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CLINICO-BIOCHEMICAL, ULTRASONOGRAPHIC AND HISTOPATHOLOGICAL CHANGES IN EXPERIMENTALLY-INDUCED HYPOTHYROIDISM IN DOGS.

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ABSTRACT

This study was carried out on 25 Mongrel dogs with age ranged from 2 to 4 years, and body weight ranged between 11-23 kg. Hypothyroidism was induced in dogs by drugs or surgical methods. The drug-induced hypothyroidism (n=5) was induced by oral dosing of Sulphamethoxazole-Trimethoprim combination (~30 mg/kg. BW of sulphamethoxazole). Five dogs were dosed with saline as control. Two surgical methods were used to induce hypothyroidism. In the first method (thyroidectomy-induced hypothyroidism), both right and left thyroid glands were surgically removed (n=5). In the second method (ligation-induced hypothyroisim), hypothyroidism was induced by surgical ligation of thyroid arterial blood supply (n=5). Five dogs were used as sham control for surgical methods. The most common clinical signs recorded in the experimental three groups were lethargy, weight gain, alopecia, and other dermatological changes. The biochemical changes included significant reduction of triiodothyroxine (TT3) and tetraiodothyroxine (TT4), increased thyroid stimulating hormone (TSH), hypercholesterolemia, hypertriglyceridemia, elevated liver enzymes (AST, ALT) and kidney function (urea and creatinine), hypocalcemia, hyperphosphatemia and hyponateremia. Ultrasonographic changes of thyroid gland in drug-induced hypothyroidism showed increase in both total and relative thyroid volume with decreased relative echogenicity. However, in ligation-induced hypothyroidism the total and relative thyroid volume and the relative echogenicity were decreased. Histopathological changes of thyroid gland (drug-induced hypothyroidism) revealed hyperplasia of glandular epithelium with papillary projection into the lumen and lymphocytic cellular infiltration, and desquamation of lining epithelium. In ligation-induced hypothyroidism, thyroid follicles were atrophied with hypertrophy of lining epithelium. These results confirmed the significant role of thyroid gland in maintaining the body metabolic equilibrium and the integrity of different organs, such as liver, kidney and skin. The early changes in TT3 and TT4 levels are considered as early makers of thyroid dysfunction in dogs.

KEY WORDS: Hypothyroidism, Ligation, Thyroidectomy, Thyroxine, Ultrasound

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INTRODUCTION

Hypothyroidism is the most common endocrinopathy of dog occurs due to impaired production and secretion of the thyroid hormones, which results in a decreased metabolic rate **[1, 13].** Hypothyroidism may occur owing to dysfunction of any part of the hypothalamic-pituitary-thyroid axis. Most cases of acquired canine hypothyroidism are caused by primary hypothyroidism due to lymphocytic thyroiditis or idiopathic thyroid atrophy **[10].** Primary hypothyroidism is the most common disorder in dogs results from problems within the thyroid gland, usually destruction of thyroid gland. The two most common causes of this disorder are lymphocytic thyroiditis, and idiopathic atrophy of thyroid gland. There are other causes of primary hypothyroidism such as neoplastic destruction, and iatrogenic causes as; surgical removal of thyroid gland, antithyroid medication, radioactive iodine treatment, and drugs as sulfamethoxazole **[14, 24, 38].** Secondary hypothyroidism is a pituitary-dependent hypothyroidism which resulted from the insufficient secretion of TSH by the pituitary gland **(10, 20, 29).** Tertiary hypothyroidism is theoretically caused by decreased hypothalamic thyrotropin releasing hormone secretion and less common in dogs **(24, 31, 35).** Congenital hypothyroidism is rare and most often results

in early postnatal death **(15, 27).** The Clinical signs of hypothyroidism including weight gain, thin hair coat, alopecia, seborrhea, weakness, and lethargy **(26).** Hypohyroidism is usually associated with biochemical changes such as hypercholesterolemia, hyperlipidema and hypertriglyceridaemia **(28, 32).** Tests currently available for diagnosing thyroid disease include; total thyroxine (TT4), total triiodothyronine (TT3), free T4 (fT4), endogenous canine thyroid stimulating hormone (cTSH), and TSH response test **(16, 21).** Ultrasonography of the thyroid gland has also been used as a diagnostic aid in the diagnosis of primary hypothyroidism **(30, 39, 42).** Histologically, there is multifocal or diffuse infilteration of the thyroid gland by lymphocytes, plasma cells, and macrophage, the remaining follicles are small, and lymphocytes, macrophages, and degenerate follicular cells may be found within vaculated colloid **(1, 10, 19, 36).**

Since very few studies were conducted on experimental hypothyroidism in pet animals, this work aim to investigate the clinical picture and determine the biochemical, ultrasonographic and histopathological changes occurring in dogs with experimentally-induced hypothyroidism

2.MATERIALS AND METHODS

2.1.Dogs

The present study was carried out on twenty five apparently healthy Mongrel dogs of an age 2-4 years and body weight 11-23 kg, during the period between February and October 2011. All dogs were dewormed with systemic anthelmentic (Ivomac super®, 1ml/50kg Bwt.), and were left for 15 days for acclimatization before the beginning of the experiment.

