow was noticed on pulse wave and colour flow Doppler of mitral valve in this group dogs before and after treatment.

4.6.2 Group II

4.6.2.1 Clinical manifestations

All the 23 dogs in this group showed similar manifestations such as bilateral alopecia, rat tail appearance, puppy like coat, dry and lustreless coat, exercise intolerance, obesity, and lethargy. Whereas other manifestations such as corneal lipidosis (3), nervous signs (6), anaemia (7), pale mucasae (5), cyanosis and myxedema (2) and goiter (2) were also noticed in hypothyroid dogs of group II. Secondary skin disorders like malasseziosis and demodicosis were also recorded in 17 dogs of this group.

Following therapy alleviation of hypothyroid manifestations such as bilateral alopecia was noticed after 25 days in 17 dogs and after 30 days in the remaining 2 dogs. Other hypothyroid associated skin and coat abnormalities like rat tail appearance, puppy like coat and dry and lustreless coat was subsided in 18, 2 and 18 dogs after 25 days and in remaining 1, 3 and 2 dogs following 30 days. Improvement in exercise intolerance, dyspnoea at rest were started after 20 days in 12 and 6 dogs, respectively and disappeared after 30 days in all the dogs of this group. Similarly, obesity and secondary skin disorders improved after 20 days in 5 dogs and in remaining 12 dogs disappearance of these signs were recorded after 30 days during the course of therapy. Signs related to corneal lipidosis, pale mucosae, anaemia, nervous system, cyanosis and myxedema improved after 20 days in 2, 4, 7 and 6 dogs and after 25 days in the remaining dogs. Whereas, goitrous swelling was partially improved in 2 dogs after 45 days following therapy (Table 12b).

However, hypothyroid dogs of this group II started clinical improvement from day 15 and complete alleviation of signs were recorded by day 30. No death was recorded in this group of dogs (Table 12).

4.6.2.2 Haematological values

The values recorded in group II dogs before and after therapy are presented below and shown in table 17 and fig. 17.

Packed cell volume (PCV)

The mean PCV of group II dogs on day 0 was 38.02 ± 1.22 per cent before therapy, that was significantly low ($P<0.05$) compared with the mean value of apparently healthy dogs (39.16 ± 0.18 per cent). A slight but a non-significant increase was seen after therapy, with the mean value reaching 38.95 ± 0.34 per cent on day 45 of therapy.

Haemoglobin (Hb)

The mean pretherapeutic Hb concentration (11.29 ± 0.14 g/dl) was non-significantly low when compared with mean values of healthy dogs (12.92 ± 0.14 g/dl). The post therapeutic level (12.42 ± 0.24 g/dl) was found to be significantly ($P<0.05$) increased on day 45.

Total erythrocyte count (TEC)

The dogs of this group showed mean TEC of 7.28 ± 0.08 x 10^6/µl on day 0, which was similar to apparently healthy dogs (7.48 ± 0.66 X 10^6/µl). This value shown a significant increase ($P<0.05$) on day 45 post therapy (7.50 ± 0.22 X 10^6/µl).

Total leucocyte count (TLC)

The mean TLC values of hypothyroid dogs of this group was 7.23 ± 0.36 X 10^3 /µl, that was similar to apparently healthy dogs (7.18 ± 0.22 X 10^3/µl) on day 0. The TLC values showed a non significant difference in these values (7.63 ± 0.30 X 10^3 /µl) after therapy on day 45.

Differential leucocyte count (DLC)

The mean neutrophil on day 0 was 68.98 ± 0.24 per cent. The mean lymphocyte, eosinophil and monocyte counts of this group of dogs were 25.62 ± 0.32,

2.98 ± 0.12 and 2.82 ± 0.36 per cent, respectively before treatment. There was no significant difference between these values in hypothyroid dogs and the values of neutrophils (67.92 ± 0.76 per cent), lymphocytes (26.66 ± 0.28 per cent), eosinophils (2.88 ± 0.52 per cent) and monocytes (2.54 ± 0.92 per cent) recorded in healthy dogs. After therapy on day 45 these values were (71.00 ± 0.14 per cent) neutrophils, (24.36 ± 0.22 per cent) lymphocytes, (2.22 ± 0.14 per cent) eosinophils and 2.42 ± 0.38 per cent monocytes.

4.6.2.3 Biochemical values

The results of estimations of various biochemical parameters in the dogs of group II before and therapy are presented below and shown in table 18and fig. 18.

Creatine Kinase MB (CKMB)

A significant increase ($P<0.01$) was recorded in the mean CKMB levels (52.28 ± 2.15 U/L) of hypothyroid dogs of this group on day 0 when compared to healthy dogs whose mean values are 24.18 ± 0.44 U/L. These values significantly ($P<0.01$) decreased and the mean value was 29.20 ± 1.35 U/L following therapy on day 45.

Lactate dehydrogenase (LDH)

In the hypothyroid dogs, the mean pre therapeutic LDH concentration was 138.34 ± 4.44 U/L that was significantly ($P<0.01$) high compared to mean concentration of healthy dogs (84.85 ± 0.26 U/L). After therapy the LDH levels were significantly decreased ($P<0.01$) with a mean value of 90.05 ± 2.90 U/L.

Alanine aminotransferase (ALT)

In dogs of this group the mean pretherapeutic enzymatic activity of ALT was 31.94 ± 1.02 U/L, which was similar to that of healthy dogs whose mean value was

31.28 ± 0.84 U/L. On day 45 following therapy the mean value (31.10 ± 0.30 U/L) was not significantly altered.

Alkaline phosphatase (ALP)

In hypothyroid dogs of group II, the ALP levels had a mean pretherapeutic value of 90.22 ± 1.02 U/L, that was significantly higher ($P<0.05$) when compared to that of healthy dogs whose mean concentration was 76.22 ± 0.24 U/L. There was a significant decrease ($P<0.05$) with respect to these levels (75.70 ± 1.13 U/L) on day 45 following therapy.

Cholesterol

In hypothyroid dogs of group II, the mean pre therapeutic cholesterol levels was 12.54 ± 1.04 mmol/L that was significantly ($P<0.01$) high compared to mean concentration of healthy dogs (5.82 ± 0.58 mmol/L). After therapy the cholesterol level was significantly decreased ($P<0.01$) to a mean value of 6.03 ± 0.48mmol/L.

Triglycerides

In dogs of group II the mean triglycerides on day 0 was 4.62 ± 1.98 mmol/L, that was significantly ($P<0.01$) high compared to mean concentration of healthy dogs (1.12 ± 0.32 mmol/L). On day 45 following therapy the mean triglycerides value (1.62 ± 0.03 mmol/L) was significantly ($P<0.01$) decreased.

Total Thyroxine (TT4)

In hypothyroid dogs, the mean pre therapeutic thyroxine levels was 0.98 ± 0.92 mcg/dl that was significantly ($P<0.05$) low compared to mean concentration of healthy dogs (3.22 ± 0.12mcg/dl). After therapy the thyroxine level was significantly increased ($P<0.05$) to a mean value of 2.80 ± 0.74mcg/dl, by day 45 when compared to pre therapeutic value.

Free thyroxine (fT4)

In group II hypothyroid dogs, the mean pre therapeutic free thyroxine levels was 0.84 ± 1.02 ng/dl on day 0 that was significantly (P<0.05) low compared to mean concentration of healthy dogs (2.82 ± 0.56ng/dl). After therapy these values were significantly increased (P<0.05) to a mean value of 2.28 ± 0.84ng/dl.

