



Studies on Canine Thyroid-stimulating Hormone (cTSH), Total Triiodothyronine (TT₃), Total Thyroxine (TT₄) and Free Thyroxine (fT₄) in Healthy and Hypothyroid Dogs

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ABSTRACT

Background: The study aimed to measure canine thyroid-stimulating hormone (cTSH) levels and their relationship with total triiodothyronine (TT₃), total thyroxine (TT₄) and free thyroxine (fT₄) in healthy and hypothyroid dogs.

Methods: The study involved 77 dogs, with 67 healthy and 10 hypothyroid. The serum concentration of cTSH was measured using a canine-specific TSH-ELISA kit, while TT₃, TT₄ and fT₄ were measured using RIA kits.

Result: The Mean serum concentration of cTSH, TT₃, TT₄ and fT₄ in healthy dogs was 2.03±0.01 µIU/ml, 0.85±0.04 nmol/l, 20.81±0.79 nmol/l and 12.59±0.35 pmol/l, respectively. Mean cTSH, TT₃, TT₄ and fT₄ concentration of hypothyroid dogs was found to be 2.16±0.03 µIU/ml, 0.93±0.07 nmol/l, 9.01±0.69 nmol/l and 5.87±0.82 pmol/l, respectively. Interquartile range (i.e. 25th to 75th percentile) for thyroid hormone (cTSH, TT₃, TT₄ and fT₄) in healthy dogs (n = 67) were 1.96-2.11 µIU/ml, 0.64-0.96 nmol/l, 16.73-25.00 nmol/l and 11.00-14.40 pmol/l, respectively and for hypothyroid dogs (n = 10) were 2.09-2.22 µIU/ml, 0.73-1.12 nmol/l, 7.50-10.29 nmol/l and 4.35-7.45 pmol/l, respectively. A significant difference (P<0.05) was recorded in healthy and hypothyroid dogs in cTSH, TT₄ and fT₄.

Key words: Canine thyroid stimulating hormone (cTSH), Enzyme-linked immunosorbent assay (ELISA), Hypothyroidism, Radioimmunoassay (RIA).

INTRODUCTION

Endocrinology studies hormones produced by endocrine glands like pituitary, thyroid, parathyroid, pancreas, adrenal and gonads, which are crucial for metabolic regulation and neural and skeletal development in fetal life; and their deficiency or surplus impacts various body systems. Hypothyroidism, a condition where the thyroid gland's ability to produce thyroid hormones decreases, can be primary, secondary, or tertiary in dogs. To assess thyroid function, serum levels of total triiodothyronine (TT₃), total thyroxine (TT₄), free thyroxine (fT₄) and thyroid stimulating hormone (TSH) must be measured. The hormonal measurement is crucial in veterinary clinical practice for identifying hypothyroidism and distinguishing it from non-thyroidal illnesses (NTIs).

Radioimmunoassay (RIA) is a sensitive *in vitro* method for assessing antigens like hormones, minerals and vitamins from biological fluids. ¹²⁵I is a commonly used radioisotope due to its long half-life (t_{1/2} = 60 days), offering a convenient and precise assay for large samples. The use of RIA in measuring hormones for veterinary clinical use has been extensively studied in India (Dadke, 2018; Roopali *et al.*, 2020, Galdhar *et al.*, 2021, Jayabhaye *et al.*, 2021, Salutgi *et al.*, 2023, Galdhar *et al.*, 2024; and Galdhar *et al.*, 2024). Canine thyroid stimulating hormone (cTSH) ELISA is a widely used diagnostic marker for canine thyroid dysfunction in veterinary clinical practice due to the unavailability of canine-specific RIA kits in India. The study aims to assess canine thyroid-stimulating hormone (cTSH) levels and their correlation with total triiodothyronine (TT₃), total thyroxine (TT₄) and free thyroxine (fT₄) in healthy and hypothyroid dogs.

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MATERIALS AND METHODS

Statutory approval

The present study was initiated after permission from the institutional ethics committee for veterinary clinical research (IEC-VCR) and institutional bio-safety committee (IBSC) of Mumbai veterinary college, Maharashtra animal and fishery sciences university (MAFSU), Mumbai-India.

Selection of healthy and hypothyroid dogs

A total of 67 clinically healthy dogs from different breeds were ethically enrolled to measure the thyroid profile from the clinical setup of Mumbai Veterinary College, Parel-Mumbai. They consisted of 43 males and 24 females, with

a mean age of 5.63 ± 0.39 years and mean body weight of 19.33 ± 0.59 kgs respectively. Health checks, anamnesis and laboratory tests (complete blood count, liver function test, kidney function test) were conducted to assess health, ensuring normal limits were met.