2.2.Experimental design

The 25 dogs were randomly divided into two groups as following:

Group I: included two subgroups:

Subgroup A: included five dogs that were given sulphamethoxazole- trimethoprim (SEPTAZOLE® suspension, Alexandria Co. For Pharmaceuticals & Chemical Industries) with a high dose of 7.5 ml / 10 kg. BW (~30 mg/kg. BW of sulphamethoxazole) PO, twice daily for six weeks **(41).**

Subgroup B: included five dogs that were given orally 7.5 ml/10 kg normal saline twice daily for 6 weeks as a control group.

Group II: included three subgroups as following:

Subgroup A: included five dogs that were subjected to experimental induction of hypothyroidism by surgical removal of thyroid gland as previously conducted **(12).**

Subgroup B: included five dogs that were subjected to experimental induction of hypothyroidism by surgical ligation of the arterial blood supply of the thyroid gland.

Subgroup C: included five dogs that were used as a control for the surgical induction of hypothyroidism (sham-operated dogs).

2.3.Clinical examination

The dogs of all groups were subjected to clinical examination and all clinical signs were recorded as previously described (18).

2.4.Biochemical analysis

Biochemical analysi was conducted weekly for 6 weeks started from the 2nd week in drug-induced hypothyroidism and for 4 weeks in surgical-induced hypothyroidism. Special kits (Bio-analytics Company) were used for determination of the levels of cholesterol, triglyceride, glucose, ALT, AST, creatinine, urea, Ca, P, Na, K, Cl. Special kits were used for determination of thyroid hormones. The serum total thyroxine concentration was measured by using a commercial magnetic separation radioimmunoassay (RIA) kit (Magic T4: Chiron Diagnostics, Halsted, Essex) previously validated for use in the dog **(17).**

Serum TT3 concentration was measured by using a commercial enzyme immunoassay kit for canine tri-iodothyronine (TT3) (ELISA Kit for Canine Tri-iodothyronine, T3) as previously described **(23).**

Serum canine TSH concentrations were measured by using a commercial immunoradiometric assay (Coat-A-Count canine TSH IRMA; Diagnostic product crop., Los Angeles, CA, USA) as previously described **(8).**

2.5.Ultrasonographic examination

Ultrasonographic examination was conducted by using Pie Medical Ultrasound apparatus with the frequency set at 8MHz. Ultrosonography was carried out weekly for 6 weeks started from the 2nd week in drug-induced hypothyroidism and for 4 weeks in surgical-induced hypothyroidism. Dogs were scanned while in dorsal recumbency by ultrasonography starting in the midline just caudal to the larynx, followed by a slow gliding motion of the probe caudally, using a longitudinal image of the common carotid artery and the trachea as landmarks in longitudinal section, then rotate the probe with 90° to obtain transverse section **(30)**. By the ultrasonography, the thyroid volume was described by the maximum width, the maximum height, and the maximum length of each lobe and were used to calculate the volume of each lobe as previously described **[30]**. The total thyroid volume was given by the sum of right and left thyroid lobe volumes. Thyroid volume was divided by the metabolic weight ($Bw^{0.75}$) of the dog to obtain the relative volume of the thyroid gland. To determine the echogenicity, the main density (MD) of thyroid lobes and main density (MD) of the cross sectional area of the adjacent sternothyroid muscle were measured by means of image analyzing software. Relative echogenicity of the thyroid gland was calculated by means of the formula, relative echogenicity =MD of thyroid gland/MD of sternothyroid muscle **(30).**

2.6.Histopathological examination

The samples for histopathological examination were collected from thyroid gland immediately after sacrifice, fixed in neutral buffered formalin 10%, dehydrated, and embedded in paraffin. The paraffin blocks were sectioned at 5-7µ thickness, and were stained with H&E stain as previously described **(40).**

2.7.Statistical Analysis

Data obtained were statistically analyzed by using student's T-test to compare induced groups to their control. The results were demonstrated as means ± standard error (SE). Results from induced groups were considered significantly different from control when $p \leq 0.05$.

3.RESULTS

3.1.The clinical signs:

The common clinical signs appeared on the three groups of the induced hypothyroidism were lethargy, obesity (Fig. 1), and dermatological changes in the form of alopecia, poor hair growth, and nodules-like form in the skin (Fig. 2).

3.2.The biochemical analysis:

The biochemical analysis of drug-induced hypothyroidism showed significant ($p \leq 0.05$) decrease in TT3 and TT4 with significant ($p \leq 0.05$) increase in TSH**.** There was a significant ($p \leq 0.05$) increase in glucose, total cholesterol, and also triglycerides. There were elevated liver enzymes (AST and ALT), increase in urea and creatinine levels**.** Serum electrolyte showed significant ($p \leq 0.05$) decrease in sodium levels while there was a significant ($p \leq 0.05$) increase in calcium, phosphorus, chloride, and non significant increase in potassium compared with its control group **(Table 1).** The biochemical analysis of thyroidectomy-induced hypothyroid showed gradual reduction in TT3, and TT4 with gradual increase in TSH with significant changes from control ($p \leq 0.05$)**.** There was a significant ($p \leq 0.05$) decrease in glucose, and significant ($p \leq 0.05$) increase in total cholesterol, triglycerides**,** liver enzymes (AST, ALT), and significant ($p \leq 0.05$) increase in urea with gradually increase in creatinine**.** Serum electrolytes showed significant ($p \leq 0.05$) increase in calcium phosphorus, chloride, and potassium with significant reduction of sodium compared with its control sham-operated group**.** The biochemical analysis of ligation-induced hypothyroidism showed significant ($p \leq 0.05$) decrease of both TT3, and TT4 and significant ($p \leq 0.05$) increase TSH**.** There was a significant ($p \leq 0.05$) decrease in glucose level, and significant ($p \leq 0.05$) increase in total cholesterol, triglycerides**,** liver enzymes (AST, ALT), and kidney function (urea and creatinine)**.** Serum electrolytes showed