Triodothyronine (T3)

In the hypothyroid dogs, triiodothyronine levels was 31.02 ± 1.02 ng/dl on day 0, that was significantly (P<0.05) low compared to mean concentration of healthy dogs (78.68 ± 0.08ng/dl). After therapy the same were significantly increased (P<0.05) to a mean value of 70.98 ± 1.14ng/dl.

Thyroid stimulating hormone (TSH)

In group dogs, the mean pre therapeutic thyroid stimulating hormone (TSH) level was 7.92 ± 0.14 ng/ml, that was significantly (P<0.01) high compared to mean concentration of healthy dogs (2.26 ± 0.42ng/ml). After therapy the TSH levels were significantly decreased (P<0.01) to a mean value of 2.68 ± 1.79ng/ml.

4.6.2.4 Thyroid ultrasonographic findings

The hypothyroid lobes of the affected dogs of group II were heterogenous (07 dogs), had an irregular thick capsule (08 dogs), ill-delineated and abnormal shape (04 dogs), smaller size (07 dogs) and were hyper echoic (05 dogs) compared with sternothyroid muscles on day 0. Where as, the mean ± SE of length, width and height of the hypothyroid lobes were 1.20 ± 0.14, 0.52 ± 0.08 and 0.50 ± 0.10 cm, respectively, that were significantly decreased (P<0.05) when compared to apparently healthy dogs (1.82 ± 0.52, 0.94 ± 0.32 and 0.72 ± 0.02 cm), respectively. These values (1.42 ± 0.22, 0.76 ± 0.10 and 0.62 ± 0.24 cm) on day 45 after therapy had no significant difference when compared to pretherapy values. The mean relative volume of thyroid gland of the hypothyroid dogs of group II was 0.16 ± 0.18cm^3 that was

significantly decreased when compared with the thyroid volume of apparently healthy dogs (0.64 ± 0.06cm^3). Whereas the mean volume of thyroid gland on day 45 was non-significantly different on day 45 (0.35 ± 0.24cm^3). However, all these parameters did not showed much improvement and did not became normal any time during the follow-up period and all thyroid glands were considered abnormal after day 45 (table 19 and fig. 19).

4.6.2.5 Echocardiographic findings

In the group II hypothyroid dogs the mean pre-treatment values of LVEdD and LVEsD were 52.36 ± 0.42 and 43.88 ± 0.64mm, that were significantly increased ($P<0.01$) when compared to the mean values of healthy dogs (37.98 ± 0.39 and 26.55 ± 0.49 mm). These dimensions after therapy (by day 45) were found to be 46.52 ± 0.27 and 38.00 ± 0.24mm, which were significantly ($p<0.05$) low from the findings recorded prior to the treatment. The other dimensions viz., LVPWd (5.24 ± 0.12mm), LVPWs (8.82 ± 0.44mm), IVSd (5.02 ± 0.88mm), IVSs (5.82 ± 0.82mm) and EPSS (9.44 ± 0.13mm) noted on day zero were also significantly different ($P<0.05$) from that of healthy dogs (6.76 ± 0.22mm, 9.27 ± 0.18mm, 5.82 ± 0.23mm, 6.97 ± 0.21mm and 4.62 ± 0.16mm). Following therapy (on day 45) there was a non-significant increase in the dimensions of LVPWd (6.23 ± 0.52mm), LVPWs (9.00 ± 0.23mm), IVSd (5.64 ± 0.25mm), IVSs (6.54 ± 0.14mm), with a significant decrease ($P<0.05$) in EPSS (7.00 ± 0.22mm) when compared to the pre treatment values (Table 20 and fig. 20). With respect to ejection fraction and fractional shortening, the values on day 0 were 31.64 ± 1.02 per cent and 15.88 ± 1.98 per cent that were significantly low ($P<0.01$) when compared to healthy dogs (65.88 ± 2.24 and 30.12 ± 0.26 per cent), respectively. However, a significant ($P<0.01$) increase to 53.66 ± 0.06 and 25.70 ± 1.02 per cent were recorded in this group dogs on day 45 post therapy. However, no

significant changes with respect to severity and intensity of turbulence and mosaic pattern of colour flow was observed on pulse wave and colour flow Doppler of mitral valve in group II hypothyroid dogs before and after treatment.

The clinical manifestations such as bilateral alopecia, rat tail appearance, puppy like coat, dry and lustreless coat and secondary skin disorders started disappearing from day 20 of therapy and were not evident by day 30 in the dogs of group II in comparison with dogs of groups I which disappeared after 30-45. Whereas, remaining signs such as exercise intolerance, dyspnoea at rest, obesity and lethargy also subsided in 15-20 days during the course of therapy in dogs of group II in comparison with the other group patients which improved only after 30 days. Few dogs which also showed corneal lipidosis, pale mucosea, anaemia, nervous signs, cyanosis, myxedema and goitre also improved after 25 days in hypothyroid dogs of group II when compared to group I dogs which improved after 35 days. Further, all the twenty three dogs included in group II for therapeutic trial showed complete recovery with no side effects when compared to death of 2 dogs in group I during the therapy period. There was no significant difference in the various haematological parameters such as, TEC, Hb, PCV, TLC and DLC values between the groups I and II (Table 21 and Fig. 21). A significant difference ($P<0.05$) of group II dogs was noticed in the values of CKMB and LDH ($P<0.01$) and cholesterol, triglycerides, total thyroxine, free thyroxine, triodothyronine and thyroid stimulating hormone ($P<0.05$) on day 45 after treatment as compared with group I. Whereas, no significant difference was noticed with the values of ALT and ALP in between the groups on day 45 (Table 22 and fig. 22). The different abnormalities associated with thyroid gland morphology and texture that revealed during thyroid lobe ultrasonography such as, irregular thick capsule, ill delineated, abnormal shape, smaller size, echogenic

difference and thyroid lobe volume both before and after therapy were not significantly different between the groups (Table 23 and fig. 23). In Group II hypothyroid dogs post therapy levels of left ventricle dimensions such as, LVEdD, LVEsD, LVPWd, LVPWs, IVSd, IVSs, EPSS, EF and FS were significantly different ($P<0.01$) in comparison with the group I on day 45 (Table 24and fig. 24).

V. DISCUSSION

Hypothyroidism is the most common hormone imbalance of dogs. Where in, the body doesn't produce enough thyroid hormones, resulting in dysfunction of thyroid gland. When a serum or blood is subjected to test show low levels of thyroid hormones, the hormone replaced given in pill form, problem solved. However, it is not so in reality. This discussion attempts to review the issues relevant to this condition and the pitfalls that keep it from being a simple problem.

The thyroid gland is an H-shape structure in dogs throat. It produces two forms of thyroid hormone: T3 is the active form of the hormone and T4 is the inactive form created to circulate in the blood stream. When T4 is absorbed out the blood stream and into tissue cells, it is converted into T3. Most of the circulating T4 is carried by blood proteins and is not available for tissue absorption; the portion that is not carried by proteins (the so-called "free T4) is the portion that is able to enter tissues for activation. More than 95 per cent of canine Primary hypothyroidism is believed to be acquired primary hypothyroidism that results from destruction of thyroid gland associated with lymphocytic thyroiditis, idiopathic thyroid atrophy or unusually thyroid neoplasia. Secondary hypothyroidism that results from deficiency of TSH is uncommon in dogs. Tertiary hypothyroidism that is associated with

deficiency of thyrotropin (TRH) has not been documented in dogs. Congenital hypothyroidism (cretinism) occurs in dogs, but rarely diagnosed as it usually results in early puppy death. Deficiency of thyroid hormones affects all organ systems causing a decrease in Basal Metabolic Rate (BMR). As the clinical signs are vague, diffuse and insidious in onset and the relatively low accuracy of most biochemical tests and the act that many factors like non thyroidal disease, drugs and normal physiological fluctuations can lower circulating thyroid hormone concentrations, many times the condition is misdiagnosed clinically (Ferguson 1994).