A total of 10 dogs of various breeds, suspected of clinical hypothyroid at the clinical setup of Mumbai Veterinary College, Parel-Mumbai, were ethically enrolled to measure thyroid profile. They consisted of 6 males and 4 females, with a mean age of 6.48 ± 1.01 years and mean body weight of 24.08 ± 3.12 kgs respectively.

Hormonal analysis

Blood samples were collected from each dog (cephalic vein or saphenous vein). Serum samples for thyroid profile estimation were stored at -20°C until the time of analysis. Thyroid hormones (TT₃, TT₄ and fT₄) were estimated using commercial RIA kits for human purposes manufactured by the Board of Radiation and Isotope Technology (BRIT), Vashi, Mumbai (India). The analysis was conducted at the Radio Isotope Laboratory, Mumbai Veterinary College, Mumbai (India). Thyroid hormones were assayed in each sample giving paired observation. TT₃ and TT₄ were measured as per the standard procedure outlined by the manufacturers. fT₄ was estimated as per the modifications recommended (Dadke, 2018). Quality control parameters viz. magnitude of control samples and recovery percentage were studied to validate every assay. cTSH was analyzed using a commercially available Canine Thyroid Stimulating Hormone ELISA kit designed for the canine purpose, supplied by Cusabio Biotech Co., Ltd. (Wuhan Huamei biotech-China).

Statistical analysis

The mean and standard error for each parameter of collected data was calculated and analyzed statistically for comparison as per the methods suggested by Snedecor

and Cochran (2004). A nonparametric statistical analysis was also used for the analysis of the results.

RESULTS AND DISCUSSION

The serum concentration of cTSH was measured using a canine-specific TSH ELISA kit, while TT₃, TT₄ and fT₄ concentrations were estimated using an RIA kit. Both assays were conducted on serum samples of healthy (n=67) and hypothyroid (n=10) dogs. All recommended quality control parameters, including the magnitude of control samples provided with the kits and percent recovery, were within the prescribed limits. The standard curve of the assays was plotted and the thyroid hormone concentrations were interpolated from the standard curve.

The mean, interquartile range (i.e., 25th to 75th percentile) and median of thyroid hormones in healthy dogs and hypothyroid dogs are presented in Fig 1 to 4. The mean serum concentration of cTSH, TT₃, TT₄ and fT₄ in healthy dogs was 2.03 ± 0.01 $\mu\text{IU/ml}$, 0.85 ± 0.04 nmol/l, 20.81 ± 0.79 nmol/l and 12.59 ± 0.35 pmol/l respectively. Mean cTSH, TT₃, TT₄ and fT₄ concentration of hypothyroid dogs was found to be 2.16 ± 0.03 $\mu\text{IU/ml}$, 0.93 ± 0.07 nmol/l, 9.01 ± 0.69 nmol/l and 5.87 ± 0.82 pmol/l respectively. A significant difference ($P < 0.05$) was recorded in cTSH, TT₄ and fT₄ of healthy and hypothyroid dogs.

Box and whisker graphs (Fig 1 to 4) were plotted for comparison of thyroid hormone concentrations between healthy and hypothyroid dogs. The 'T' bar represents the data which is equal to the range. In the present study, the observed range in healthy dogs for cTSH, TT₃, TT₄ and fT₄ was (1.79 to 2.19 $\mu\text{IU/ml}$), (0.37 to 1.23 nmol/l), (5.14 to 37.00 nmol/l), (6.10 to 19.00 pmol/l) and for hypothyroid dog was (2.08 to 2.26 $\mu\text{IU/ml}$), (0.61 to 1.32 nmol/l), (6.44 to 14.16 nmol/l), (2.10 to 10.00 pmol/l) respectively.