significant ($p \leq 0.05$) increase in calcium phosphorus, chloride, and potassium with significant reduction of sodium compared with its control sham-operated group **(Table 3).**

3.3.Ultrasonographic examination:

US of thyroid gland of drug-induced hypothyroidism showed significant ($p \leq 0.05$) increase in both total thyroid volume and relative thyroid volume with significant ($p \leq 0.05$) decrease in relative echogenicity compared with control group **(Table 2, Figures 3- 6).**

US of thyroid gland of ligation-induced hypothyroidism showed significant ($p \leq 0.05$) decrease in total and relative thyroid volume and also the relative echogenicity **(Table 2, Fig. 7-10).**

3.4.Histopathological examination:

The histopathological changes of thyroid gland of drug-induced hypothyroidism **(Fig. 13)** showed hyperplasia of glandular epithelium with papillary projection into the lumen and lymphocytic cellular infiltration. Some syncytial epithelial cells are found in some glands with desquamation of lining epithelium and necrotic cellular debris in their lumens compared with normal microscopic appearance of the thyroid gland of control group **(Fig. 11).**

The histopathological examination of thyroid gland of ligation-induced hypothyroidism (Fig. 14) showed some thyroid follicles showed cystic dilatation with retained secretion in their lumen and flattened lining epithelium, and the adjacent thyroid follicles were atrophied with hypertrophied lining epithelium compared with sham-operated group **(Fig. 12).**

4.DISCUSSION

The clinical signs appeared on the three groups of the induced hypothyroidism were lethargy, weight gain, and dermatological changes included alopecia, poor hair growth, and nodules like form in the skin. These findings were similar to previous studies **(4, 26, 27).**

The significant decrease in TT3 and TT4 in the three groups indicates the successful induction of hypothyroidism in dogs by any of the three methods (drug induced, thyroidectomy and ligation of thyroid arterial blood supply). In reponse to decreased thyroid hormone, the TSH was significantly increased. It is well known that there is a negative feed back regulation between thyroid hormones and TSH secreated by posterior pituitary **(6, 24)**. Therefore, reduction of thyroid hormones stimulates the secretion of TSH. The changes in thyroid hormones from the second week indicate the they can be used as early markers for thyroid dysfunction in dogs.

The significant increase in glucose level in drug-induced hypothyroidism by potentiated sulfonamide drugs was consistent with other study **(2).** The increase in blood glucose level might by be attributed to the resistant antagonistic action of potentiated sulfonamides on insulin receptors (2). However, the significant decrease in glucose level in the 2^{nd}, 3^{rd} and the 4^{th} week of thyroidectomy-induced and ligation induced groups could be attributed to the role of thyroid hormones in the mobilization and utilization of glycogen stores and increase protein synthesis and also glucose metabolism and gluconeogenesis **(Plumb, 1999).** The significant increase of cholesterol and triglycerides in the three groups of induction might be attributed to decrease of thyroid hormones that lead to decrease synthesis and degradation of cholesterol resulting in increase in cholesterol level in the serum **(9, 11, 24, 27, 28).** Elevated liver enzymes (AST, ALT) could be attributed to the impairement of the liver function which associated with hypothyroidism **(22, 25, 34, 37).** Electrolyte analysis showed significant increase in calcium and phosphorus in induced-hypothyroidism groups compared to control. These changes might be attributed to impairement of calcitonin production from thyroid hormone **(5),** and metabolic derangements induced by thyroid hormone deficiency that altered calcium homeostasis **(33).** The significant decrease in sodium with

Table 1 Changes in thyroid hormones, metabolites and electrolytes in drug-induced hypothyroidism compared with control.