In the present study out of a total 10,172 dog examined, 182 dogs were showing the signs suggestive of hypothyroidism such as bilateral alopecia, obesity, exercise intolerance and dyspnoea at rest out of which 47 were diagnosed for primary hypothyroidism. The diagnostic protocol is based on the classical clinical manifestations and by the haematobiochemical, ECG, radiological, ultrasonographic and echocardiographic findings. The skin samples were also tested for the presence of secondary invaders such as *Malassezia pachydermatis* and *Demodex canis.* The overall prevalence of hypothyroidism in dogs was found to be 0.46 per cent and the prevalence among the dogs exhibiting clinical manifestations suggestive of hypothyroidism was 30.92 per cent. Among the dogs, 1 (2.13 per cent) had congenital and the remaining 46 (97.87 per cent) had acquired hypothyroidism.

The prevalence rate of hypothyroidism in dogs recorded in the present study is in agreement with Mooney (2003), who reported that the prevalence of canine hypothyroidism is from 0.2 to 0.8 per cent. However, higher prevalence rate was also reported by Borku and Atkas (2007) as 1:156 to 1:500. The variation could be due to the different populations being screened in each study with respect to age, sex, breed and geographical location. In the present study the breed wise prevalence was

recorded as 2.13 per cent to 25.53 per cent. The highest rate of occurrence was recorded in Labrador retriever (25.53 per cent) followed by Spitz (23.40 per cent), German shepherd (19.15 per cent), Golden retriever (12.77 per cent), non-descript breed and Doberman pinscher (8.15 per cent). Whereas, the lowest rate of prevalence was recorded in Daschund and Pug (2.13 per cent). Golden retriever and Doberman pinscher are the most common breeds reported to be at higher risk for hypothyroidism. Primary acquired hypothyroidism is commonly noticed in Cocker spaniels, Daschunds, Doberman pinscher and Golden retriever. Where as congenital hypothyroidism is common in German shepherds (Benzamin, 1998). Panciera (1994) documented that a variety of dog breeds are at high risk for hypothyroidism such as German shepherd, Doberman pinscher, Grey hound, Daschund and Boxer. The disease also occurs in mixed breed dogs. Where as cretinism or dwarfism, a congenital hypothyroidism that arises because the hypothalamus in the brain does not produce enough TRH is commonly noticed in Giant schnauzers and Boxers. The findings in the present study are in partially agreement with the above author as the prevalence of hypothyroidism was recorded maximum in German shepherd followed by Spitz, Labrador retriever, Golden retriever, Doberman pinscher and Daschund. Satish Kumar et al., (2007) reported that Spitz followed by Labrador and German shepherd were at higher risk for hypothyroidism. The highest prevalence in German shepherd and Spitz recorded in the present study could be attributed to the population of the said breed in the investigating area. The highest age wise prevalence of 61.70 per cent was recorded in dogs between 5-10 years and lowest prevalence of 6.83 per cent in dogs less than 5 years. In general the primary hypothyroidism in the present study was recorded in dogs aged between 6 months to 14 years. The present findings are in agreement with Panciera (1999) who documented that the mean age at

diagnosis of hypothyroidism is 7 years, with a range of 0.5 to 15 years. Satish Kumar et al., (2007) stated that hypothyroidism is commonly affected in dogs between 4 to 15 years of age and Krishnamurthi and Rajan (2002) presented bilateral symmetrical alopecia, hyperpigmentation associated hypothyroidism in six castrated dogs aged between 3 to 5 years.

Neutered males and females were reported to be at increased risk for developing hypothyroidism compared with sexually intact animals. Satish Kumar et al., 2007 reported spayed females and castrated male dogs were at higher risk of primary hypothyroidism. In the present investigation the highest prevalence of 40.43 per cent and 31.91 per cent was recorded in neutered female and male dogs, respectively that were relatively low in intact dogs. The findings in the present study are in agreement with the above authors. The relative variation in the prevalence of hypothyroidism among castrated and uncastrated dogs could be associated with endocrine and hormonal disturbances.

The primary clinical manifestations noticed in the present investigation were skin and coat abnormalities such as, bilateral alopecia, rat tail appearance, puppy like coat, lustreless dry, hyperpigmented coat. These findings are in agreement with Satish Kumar et al., (2007) and Krishna murthy and Rajan (2002) who opined that patchy or extensive alopecia, dry brittle hair coat, scaly lesions, hyperpigmentation and bilateral symmetrical skin lesions are characteristic of hypothyroidism. Whereas, Fialkovikova et al., (2008) reported non-pruritic alopecia (alopecia X) on ventral thighs (bald thigh syndrome), rat tail appearance with brittle, dry and change in coat colour and scaling of skin are the characteristic skin and coat abnormalities of thyroid malfunction in dog. The clinical signs of the disease vary greatly because of the myriad of systems the thyroid hormone impacts. It is a single endocrinological disease suspected most

commonly in canine suffering from alopecia (Doering and Jensen, 1973). Most owners are alerted to a problem when changes in the dogs coat occur. In fact this is the first symptom. The classical signs include alopecia on both sides of trunk or specifically on the tail i.e., the "rat tail". Hair regrowth is usually slow and may come in dry, dull or differently coloured. The skin may also change in colour and become greasy, strong, smelling and thick. Often the outer hairs break off, leaving a short, softer undercoat, classically observed as "puppy-like coat". In the present study clinical signs related to dermatological changes were recorded in 68–82 percent of hypothyroid dogs. Panciera (1999) documented that dermatological changes occur in 60 per cent to 80 per cent of hypothyroid dogs and alopecia is usually bilaterally symmetric and is first evident in areas of wear, such as lateral trunk, ventral thorax and tail. Fading of coat colour may also occur and failure of hair re growth after hair clipping is common. Another classical finding in hypothyroid dogs is a thickening of some tissues, especially of the face and head. The skin in particular, thickens leading to more skin folds and that is classically referred to as "tragic face". The thickening is called as myxedema which occurred in 8.51 percent of patients of the present study. These findings are in agreement with Satish Kumar et al., (2007) who reported it as 8.85 percent. Myxedema (cutaneous mucinosis) is a rare dermatological manifestation of hypothyroidism characterized by non-pitting thickening of the skin especially of the eyelids, cheeks and forehead and is associated with the deposition of hyaluronic acid in the dermis (Kelly and Hill, 1984). Whereas, Doering and Jensen (1973) opined that some times myxedema may be attributed to accumulation of excessive amounts of mucopolysacharides and protein in the dermis that may also occur in facial nerves resulting in facial paralysis. Similar findings of drooping of eyelids and ear associated with facial paralysis was also noticed in the present study.

Even though dermatological manifestations were the common findings that occurred in over 80 per cent of the cases of the present study, about 76 per cent of them were also found to be associated with secondary skin infestations, such as *Malasseziosis, Demodicosis* and bacterial pyoderma. These findings are in accordance with Panciera (1994) who narrated that secondary skin disorders such as malassezia infection, bacterial pyoderma, sebbhoric otitis externa are commonly associated skin disorders with primary hypothyroidism in dogs.