The box represents the middle half of the data. The present study reports the interquartile range (25th to 75th

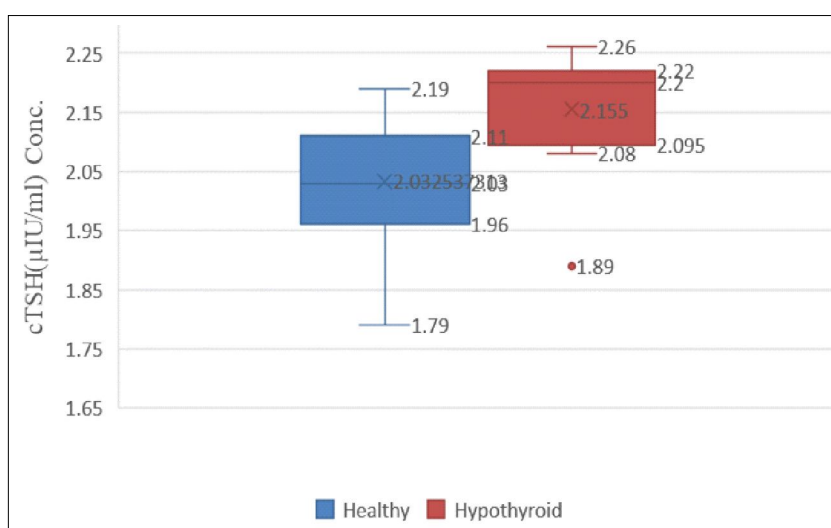


Fig 1: Comparison of cTSH $\mu\text{IU/ml}$ between healthy and hypothyroid dogs.

percentile) for cTSH, TT₃, TT₄ and fT₄, as 1.96 to 2.11 µIU/ml, 0.64 to 0.96 nmol/l, 16.73 to 25.00 nmol/l, 11.00 to 14.40 pmol/l, for healthy dogs; and (2.09 to 2.22 µIU/ml), (0.73 to 1.12 nmol/l), (7.59 to 10.29 nmol/l) and (4.35 to 7.45 pmol/l) for hypothyroid dogs, respectively. The Horizontal bar in the box is the median of the data. In the present study, the observed median for cTSH, TT₃, TT₄ and fT₄ in healthy dogs was 2.03 µIU/ml, 0.86 nmol/l, 19.30 nmol/l and 13 pmol/l and for hypothyroid dogs was 2.20 µIU/ml, 0.92 nmol/l, 8.62 nmol/l and 5.5 pmol/l respectively. The mean, interquartile range (i.e. 25th to 75th percentile) and median values of circulating thyroid hormone (TT₃, TT₄ and fT₄) levels stated in the present study for healthy dogs are in agreement with the reference range established by Galdhar *et al.* (2024).

The present study reports, a statistically significant ($p < 0.05$) difference in the mean concentrations of cTSH, TT₄ and fT₄ of healthy and hypothyroid dogs, whereas a non-significant difference ($p < 0.05$) was found in the mean concentration of TT₃ value of healthy and hypothyroid dogs. This finding was in correlation with the observation reported by Panciera (1994), who explained that under stimulation by TSH, the thyroid gland preferentially secretes T₃ and much of the circulating T₃ is derived from peripheral deiodination of T₄.

In the case of cTSH healthy dogs ($n=67$), the percentile range (25th to 75th percentile) was 1.96 to 2.11 µIU/ml while in the case of hypothyroid dogs ($n=10$), the percentile range (25th to 75th percentile) was 2.09 to 2.22. Out of 10 hypothyroid dogs, 7 (70%) dogs had an orientation range

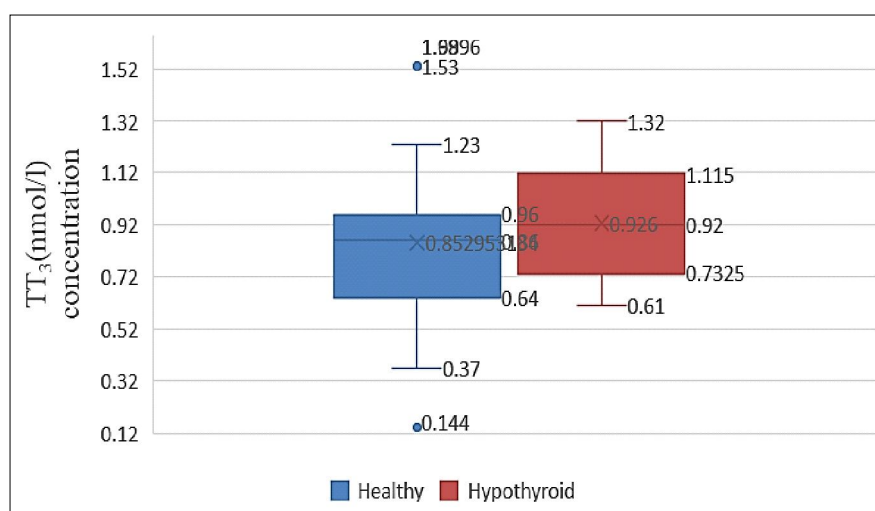


Fig 2: Comparison of TT₃ (nmol/l) between healthy and hypothyroid dogs.