Parameters	2nd week		3rd week		4th week		5th week		6th week	
	Control	Drug-induced	Control	Drug-induced	Control	Drug-induced	Control	Drug-induced	Control	Drug-induced
TT3 (nmol/L)	1.67±0.16	0.98±0.05*	1.63±0.17	1.02±0.06*	1.47±0.17	0.68±0.03*	1.54±0.14	0.48±0.03*	1.65±0.15	0.32±0.03*
TT4 (nmol/L)	10.72±0.58	11 ±0.68	10.82±0.78	9.39±0.49	10.02±0.66	6.45±0.86*	10.36±0.67	6.14±1.22*	10.73±0.52	4.34±0.51*
TSH (mU/L	2.61±0.2	5.95±1.04*	3.02±0.11	5.8 ±1*	3.24±0.08	14.3±3.57*	2.95±0.03	20.7±1.99*	2.82±0.05	28.9±0.63*
Glucose (mg/dl)	81±1.33	103±8*	76.7±1.1	96±6*	82.3±1.6	122±2.67*	79.3±1.1	120±7.33*	83.3±1.6	123.7±4.4*
Cholesterol (mg/dl)	204±7.33	342±11.3*	213 ±6	383.3±10.4*	209.3±4.9	393.7±14.2*	210 ±5.3	386±15.3*	214±4.7	412±12.7*
Triglyceride (mg/dl)	62±2	115±16 *	66.7±1.8	132.7±26.2*	65±1.33	137.3±13.1*	65.7±1.6	127.7±10.2*	71±1.33	147±8.7*
AST (mg/dl)	31±1.33	34.7±1.1	34.3±1.8	39.3±1.1*	33±1.33	74.7±2.4*	35.3±1.1	66.7±5.6*	39.3±1.1	49±1.33*
ALT (mg/dl)	26.7±3.78	66.7±6.4 *	34.3±4.22	82.3±14.2*	31±2.67	99.7±8.4*	34.3±2.67	110.7±8.4*	39±3.33	125.7±5.1*
Urea (mg/dl)	30.7±1.1	42±0.67*	32 ±0.67	40±0.67**	33.3±0.89	40.3±1.1*	30.3±0.44	41±2*	34 ±0.67	48±0.67*
Creatinine (mg/dl)	0.68±0.02	0.92±0.03*	0.71±0.03	0.94 ±0.11	0.76 ±0.03	1.04±0.18	0.74±0.04	1.02±0.06*	0.69±0.02	1.17±0.04*
Calcium (mg/dl)	8.98 ±0.09	9.72 ±0.54	8.77 ±0.11	9.12 ±0.72	8.9 ±0.14	10.06 ±0.7 *	9.1 ±0.09	12.68 ±0.49 *	9.28±0.1	13.84
Phosphorus (mg/dl)	3.35 ±0.3	4.12 ±0.18 *	3.71 ±0.16	4±0.14	3.62 ±0.19	4.18 ±0.09 *	3.31 ±0.19	5.7 ±0.13 *	2.97 ±0.09	6.47±0.37*
Potassium	4.27 ±0.33	4.31 ±0.06	4.39 ±0.32	4.55 ±0.05	4.2±0.3	4.89 ±0.32	4.07 ±0.3	4.78 ±0.25	3.96 ±0.24	4.38 ±0.18
Sodium (mmol/L)	142.7 ±2.9	149.7 ±1.1 *	128.7 ±2.4	154.7 ±1.1 *	142.7±4.4	160.3 ±8.2	151.3 ±2.9	137.7 ±1.8 *	160.3 ±1.6	155 ±4.67
Chloride (mmol/L)	87.3 ±7.1	121.3 ±2.4 *	83.7 ±4.9	132.3 ±7.6 *	88±4.7	136.3 ±2.2 *	90.7 ±3.8	126.3 ±22.2	89.7 ±3.8	136 ±16.7 *

Data are presented as mean ±S.E.. * Means significantly different from control at P≤0.05.

Table 2 Ultrasonographic changes of thyroid gland in drug-induced hypothyroidism compared with control.

Parametrs	2nd week		3rd week		4th week		5th week		6th week	
	Control	Drug-induced	Control	Drug-induced	Control	Drug-induced	Control	Drug-induced	Control	Drug-induced
Total volume (ml)	2.04 ±0.24	2.52 ±0.21	2.12 ±0.17	3.2 ±0.45 *	2.39 ±0.42	4.15 ±0.52 *	2.25 ±0.18	3.56 ±0.45 *	2.38 ±0.2	3 ±0.34
Relative vol. (ml/ $kg^{0.75}$)	0.27 ±0.02	0.35 ±0.02 *	0.28 ±0.02	0.43 ±0.03 *	0.31 ±0.06	0.52 ±0.05 *	0.29 ±0.03	0.42 ±0.05 *	0.3 ±0.03	0.34±0.05
Relative echog. (%)	1.11 ±0.45	0.68 ±0.02 *	1.08 ±0.15	0.49 ±0.04 *	0.99 ±0.25	0.44 ±0.02 *	1.26 ±0.25	0.37 ±0.03 *	1.23 ±0.13	0.24±0.04 *

Data are presented as mean (±S.E.). * Means significantly different from control at P≤0.05.

Table 3 Changes in thyroid hormones, metabolites and electrolytes in drug-induced hypothyroidism compared with sham-operated group