Thyroid system plays a greater role in regulating several functions and influences the immune system and hence, when it is depressed or compromised, the whole body becomes more and more vulnerable to the insults (Chen and Loch, 1997). This could be attributed to the secondary skin infections reported in the present study. Whereas, Mayr (2007) and Bansal et al., (2007) opined that hypothyroid dogs are predisposed to recurrent bacterial infections of the skin such as folliculitis, pyoderma and furunculosis. *Malassezia* species infections and demodicosis are associated with hypothyroidism in dogs. Pruritus an unusual manifestation of hypothyroid dogs may occur with these concurrent infections. whereas Chastain and Panciera, (1995) documented that hypothyroidism may cause impaired neutrophil and lymphocyte function thereby causing abnormal systemic immune responses and alterations in local immunity resulting in pyoderma and sebhorrhoea. In the present study, infection with *Malassezia pachydermatis, Demodex canis* were noticed in 20 and 7 dogs, respectively and pyoderma among 4 dogs. The findings of present investigation are in accordance with the above authors.

Apart from dermatological abnormalities, signs related to decrease metabolic rate such as, obesity, lethargy, exercise intolerance, cold intolerance, dyspnoea at rest and weakness were also recorded in 25-78 per cent of hypothyroid dogs of the present

study. These findings are in accordance with Greco et al., (1998) who documented similar signs related to decrease metabolic rate. They further opined that the degree of obesity is usually moderate, although obesity is the presenting complaint in dogs of decreased thyroid function. The presence of lethargy is frequently over looked, as owners may notice abnormality only after thyroid hormone supplementation resulting increased activity and alertness (Panciera, 1994). These abnormalities could be attributed to general metabolic derangements that occur in impaired thyroid function or may be an indication of a neuropathy or myopathy (Mayr, 2007). The neuromuscular signs observed in the present study that account for about 25 percent were facial paralysis, seizures and paraplegia. The findings are in opinion with Jaggy et al., (1994), who stated that in hypothyroidism, nerves do not conduct electrical impulses normally. This may account for some of the general weakness, exercise intolerance, lethargy and listlessness observed in hypothyroidism. Sometimes single nerves (focal neuropathy) can get entrapped as they exit the skull or spinal cord as they (like other tissues) swell with myxedema. Pressure on these nerves can lead to paralysis of facial muscles and/or head tilt, bizarre eye motions and balance disruptions. Seizures, disorientations and circling may occur due to severe hyperlipedemia or cerebral atherosclerosis. Whereas, Budsberg et al., (1993) documented that unilateral lameness reported in hypothyroid dogs could be a manifestation of generalised neuropathy.

Abnormalities of cardiovascular system such as bradycardia, low voltage R amplitude, P amplitude, arrythmias and reduced left ventricular functions were noticed in hypothyroid dogs of the present study. The findings are in agreement with Gaalova et al., (2008) and Varshney et al., (2007) who opined that low heart rate, brady arrythmias, low voltage QRS complexes and first degree AV block are the

common electrocardiogram changes of cardiovascular system associated with hypothyroid dogs. Cardiovascular abnormalities in hypothyroid dogs are common but significant clinical signs rarely result. Bradycardia, weak pulses and low voltage electrocardiogram complexes, arrythmias, first degree AV block and atrial fibrillation have been associated with hypothyroidism in dogs (Panciera, 1994). Auscultation of thorax of the present hypothyroid dogs revealed, cardiac murmur and/or arrythmias that was in agreement with the above authors.

Hypothyroid dogs of present investigation also showed few general signs like corneal lipidosis (10.64 per cent), pale mucosa (19.25 per cent), anaemia (27.6 per cent), cyanosis (8.51 per cent) and goitre (10.64 per cent). The findings are in accordance with Gaalova et al., (2008) who opined that hypothyroidism interferes with the electrical fibres that more or less provide the wiring of the heart. The rhythmic contractions of the heart muscle, as normally stimulated by these electrochemical fibres, abnormal rhythms or slow heart rate and consequently dyspnoea, pale mucosa, anaemia and cyanotic tongue also occur in as many as 26 per cent of hypothyroid dogs. Whereas, Durieux et al., (2008) and Williams et al., (2007) documented that ocular changes are not common in hypothyroidism but the high levels of blood cholesterol and circulating fat can sometimes lead to ocular changes. Corneal dystrophy, an abnormal change in the clear covering of the eye, is such sign. This finding is usually represented as a small white spot (sometimes a white circle) on the eye surface. In the present study corneal lipidosis is the only ocular abnormality recorded and could be attributed to the said reason.

Hypothyroid dogs of the present study also showed various systemic illness resulting in disorders associated with cardiovascular, nervous, renal, musculoskeletal systems and diabetes mellitus. These findings are in agreement with Vressler et al.,

(2003); Philips and Harkane, (2003) and Hess and Ward, (1998) who opined that these are the common systemic illness that occur concurrently during thyroid malfunctions in dogs. Whereas Hess et al., (2003) documented the association between diabetes mellitus, hypothyroidism and atherosclerosis in dogs and concluded that diabetes mellitus and hypothyroidism are more prevalent in dogs with atherosclerosis compared to dogs without this condition.

Hypothyroid dogs of the present investigation also showed lameness (10.64 per cent) of varied intensity. This finding is corroborated with, Franch et al., (2004) and Rossmeisl, (2009) who documented severe bone destruction of talus and a periosteal reaction of the calcaneous and popletial lymphadenopathy, mild crepitous and swelling of left tarsal joint of hypothyroid dog. Chronic canine hypothyroidism resulted in substantial but subclinical phenotypic myopathic changes indicative of altered muscle energy metabolism and depletion of skeletal muscle carnitine. Paradis et al., (2003) stated the effects of moderate to severe osteo-arthritis on canine hypothyroidism and concluded that osteoarthritis need not to be considered a factor influencing thyroid function evaluation in dogs.

In the present study, haematological findings of the hypothyroid dogs of both the groups revealed normocytic, normochromic, non-regenerative anaemia. However, a significantly low ($P<0.05$) levels of PCV along with a non-significantly different TEC, Hb, TLC and DLC were noticed among hypothyroid. Whereas, serum cholesterol (11.88 ± 1.22 and 12.54 ± 1.04mmol/L), triglycerides (4.98 ± 1.84 and 4.02 ± 1.98 mmol/L), CKMB (49.46 ± 2.34 and 52.28 ± 2.15U/L), LDH (134.42 ± 3.25 and 138.24 ± 4.44U/L) and ALP (92.24 ± 0.88 and 90.24 ± 1.02U/L) were significantly ($P<0.05$) increased in hypothyroid dogs of GI & GII, respectively. However these values decreased significantly ($P<0.05$) following therapy on day 45.

These findings are in agreement with Andronic et al., (2008) and Rossmeisl (2009) who reported that a mild non-regenerative anaemia occurs in 30 per cent of hypothyroid dogs. They further documented that hypercholesterolemia occur in 75 per cent of hypothyroid dogs, whereas, hypertriglyceridemia occur in up to 88 per cent. In rare cases hyperlipedemia may lead to atherosclerosis. Less common abnormalities include mild increase in alkaline phosphatase, alanine amino transferase and creatine kinase activities. However, Panciera (1994) opined that haematobiochemical abnormalities are neither specific to hypothyroidism nor consistently found, so their use in diagnosis is limited. Whereas, Suraniti et al., (2008), Bansal et al., (2007) and Mooney (2003) documented that increased levels of serum cholesterol, creatine kinase MB and alkaline phosphatise activities were the major biochemical alterations noticed in hypothyroid dogs apart from non-regenerative anemia.