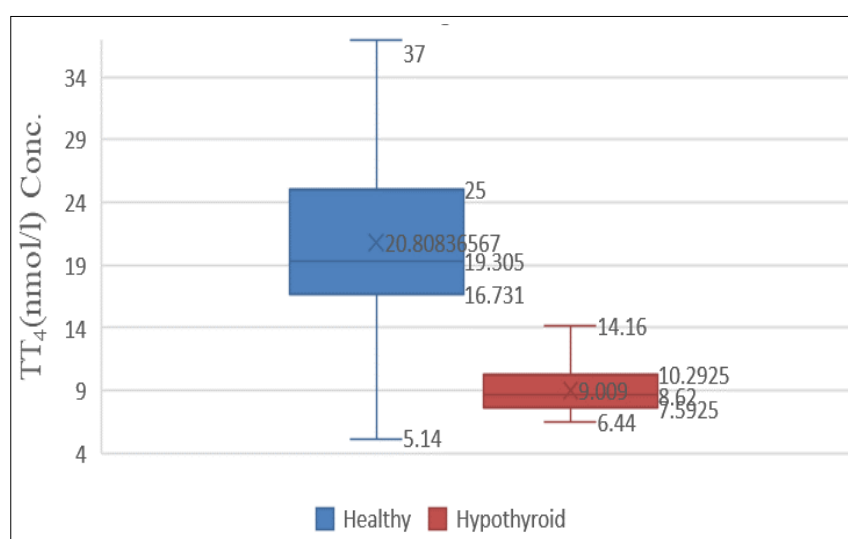


Fig 3: Comparison of TT₄ (nmol/l) between healthy and hypothyroid dogs.

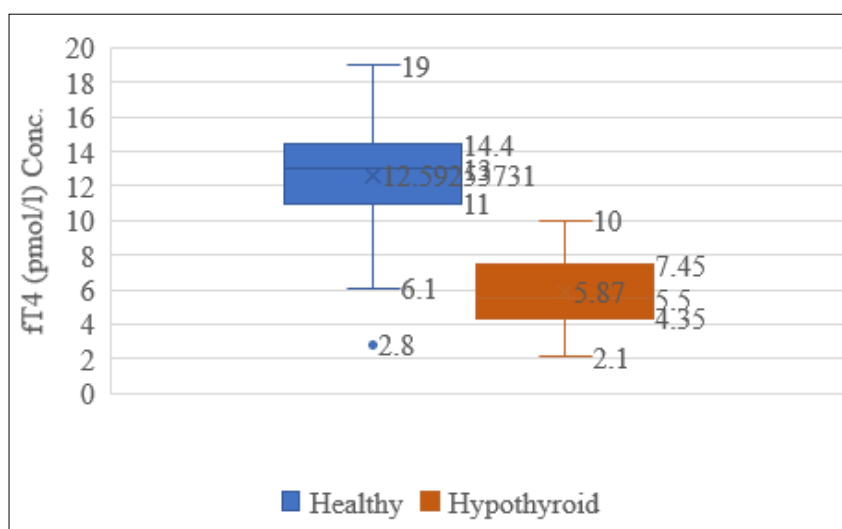


Fig 4: Comparison of FT₄ (pmol/l) between healthy and hypothyroid dogs.

above that of the reference range of healthy dogs and 3 (30%) dogs had a reference range the same as that of the reference range of healthy dogs. This suggests that many dogs in true hypothyroidism did not have elevated cTSH levels, as one would expect. These findings support the observations reported by Baker (1997) and Dixon (2001). According to Dixon (2001), the combination of reduced TT₄ and increased canine TSH values is highly specific for hypothyroidism. However, some naturally occurring cases of canine hypothyroidism had cTSH concentration within the reference range due to later diagnosis or prolonged periods of low thyroid hormones. This might result from disruption of the feedback pathway by down-regulation or exhaustion of TSH production by pituitary thyrotropes.

CONCLUSION

The present study reports the status of thyroid hormone levels (cTSH, TT₃, TT₄ and fT₄) in healthy and hypothyroid dogs. The study found a significant difference in cTSH, TT₄ and fT₄ concentrations between healthy and hypothyroid dogs, while TT₃ concentration showed the non-significant difference. In hypothyroid dogs, 70% had an orientation range above the healthy reference range for cTSH, while 30% had a reference range identical to the healthy range. This suggested that many dogs with true hypothyroidism did not exhibit elevated cTSH levels.

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Conflict of interest

The authors declare that there is no conflict of interest.

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