Parameters	1st week			2nd week			3rd week			4th week		
	Sham	TIH	LIH	Sham	TIH	LIH	Sham	TIH	LIH	Sham	TIH	LIH
TT3 (nmol/L)	1.59±0.08	0.69 ±0.12	1±0.12*	1.38±0.05	0.41±0.08*	0.7±0.08*	1.05±0.04	0.42±0.06*	0.37±0.03*	1.76±0.03	0.13±0.04*	0.23±0.04*
TT4 (nmol/L)	9.77 ±0.36	8.16±0.44*	8.68 ±0.68	8.47 ±0.36	5.59 * ±0.56	6.07 ±1.1	8.18 ±0.38	5.83±0.56*	3.68±0.45*	8.61±0.37	4.09±0.53*	2.93±0.32*
TSH (mU/L	4.06 ±0.64	5.48 ±0.49	5.57 ±0.93	3.47 ±0.64	12.1±0.55*	6.55 ±1.43	3.29 ±0.65	37.1±1.78*	18.5±0.36*	3.7±0.66	46.6±1.38*	30.5±2.02*
Glucose (mg/dl)	100 ±6.7	90 ±8	109 ±9.3	105.3 ±6.4	71.7 ±6.4 *	110 ±8	110.3 ±6.4	67 ±6.67*	102±8	99±6.9	59±4*	76.6±5.6*
Cholesterol (mg/dl)	209.7±19.6	205 ±4.7	216.7±10.4	217.7±17.1	193.3 ±3.6	216.3±15.78	228.7±14.9	221.7 ±2.2	233.3±18.4	204±6	235.3±9.6 *	254±18.7*
Triglyceride (mg/dl)	94.3 ±6.4	109.3 ±2.9 *	83.7 ±4.2	96 ±1.3	121 ±4 *	78±2 *	88.7 ±11.8	137.7 ±8.4 *	84.7±1.8	87 ±4	145±9.3 *	121.7±2.4*
AST (mg/dl)	51.3 ±1.1	66 ±6 *	98.7±2.4*	49 ±0.67	70 ±2.7*	83 ±4 *	42.3 ±0.44	76.7 ±3.1*	84.7±3.8*	40.3±0.44	82.3±2.2*	89±2.7*
ALT (mg/dl)	68 ±3.3	83 ±4.67	99 ±12.7*	79.3 ±1.8	85.3 ±4.2	95.3 ±9.6	82.67±2.89	88.3 ±4.9	91.7±4.9	78.7±2.4	92±3.33*	97 ±4 *
Urea (mg/dl)	39.7 ±1.1	43 ±0.67*	37.7 ±2.2	42.7 ±1.8	49.3 ±1.1*	38.7 ±4.22	45 ±1.8	51.3 ±1.1*	47.3 ±2.2	47.7 ±1.1	53.7 ±1.1*	47.7±2.9
Creatinine (mg/dl)	0.79 ±0.06	0.74 ±0.02	0.95 ±0.06	0.87 ±0.05	0.88 ±0.05	0.98 ±0.05	0.91 ±0.04	1.16 ±0.05 *	1.02 ±0.05	0.88 ±0.02	1.19 ±0.05 *	1.11 ±0.06 *
Calcium (mg/dl)	8.88 ±0.09	10.07±0.24*	8.46 ±0.99	8.18 ±0.13	4.67 ±0.5*	6.89 ±0.78	8.43 ±0.19	4.69±0.38*	4.12±0.17*	9.16±0.11	4.12±0.16*	3.72±0.14*
Phosphorus (mg/dl)	3.26 ±0.04	4.02 ±0.16*	4.6±0.13*	3.27 ±0.06	7.6 ±0.6*	3.35 ±0.51	3.09 ±0.02	7.34 ±0.6 *	7.17±0.5 *	2.73±0.07	8.84±1.04*	8.5±0.4*
Potassium	3.37 ±0.25	3.68 ±0.11	4.26 ±0.38	4.53 ±0.25	5.02 ±0.09	4.57 ±0.33	4.04 ±0.13	5.72±0.07*	4.9 ±0.25 *	5.08±0.1	5.85±0.07 *	5.86±0.1*
Sodium (mmol/L)	152 ±4	152 ±3.33	144.3 ±4.2	158.7 ±3.6	157 ±3.33	137 ±1.3*	154 ±3.3	137.7 ±3.1 *	149±1.3	139±1.3	141.7±3.1	137±4.7
Chloride (mmol/L)	83.7 ±2.2	98.7 ±2.9 *	110.3±3.8*	88.7 ±1.6	106 ±3.3 *	128 ±2 *	80.7 ±1.1	98 ±0.7 *	139.3±2.9*	100.7±1.8	131 ±2 *	135.3±1.1*

Data are presented as mean ± S.E.. * Means significantly different from control at P≤0.05. TIH: thyroidectomy-induced hypothyroidism, LIH: ligation-induced hypothyroidism.

Table 4 Ultrasonography of thyroid gland of ligation group compared with sham-operated group.

Parameters	1st week		2nd week		3rd week		4th week	
	Sham	Ligation	Sham	Ligation	Sham	Ligation	Sham	Ligation
Total volume (ml)	2.66 ±0.23	1.21 ±0.26 *	2.82 ±0.08	0.94±0.22 *	2.14 ±0.08	0.75 ±0.14 *	2.51±0.1	0.62 ±0.13 *
Relative vol. (ml/ $kg^{0.75}$)	0.3±0.02	0.14 ±0.04 *	0.31 ±0.01	0.11 ±0.03 *	0.23 ±0.004	0.08 ±0.02 *	0.27±0.004	0.07±0.02 *
Relative echog. (%)	0.8 ±0.27	0.49±0.02	0.69 ±0.08	0.35 ±0.01 *	0.94 ±0.11	0.3±0.02 *	0.79±0.02	0.18 ±0.05 *

Data are presented as mean ±S.E.. * Means significantly different from control at P≤0.05. TIH: thyroidectomy-indecued hypothyroidism LIH: ligation-induced hypothyroidism.