With respect to thyroid profile, significantly low ($P<0.05$) levels of serum total thyroxine (0.82 ± 1.06 and 0.98 ± 0.92 mcg/dl), free thyroxine (0.44 ± 1.26 and 0.84 ± 1.02 ng/dl) and triiodothyronine (28.54 ± 1.34 and 31.02 ± 1.02 ng/dl) and elevated levels of thyrotropin (7.08 ± 1.02 and 7.92 ± 0.14 ng/ml) were recorded in hypothyroid dogs of groups I and II, respectively. However, these values significantly differed following therapy by day 45 in all the dogs of both the groups of present study. These findings are in agreement with Mooney (2003) who stated that the diagnosis of hypothyroidism is easily achieved by demonstration of low circulating TT4 with elevated TSH concentrations. However, in some the combination does not occur and other diagnostic tests such as fT4 and thyroglobulin autoantibody analysis are required. Whereas, Suraniti et al., (2008) reported normal range of fT4 but with highly elevated TSH in Doberman pinscher dogs between 6-8 years suffering with hypothyroidism. In the present study low levels of TT4, fT4 and T3 with an elevated

level of TSH was recorded. Measurement of canine-thyrotropin had an excellent specificity and suggests that the measurement of TSH is a valuable tool in confirming canine hypothyroidism. However, due to low sensitivity of TSH assays it can not be recommended to exclude the disease (Boretti and Reusch, 2004). The authors concluded that in most of the hypothyroid dogs T4 is decreased but with the presence of autoantibodies to T4 the values can be normal or increased. Total T4 concentrations do not differ significantly between males and females but are higher in small dogs than in medium and large breed dogs (Jagpreeth et al., 2006). Similar findings were noticed in the present study.

Protein bound hormone acts as a reservoir to maintain concentration of free hormone in plasma despite fluctuations in release of metabolism of T3 and T4, or in plasma protein concentrations. Thus free hormone concentrations are less affected by changes in protein concentrations and binding than one total hormone measurements. Because only free hormone can enter cells and bind to receptors, measurements of fT4 should give a more accurate representation of thyroid function. In humans, fT4 concentrations often remains normal during nonthyroidal illness, but this not always occur in dog. In the present investigation, estimation of free thyroxine levels were also included and found that these values are significantly lower ($P<0.05$) in hypothyroid dogs compared to healthy dogs and however, these values increased significantly following L-thyroxine supplementation. Boretti and Reusch, (2004) documented that 13 per cent to 38 per cent of hypothyroid dogs have a TSH concentrations within the reference range, that could be attributed to secondary or tertiary hypothyroidism, fluctuations in TSH concentrations, and effect of drugs or concurrent illness. In addition, the TSH assay may not detect all isoforms of circulating TSH. Specificity of TSH alone for diagnosis of hypothyroidism is lower

than measurement of fT4 or TT4 because TSH concentrations are increased in 7 per cent to 8 per cent of euthyroid dogs. Reasons for increased TSH with a normal TT4 include early hypothyroidism and effects of drugs.

30 hypothyroid dogs presenting the signs of reduced metabolic rate were subjected to 2D echocardiography and diagnosed for DCM. The mean values of various left ventricle dimensions such as, LVEdD (51.88 ± 0.26 and 52.36 ± 0.42 mm), LVEsD (44.12 ± 0.24 and 43.88 ± 0.64 mm) and EPSS (9.12 ± 0.80 and 9.44 ± 0.13mm) were significantly increased ($P<0.01$) in group I and II of hypothyroid dogs. Whereas, LVPWd, LVPWs, IVSd and IVSs (5.66 ± 0.54 and 5.24 ± 0.12 mm, 8.12 ± 0.58 and 8.82 ± 0.44mm , 4.96 ± 0.29 and 5.02 ± 0.88 mm, 5.88 ± 0.54 and 5.82 ± 0.82mm) also increased significantly ($P<0.05$). With respect to ejection fraction and fractional shortening the values were (33.46 ± 1.22 and 31.64 ± 1.02 per cent and 14.16 ± 2.04 and 15.88 ± 1.98 per cent) in hypothyroid dogs of group I and II, respectively that were significantly decreased ($P<0.05$) as compared to healthy dogs. However, all these left ventricular dimensions improved significantly ($P<0.05$) following levothyroxine supplementation. The findings are in agreement with Panciera (1994), who suggested that hypothyroidism in dogs results in reversible impairment of left ventricular functions and hence, echocardiographic measurements of left ventricular, including fractional shortening, were found to be reduced in hypothyroid dogs.

Fialkovicova et al., (2008) stated that thyroid function should be evaluated in all dogs with evidence of impaired left ventricular function, especially those with recent evidence of weight gain, exercise intolerance and dyspnoea at rest. Abnormalities of the cardiovascular system such as sinus bradycardia, weak apex beat, low QRS voltages, and inverted T waves occur in hypothyroid dogs. Reduced left ventricular

pump function has also been documented and hypothyroidism may exacerbate clinical signs in dogs with underlying cardiac disease. Although hypothyroidism rarely causes clinically significant myocardial failure in dogs, dilated cardiomyopathy and hypothyroidism may occur concurrently. In the present study out of total 47 dogs diagnosed for thyroid dysfunction, based on clinical and thyroid profile, ECG and 2D-echocardiography confirmed 30 hypothyroid dogs with concurrent DCM. These were exhibiting severe exercise intolerance, dyspnoea at rest and were obese with / without classical skin abnormalities. Hence, it is opined that hypothyroid is an important factor in differential diagnosis in large breed dogs with DCM. These findings are in accordance with the above authors. Gaalova et al., (2008) and Mac Gregor et al., (2004) stated that cardiomegaly, pleural effusion or pulmonary edema is the common thoracic radiographic abnormalities associated in thyroid dysfunction dogs. X ray findings in the present study revealed cardiomegaly (51.06 per cent) and auricular bulge (12.77 per cent). Whereas, X-ray of neck of goitre dog revealed as soft tissue swelling of thyroid lobes at the caudal portion of larynx. Similarly, inflamed carpal joint was noticed on skiagram of hypothyroid dog exhibiting lameness of forelimbs. These findings could be attributed to overweight associated with obesity of thyroid dysfunction dog and are in accordance with Paradis et al., (2003).

Because of the relatively low accuracy of most commonly used biochemical tests and to rule out false positive diagnosis thyroid ultrasound was also performed to access the size, shape and texture differences of thyroid lobes of hypothyroid and healthy dogs. The mean ± SE of length (1.12±0.05 and 1.20±0.14cm), width (0.68±0.32 and 0.52± 0.08cm), height (0.60±0.10 and 0.50±0.10cm) and volume (0.21±0.02 and 0.16±0.24cm^3) of thyroid lobes in group I and II dogs, respectively, were significantly ($P<0.05$) decreased. However, a non-significant difference was

noticed with these values after therapy. These findings are in agreement with Marco and Larson (2006) who documented significantly lower total thyroid gland volume compared to healthy dogs. Whereas, Bromel et al., (2005) opined that ultrasonographic findings in hypothyroid dogs were more variable viz., round to oval shape with hypoechoic texture of thyroid of dogs with thyroid malfunction. The thyroid lobes in the present hypothyroid dogs were sonographically smaller, hypoechoic, heterogeneous, misshapen or ill delineated. These findings are in agreement with Taeymans et al., (2007) who opined that the measurement of the length was the most difficult of the three measurements to obtain because of the difficulty in identifying the sharp caudal end point of the gland. Also, as the edge of the pathologic glands was ill-defined at some places, defining the shape and the capsule de-lineation of the gland was based on a more subjective interpretation than the determination of the relative echogenicity and homogenecity of the gland parenchyma. Diagnostic ultrasound of the canine thyroid gland is relatively easy to perform, usually doesn't require sedation of the patient, gives a quick result compared to biochemical blood test, and has been proven to be a sensitive test in the diagnosis. It can therefore be considered as an effective additional test in the diagnostic protocol in hypothyroid dogs.