Fig. 1. Obesity in a dog with drug-induced hypothyroidism

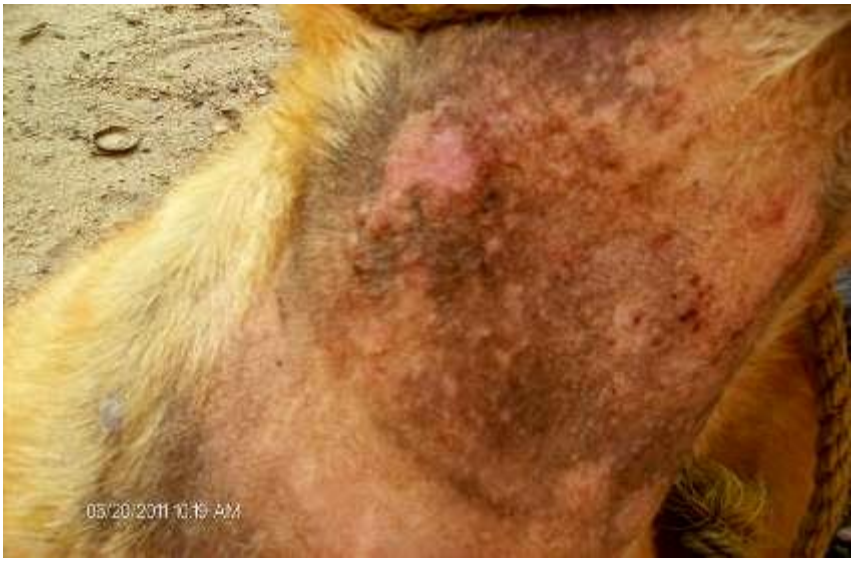

Fig. 2. Skin nodules and alopecia at the neck in drug-induced hypothyroid dog

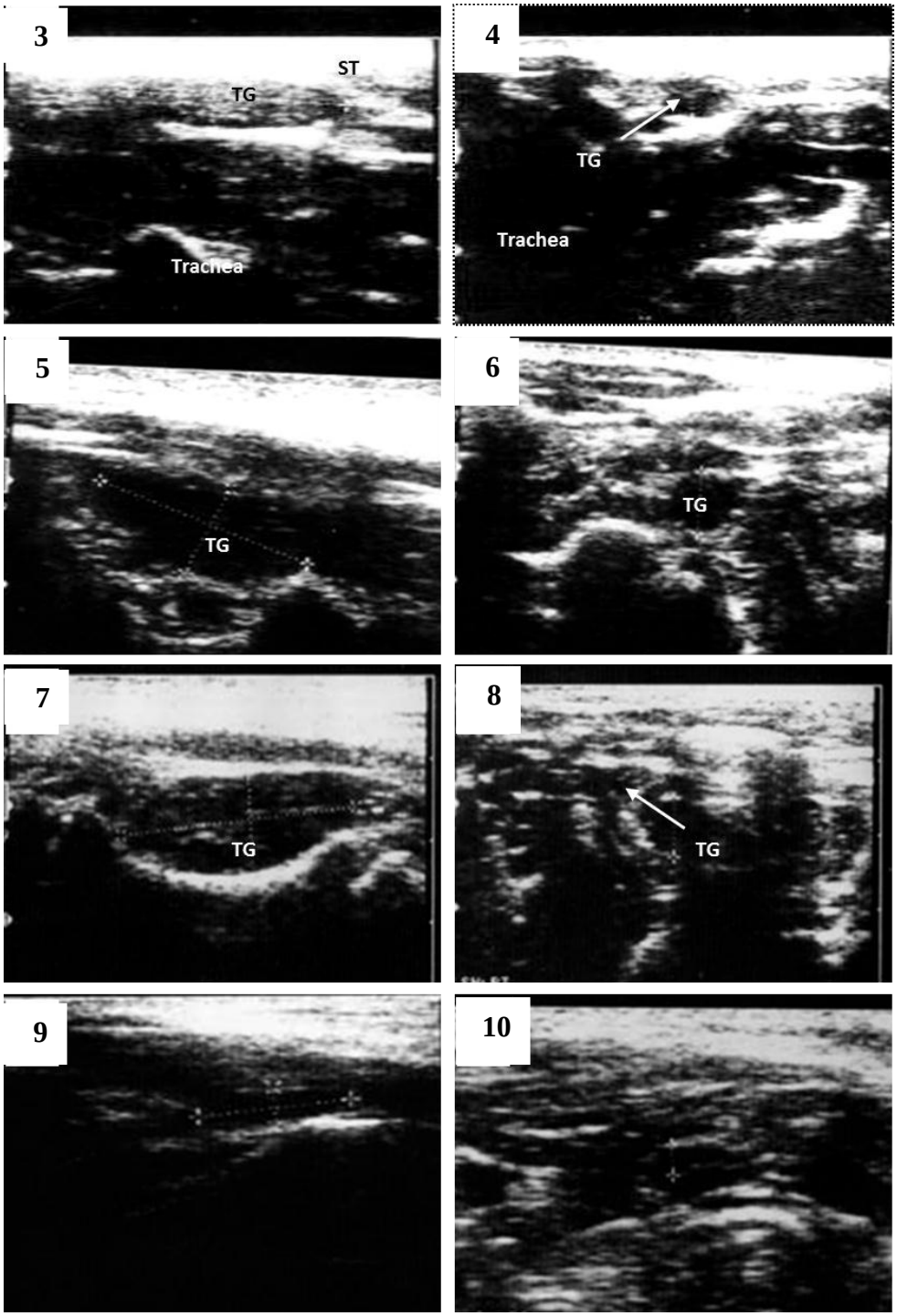

Figure 3. Ultrasonographic (US) image of thyroid gland (TG) in control group showing hyperechoic area compared to sternothyroid (ST) muscle in longitudinal section. Figure 4. US of thyroid gland in control group showing oval shape in transverse section (TS). Figure 5. The thyroid gland in drug-induced hypothyroid dog (6^{th} week) is hypoechoic with increase volume in longitudinal section (LS). Figure 6. The thyroid gland in drug-induced hypothyroid dog (6^{th} week) has oval shape and increase volume in TS. Figure 7. The thyroid gland of sham-operated dog showing isoechoic compared with sternothyroid muscle in LS. Figure 8. The thyroid gland of sham-operated dog has oval shape in