In the present study, therapy of Group I hypothyroid dogs was carried out using levothyroxine @20 mcg/kg bw for 45 days. Whereas, L-carnitine @ 30 mg/kg bw was also included along with levothyroxine for group II dogs. The clinical manifestations such as bilateral alopecia, rat tail appearance, puppy like coat, dry and lustreless coat and secondary skin disorders started disappearing from day 20 of therapy and were not evident by day 30 in the dogs of group II in comparison to group I which disappeared after 30-45 days. Levothyroxine supplementation (Tab.

Eltroxine) was found to be highly efficacious in alleviating the hypothyroid signs such as increased physical activity, normalization of weight, growth of hair and shine in hair coat with improving serum T4 and T3 levels.

The treatment with Eltroxine tablets caused complete recovery from dermatitis and skin lesions after two courses in four of six dogs studied and the texture of skin and hair coat also returned to normalcy (Krishnamurthy and Rajan, 2002). The present findings with respect to improvement in clinical manifestations are in accordance with the afore said authors.

Whereas, signs associated with decreased metabolic rate disappeared in 15-20 days and after 30 days, respectively in dogs of group II and I. Some dogs which also showing corneal lipidosis, pale mucosea, anaemia, nervous signs, cyanosis, myxedema and goitre improved after 25 days in hypothyroid dogs of group II when compared to group I which improved after 35 days. Further, all the twenty three dogs included in group II for therapeutic trial showed complete recovery with no side effects when compared to death of 2 dogs in group I during the therapy period. A significant difference ($P<0.05$) of CKMB and LDH ($P<0.01$) and cholesterol, triglycerides, total thyroxine, free thyroxine, triodothyronine and thyroid stimulating hormone ($P<0.05$) of GII dogs was noticed on day 45 after treatment as compared with group I. The different abnormalities associated with thyroid gland morphology and texture that revealed during thyroid lobe ultrasonography such as, irregular thick capsule, ill delineated abnormal shape, smaller size, echogenic difference and thyroid lobe volume both before and after therapy were not significantly different between the groups. In Group II hypothyroid dogs, post therapy levels of left ventricle dimensions such as, LVEdD, LVEsD, LVPWd, LVPWs, IVSd, IVSs, EPSS, EF and FS were significantly different ($P<0.01$) in comparison with the group I on day 45. Faster

improvement in clinical manifestations, thyroid profile, biochemical profile, ultrasonographic findings of thyroid gland and echocardiographic features and rapid recovery of hypothyroid dogs among group II could be attributed to the L-carnitine supplementation for those dogs. Fialkovikova et al., (2008) reported a case of dilated cardiomyopathy and hypothyroidism in a Great Dane and opined that pimobendon @ 0.2mg/kg.bw once daily along with levothyroxine supplementation is effective in treating such concurrent cardiac conditions in hypothyroid dogs. The cardiac changes that develop in dogs with hypothyroidism are important because of the high incidence of hypothyroidism and heart disease. Any alteration in cardiac function induced by hypothyroidism could result in worsening of pre-existing cardiac complaint, leading to death. Hence, use of antiarrythmias, ionotropes and levocarnitine along with thyroid supplementation help faster recovery (Panciera, 1994). Whereas, Gaalova et al., (2008) opined that thyroid hormones have direct and indirect effect on heart and hence supplementation of levothyroxine for considerable period helps to disappear the signs. The findings of the present study are in agreement with above authors.

Some changes in structure and function of the heart are effected rapidly following thyroid hormone supplementation, but their importance in dogs is unknown. Hypothyroidism in rats induces a change from the (alpha-myosin) V_1 to the (beta-myosin) V_3 isoenzyme, which contains less ATPase activity and thus is associated with decreased myocardial contractility. Because the canine heart contains only the beta-myosin (V_3) isoenzyme, other factors may be more important in the pathogenesis of myocardial hypocontractility in hypothyroid dogs. Infiltration of myocardium with mucopolysacchrides or impaired coronary arterial vasodilatory ability, possibly secondary to increased alpha-adrenergic activity or atherosclerosis, might account for prolonged impairment of cardiac function. In addition, alterations in peripheral

circulation may return to normal more slowly. Some markers of hypothyroidism at the tissue level change slowly following levothyroxine supplementation, and normalization of many clinical abnormalities often requires months of thyroid hormone supplementation. Because a complete turnover of protein occurs in the heart approximately every 3 weeks, the mean duration of levothyroxine administration in the present study of 45 days seems adequate to allow resolution of most of the cardiac changes. In the present investigation, even though levothyroxine supplementation helped resolve clinical signs and physical condition, dogs of group II which were also treated with L-carnitine, showed faster recovery. This response could be attributed to the added effect of Levocarnitine which might result in improved nutrition and energy required for the myocardium and there by causing improved myocardial contractility (Dove 2001) among DCM associated hypothyroid dogs of group II. Roudebush and Freeman (1999) documented that myocardial carnitine levels were low in dogs suffering with DCM and these dogs have high oxidative stress and alterations in the endogenous antioxidant system and hence, its supplementation could help quick recovery. Further low thyroid functions may indicate a need for carnitine to help overcome low energy levels and the tendency to gain weight and hence indicated in hypothyroid dogs. Levocanitine, an amino acid that is synthesized in the liver and kidneys from lysine and methionine in the presence of ascorbate. Dietary supplementation of L-carnitine improves nitrogen retention increasing lean mass and reducing fat mass, cholesterol, triglycerides, fatigue and hence indicated in increased cholesterolemia, increased triglyceridemia and obesity. This could be attributed to enhancing fatty acid oxidation and energy availability for protein synthesis during times of need. Its primary role is to help fatty acids into energy producing units in the cells-the mitochondria where they can be converted to energy. This is a major source

of energy for muscles including myocardium and hence includes muscle function and exercise capacity thereby physical activity and exercise intolerance (Weinsier et al., 1984). Because protein synthesis is reduced in hypothyroidism, cardiac proteins may be altered more slowly in the early part of treatment of hypothyroidism.

Hence, it may be concluded from the present findings that the dogs suffering with primary hypothyroidism are presented with skin and coat or decreased metabolic rate abnormalities or both. Those hypothyroid dogs that are presented with cardiovascular disorders (manifested by decreased metabolic rate) need a thorough diagnostic procedure to be followed, particularly 2D echocardiography to confirm the associated DCM and such patients can be effectively treated with additional supplementation of levocarnitine along with levothyroxine for faster clinical recovery.

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APPENDIX - B
ESTIMATION OF TOTOL CHOLESTEROL

Principle:- Enzymatic determination of total cholesterol according to the following reactions.

Cholesterol ester + H_2O ----------> Cholesterol + Fatty acids
Cholesterol +O_2 ----------> 4- Cholesterol-3-one + H_2O_2
$2H_2O_2$ + 4- Aminoantipyrine ----------> Quinoneimine + $4H_2O$

Procedure :- Pipette in a clean dry test tube labelled as Blank (B), Standard (S) and Test (T)

Enzyme	B	S	T
Reagent	1ml	1 ml	1 ml
Deionized water	0.01 ml	-	-
Standard	-	0.01 ml	-
Serum/Plasma	-	-	0.01 ml

Mix and read the density (OD) at 500nm against blank after 5 min incubation (37^0C). The final colour is stable for at least 1 hour.

APPENDIX-C
ESTIMATION OF TRIGLYCERIDES

Principle :- Determination of triglycerides after enzymatic splitting with lipoprotein lipases. Indicator is quinoneimine, which is generated from 4-cholophen by hydrogen peroxide under the catalytic action of peroxidise.

Triglycerides+H_2O --LPL-----→ glycerol+fatty acids
Glycerol +ATP-------GK-----→glycerol-3-phosphate+ADP
Glycerol-3-phosphate+O_2------GPO-----→Dihydroxyacetone-phosphate+H_2O_2

H_2O_2+4-Aminoantipyrine+p-chlorophenol------POD---→Quinoneimine+HCl+4H_2O

Method	kinetic
Slope of reaction	Increasing
Wavelength	505nm
Flow cell temperature	37^0C
Sample	serum/plasma
Sample volume	10µl
Reagent volume	1000µl
Incubation	10 minutes at 37^0C
Standard concentration	200mg/dl
Unit	mg/dl
Normal range	0-150mg/dl
Linearity	upto 1000 mg/dl

Pipette into tet tubes	Blank	Standard	Test
Standard	-	10 µl	-
Sample	-	-	10 µl
Reagent	1000 µl	1000 µl	1000 µl

Mix well and incubate for 10 min. at 37^0C. Read the absorbance at 505 nm against reagent Blank.

APPENDIX-D
ESTIMATION OF ALKALINE PHOSPHATASE

Kinetic determination of Alkaline phosphatise (ALP) based upon DGKC and SCE recommendation

p-Nitrophenyl phosphate + H_2O----------> p-Nitrophenol + Inorganic phosphate

Under alkaline conditions a colorless p-Nitrophenol is converted to 4-nitrophenoxide which develops a very intense yellow color. This intensity is proportional to the activity of alkaline Phosphatase in the sample.

Procedure :-

Method	kinetic
Slope of reaction	Increasing
Wavelength	405nm
Delay time	60sec
Measuring time	180sec
Temperature	37^0C
Sample volume	20µl
Working reagent Vol	1000µl
Factor	2750
Normal range	64-306 U/L
Linearity	upto 700 U/L

Test procedure:-

Sample	20µl
Working reagent	1000µl

Mix well, and incubate for 1 min. at 37^0C. Read Absorbance and at the same time start the stop watch. Read the absorbance again exactly after 1, 2 & 3 min.

APPENDIX-E
ESTIMATION OF ALANINE AMINO TRANSFERASE (ALT)

Method : Modified IFCC method

Principle :-

L-Alanine+2-Oxoglutarate-<ALT>---→L-Glutamate+Pyruvate

Pyruvate+NADH+H^+----<LDH>--------→D-Lactate+NAD^+

Reagents :- R1: TRIS Buffer Ph 7.5 100mmol/L
L-Alanine 500mmol/L
LDH ≥1200U/L

R2: 2-Oxoglutarate 1500mmol/L
NADH 0.18mmol/L

Working reagent: Mix 4 parts of R1+ 1part of R2

Method	kinetic
Slope of reaction	decreasing
Wavelength	340nm
Delay time	60sec
Delta time	60sec
No of readings	3
Temperature	37^0C
Sample	serum/plasma
Sample volume	100µl
Working reagent Vol	1000µl
Unit	U/L
Factor	1745
Normal range	upto 42 U/L
Linearity	upto 350 U/L

Procedure: a sterile test tube labelled test was taken.

Pipette in to test tube	Test
Working reagent	1000 µl

Sample	100 µl

The contents of the test tube were mixed well and incubated for 1 minute at 37^0C and then absorbance was read on biochemical auto analyser.

APPENDIX-F
ESTIMATION OF SERUM ASPARTATE AMINO TRANSFERASE(AST)

Principle:- Optimized UV-test according to IFFCC (International Federation of Clinical Chemistry and laboratory Medicine) [modified]
L-Alanine +2-Oxogilutrate<ALT>L-Glutamate+ Pyruvate Pyruvate + $NADH+H^+$ <LDH>D-Lactate+NAD^+

Reagents :- R1: TRIS Buffer PH 7.5 100mmol/L
L-Alanine 500mmol/L
LDH ≥1200U/L
R2: 2-Oxoglutarate 15mmol/L
NADH 0.18mmol/L

Method	kinetic
Slope of reaction	decreasing
Wavelength	340nm
Delay time	60sec
Delta time	60sec
No of readings	3
Temperature	37^0C
Sample	serum/plasma
Sample volume	100µl
Working reagent Vol	1000µl
Unit	U/L
Factor	1745
Normal range	1745U/L

Procedure: A sterile test tube labelled test was taken.

Pipette in to test tube	Test
Working reagent	1000 µl
Sample	100 µl

Mix, read absorbance after 1 min and start stop watch. Read absorbance again after 1, 2 and 3 min at 37^0C.

APPENDIX-G
MEASUREMENT OF CREATINE KINASE-MB

Principle:- The sample is incubated in the CKMB reagent which includes the anti CK-M antibody. CK-B catalyses the reversible phosphorylation of ADP, in the presence of creatine phosphate to form ATP and creatine. The auxillary enzyme hexokine (HK) catalyses the phosphorylation of glucose by the ATP format, to produce ADP and glucose-7 phosphate is oxidised to 6- phosphogluconate with the concomitant production of NADH.The rate of NADH formation measured at 340 nm is directly propotional to serum CK-B activity.

Reagent: CKMB reagent
Reagent preparation: Reconstitute each vial of CK-MB reagent with the volume of distilled or deionised water specified on the vial label. Swirl to dissolve.

General system parameters:

Method	kinetic
Slope of reaction	Increasing
Wave length	340nm
Temp	37ºc
Sample volume	50µl
Reagent volume	1000 µl
Delay time	300sec
Unit	µl

Procedure:

Reagent	1000µl
Sample	50µl

Mix and incubate at room temperature for 5 minutes. Then measure the absorbance against distilled water.

APPENDIX-H
MEASUREMENT OF LACTATE DEHYDROGENASE

Principle:- LDH catalyses the oxidation of lactate to pyruvate in the presence of NAD which is subsequently reduced to NADH. The rate of NADH formation measured at 340nm is directly proportional to serum LDH activity.
Reagent:lactate dehydrogenase reagent
Reagent preparation:
Reconstitute reagent with volume of distilled or deionised water stated on the vial label. Invert gently to dissolve.

General system parameters:

Method	kinetic
Slope of reaction	Increasing
Wave length	340nm
Temp	37ºc
Sample volume	25µl
Reagent volume	1000 µl
Delay time	60sec
Unit	µl

Procedure: Reagent 1000µl
Sample 25µl

Mix and incubate at room temperature for 60 seconds. Then measure the absorbance against distilled water.

APPENDIX-I
THYROXINE T4

The EIA-THYROID –T4 TOTAL is a one step immunoassay to determine the presence of total thyroxine (total T4) in serum using competitive microplate enzyme immunoassay.

Plates coated with anti-T4 antibodies. Serum reference, patient specimen, or control is first added to microplate well. Enzyme –T4 conjugate is added. Thyroxine present in the sample competes with enzyme-thyroxine conjugate for binding with anti-T4 coated microplate to form an antigen-antibody complex.

Unbound conjugate is removed by washing. The enzyme activity in the antibody-bound fraction is inversely proportional to the native thyroxine concentration. The enzyme activity is revealed by a colour change in TMB- substrate solution.