transverse section (TS). Figure 9. The thyroid gland of ligation-induced hypothyroidism dog (4^{th} week) showing decreased volume and hypoechogenicity compared with sternothyroid muscle in LS. Figure 10. The thyroid gland of ligation-induced hypothyroid dog (4^{th} week) has oval shape and decreased volume in TS.

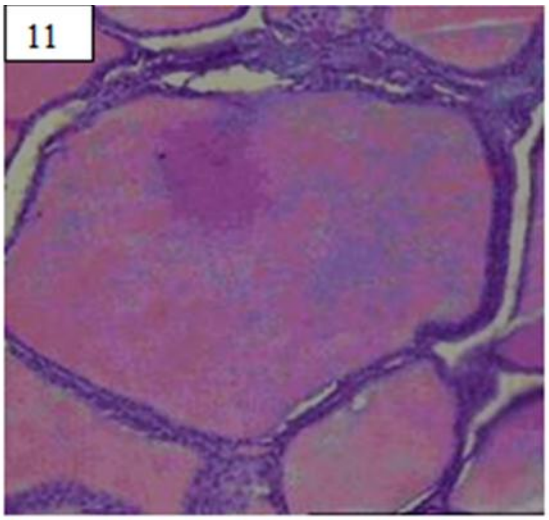

Figure 11. A photomicrograph of thyroid gland of a dog in control group showing normal microscopic appearance of thyroid gland.

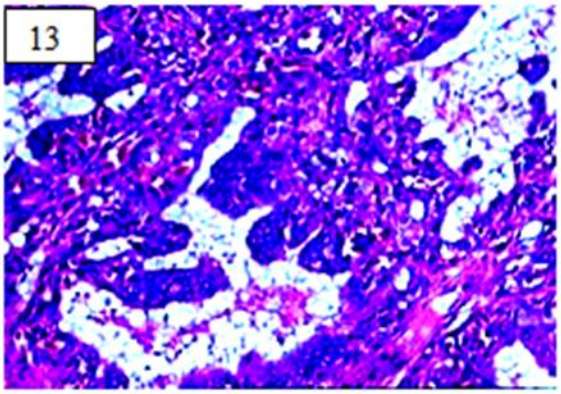

Figure 12. A photomicrograph **of** thyroid gland of drug-induced hypothyroid dog showing hyperplasia of glandular epithelium with papillary projection into the lumen and lymphocytic cellular infiltration. Some syncytial

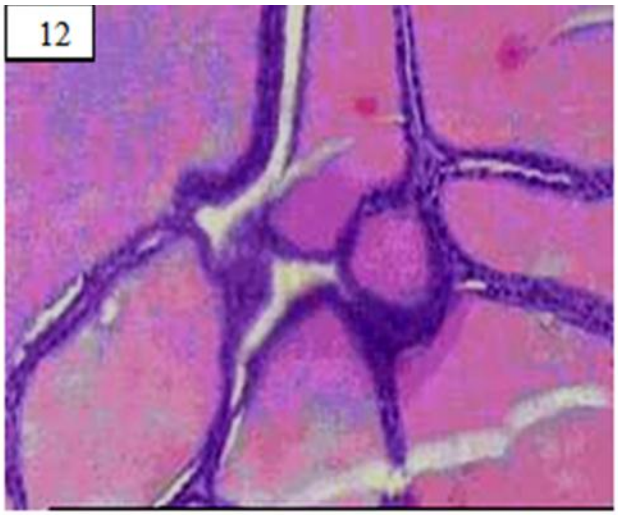

Figure 13. A photomicrograph of thyroid gland of sham-operated dogs showing normal microscopic appearance of thyroid gland.