Preparation of the reagents:-

ready to use reagents:

T4 antibody coated microtitre wells

calibrators

control serum

TMB-substrate

Stopping reagent

Reagents to reconstitute:

working washing solution:- Thoroughly shake washing solution concentrate. To make working washing solution take required amount of concentrate and mix with purified water (1:25 ratio) in a separate vial. Thoroughly mix the solution. Working washing solution may be stored for 3 days at 2-8^{0}C temperature.

3.Working conjugate solution:-

Working conjugate solution must be prepared before usage. To make working conjugate solution take equal amounts of conjugate and buffer ANS in a separate vial. Thoroughly mix the solution. The reagent may not be stored.

Test procedure:-

Note :- Before use, allow reagents to reach room temperature (18-24^{0}C) for 30min.

1. Bring all the components and clinical specimens to be tested to room temperature.
2. Format the microplate wells for each calibrators, control serum and patient specimen to be assayed in duplicate. Leave unused microtitre wells in a

package and do not remove silica gel drier, and then place the package into self-sealing plastic bag, seal and store at 2-8^{0}C for 1 month.

3. Pipette 25µl of the appropriate calibrators, control serum or specimen into assigned well.
4. Add 200µl of working conjugate solution to all the wells.
5. Incubate on a thermoshaker (500-800rpm) for 30 minutes at (37+0.5)^{0}C
6. Wash the wells 5 times with 300µl of working washing solution per well and tap the plate firmly against absorbent paper to ensure that it is dry.
7. Pipette 100µl of TMB- substrate into each well at timed intervals.
8. Incubate for 15-20min at room temperature in the dark.
9. Add 150µl of stopping reagent into each well at same timed intervals as in step 7. Gently mix for 5-10sec.
10. Read the absorbance on the microplate reader at 450nm within 20min after stopping reaction.

APPENDIX-J
TRIIODOTHYRONINE (T3)

Principle of the test:- In the T3 EIA, a second antibody (goat anti-mouse IgG) is coated on microtiter wells. A measured amount of patient serum, a certain amount of T3 conjugated with horseradish peroxidise are added to the microtiter wells. During incubation, the mouse anti-T3 antibody is bound to the second antibody on the wells, and T3 and conjugated T3 compete for limited binding sites on the anti-T3 antibody. After a 60-minute incubation at room temperature, the wells are washed 5 times by water to remove unbound T3 conjugate. A solution of TMB reagent is then added and incubated for 20 minutes, resulting in the development of blue colour. The colour development is stopped with the addition of stop solution, and the absorbance is measured spectrophotometrically at 450nm. The intensity of the colour formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled T3 standards assayed in the same way, the concentration of T3 in the unknown sample is then calculated.

Reagent preparation:-

1. All reagents should be allowed to reach room temperature (18-25^{0}C) before use.
2. To prepare Working T3-HRPO Conjugate Reagent, add 0.1ml of T3-HRPO Conjugate concentrate (11x) to 1.0 ml of T3 conjugate Diluent (1:10 dilution), and mix well.

Note:- Prepare only the amount of conjugate that is required each time. Working conjugate reagent should be used within 24 hours. Discard the excess after use.

Assay procedure:-

1. Secure the desired number of coated well in the holder. Make data sheet with sample identification.
2. Pipette 50µl of standard, samples, and controls into appropriate wells.
3. Dispense 50 µl of the Antibody Reagent into each well. Mix thoroughly for 30 seconds.
4. Add 100µl of working conjugate reagent into each well. Mix thoroughly for 30 seconds. It is important to have a complete mixing in this step.
5. Incubate at room temperature for 60 minutes.
6. Remove the incubation mixture by flicking plate contents into a waste container.

7. Rinse and flick the microtiter wells 5 times with distilled or deionized water. (please do not use the tap water.)
8. Strike the wells sharply onto absorbent paper to remove residual water droplets.
9. Dispense 100µl TMB Reagent into each well. Gently mix for 10 seconds.
10. Incubate at room temperature in the dark for 20 minutes without shaking.
11. Stop the reaction by adding 100µl of stop solution to each well.
12. Gently mix for 30 seconds.

*It is important to make sure that the blue colour changes to yellow colour completely.

13. Read OD at 450nm with a microtiter reader within 15minutes.

APPENDIX-K
THYROID STIMULATING HORMONE (TSH)

The Teco Thyroid Stimulating Hormone ELISA test kit is intended for the quantitative determination of thyroid stimulating hormone (TSH, Thyrotropin) concentration in serum or plasma.

Principle:-

The essential reagents required for an immunoenzymometric assay include excess amount of antibodies (both enzyme conjugated and immobilized) with high affinity, high specificity and contain different epitopes with distinct recognition and native antigen. In this assay procedure, the immobilization takes place at the surface of microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-TSH antibody.

Upon mixing, a reaction results between the native antigen contained in the serum, the monoclonal biotinylated antibody and enzyme-labelled antibody, without competition or steric hinderance, to form a soluble sandwich complex.

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

Reagent preparation:-

1. All the reagents should be allowed to reach room temperature (18-25^{0}c) before use.
2. Working wash solution:- Add 20ml of wash solution concentrate to 1000ml of deionized water, and mix well. The amount of working wash solution depends on the assay procedure described below. The working wash solution is stable at room temperature for at least 7 days.

Assay procedure:-

1. Secure the desired number of coated wells in the holder. prepare data sheet with sample identification .
2. Pipette 50µl of standards, sample and controls into each well.
3. Pipette 100µl of conjugate reagent into each well. Mix thoroughly for 30 seconds.
4. Incubate at room temperature for 60minutes.
5. Discard the contents of the wells by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
6. Pipette 300µl of working wash solution, decant or aspirate. Repeat 2 additional times for a total of 3 washes. An automatic ELISA washer may be used.
7. Add 100µl of TMB reagent into each well. Gently mix for 10 seconds.
8. Incubate at room temperature in the dark for 15 minutes without shaking.
9. Pipette 50µl of stop solution to each well and gently mix for 10-20 seconds. It is critical to make sure that the blue colour change to yellow colour completely.
10. Read the absorbance at 450nm for each well.

SRI VENKATESWARA VETERINARY UNIVERSITY
COLLEGE OF VETERINARY SCIENCE, RAJENDRANAGAR, HYDERABAD – 30
Department of Veterinary Clinical Medicine
CANINE HYPOTHYROIDISM ASSESSMENT – DATA SHEET

CLIENT:_____

CASE No. _____ DATE: _____ BREED: _____ SEX: _____ AGE: _____

HISTORY: ASSESSMENT	CLINICAL ASSESSMENT	PHYSICAL
	Skin: Hair: Alopecia: Rat tail: Lesions: Myxedema: Corneal lipidosis: Physical condition: Dyspnoea at rest: Cyanosis: Exercise intolerance:	Arrhythmias: Bradycardia: Tachycardia: Murmurs: CRT: Lungs

SECONDARY COMPLAINTS	ELECTRO CARDIOGRAPHY:	HEMOGRAM:
		Day 0
Day30		
Skin complaints:	ECHO CARDIOGRAPHY:	TEC: Hb:
Systemic complaints:	CHEST X-RAY:	PCV:

TLC:

THYROID ULTRASOUND:

THYROID PROFILE:

	Day 0	Day 30
COMPLAINT		
T3: ( )		
T4: ( )		
TSH: ( )		
BIOCHEMISTRY:		
Cholesterol:		
ABSCENT		
Triglycerides:		
ALP:		

DIAGNOSIS:

HYPOTHYROIDISM / ASSOCIATED

TREATMENT:

CLINICAL RESPONSE: PRESENT /