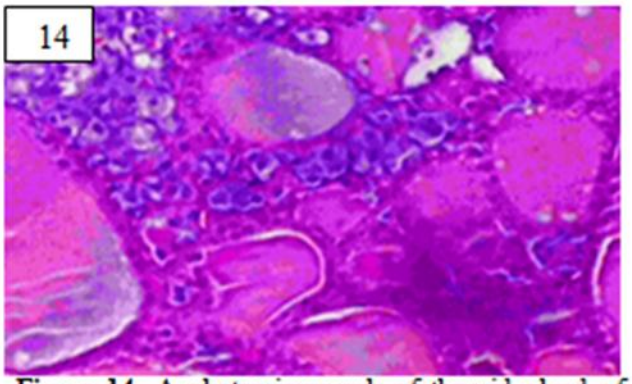

Figure 14. A photomicrograph of thyroid gland of ligation-induced hypothyroid dog. Note the presence of cystic dilatation in some thyroid follicles with retained secretion in their lumen and flattened lining epithelium. The adjacent thyroid follicles were atrophied with hypertrophied lining epithelium.

significant increase in potassium and chloride could be attributed to reduction of glomerular filteration rate (GFR) secondary to hypothyroidism that leads to increase the excretion of sodium resulting in decrease serum sodium level and elevation of chloride and potassium levels **(7).**

The ultrasonographic examination of thyroid gland of drug-induced hypothyroidism showed significant increase in total volume and relative volume that may be attributed to the effect of sulphonamides that have a goitrogenic effect on thyroid gland leading to increase thyroid volume **(6).** The decreased relative echogenicity might be due to decreased thyroid gland production of thyroid hormones **(3, 30, 39).** On the other hand, the significantl decrease in total volume, relative volume and relative echogenicity in ligation-induced group might be attributed to the ligation of arterial blood supply that causes thyroid gland ischemia leading to decreased volume and homogenous parenchyma **(3, 30, 39).**

Histopathology showed hyperplasia of glandular epithelium with papillary projection into the lumen and lymphocytic cellular infiltration. Some syncytial epithelial cells are found in some glands with desquamation of lining epithelium and necrotic cellular debris in microscopic

examination of thyroid gland of drug-induced hypothyroid dog. Similar results were observed in other studies **(1, 10, 17, 34, 36).** Some thyroid follicles showed cystic dilatation with retained secretion in their lumen and flattened lining epithelium, and the adjacent thyroid follicles were atrophied with hypertrophied lining epithelium in microscopic examination of thyroid gland of ligation group **(13).**

CONCLUSION

Based upon the results of this work, it can conclude that hypothyroidism can be successfully induced under experimental conditions in dogs by administrating Sulphamethoxazole-Trimethoprim combination at dose of 30 mg/kg. B.W., PO, twice daily for six weeks. Hypothyroidism can also be successfully induced by surgical removal of thyroid gland (thyroidectomy), and by surgical ligation of thyroid arterial blood supply. Hypothyroidism by all methods produced clinical, biochemical, ultrasonographic, and histopathological changes when compared with the control groups. Hypothyroidism causes changes in the biochemical parameters soon after induction. Therefore TT3 and TT4 together with other biochemical changes could be used to monitor the early detection of thyroid dysfunction. These results highlight the significant role of thyroid gland in maintaining the body metabolism equilibrium and the integrity of many biological organs including liver, kidney, and skin.

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التغيرات السريرية والبيوكميائية والهستوباثولوجية والتغيرات فى الفحص بالموجات الفوق صوتية للغدة الدرقية للإحداث التجريبى لنقص هرمونات الغدة الدرقية فى الكلاب.

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تم إحداث الإنقاص التجريبى لهرمونات الغدة الدرقية بعدة طرق وذلك فى ثلاثة مجموعات وهى كالأتى: المجموعة الأولى وفيها تم الإنقاص المحدث لهرمونات الغدة الدرقية بإستخدام الأدوية (السلفاميثوكسازول-الترايميثوبريم) لمدة ست أسابيع متصلة. المجموعة الثانية وفيها تم الإنقاص المحدث لهرمونات الغدة الدرقية عن طريق ازالة الغدة الدرقية جراحيا. المجموعة الثالثة وفيها تم الإنقاص المحدث لهرمونات الغدة الدرقية عن طريق ربط الأوعية الدموية المغذية للغدة الدرقية جراحيا. أكثر الأعراض المرضية ظهورا بعد الإنقاص المحدث فى الثلاث مجموعات السابق ذكرهم هى: الخمول، الزيادة المفرطة فى الوزن، تساقط الشعر، بالأضافة الى بعض التغيرات الجلدية. أما التغيرات البيوكميائية إشتملت على نقص فى هرمونات الثيروكسين وزيادة الهورمون المنشط للثيروكسين وزيادة الكوليستيرول، زيادة الدهون الثلاثية، زيادة انزيمات الكبد (AST&ALT) ووظائف الكلى، إنخفاض مستوى الصوديوم والكالسيوم وزيادة مستوى الفسفور. إشتملت التغيرات فى الفحص بالموجات الفوق صوتية للغدة الدرقية لمجموعة الإنقاص المحدث بإستخدام الأدوية على زيادة فى كلا من الحجم الكلى والحجم النسبى وإنخفاض نسبة الكثافة النسبية للغدة الدرقية بينما فى مجموعة الإنقاص المحدث عن طريق ربط الأوعية الدموية المغذية للغدة الدرقية حدث تناقص فى الحجم الكلى والحجم النسبى والكثافة النسبية للغدة الدرقية. أظهرت التغيرات الهستوباثولوجية للغدة الدرقية فى مجموعة النقص المحدث بإستخدام الأدوية زيادة اعداد خلايا الغدة تضخم الغدة الدرقية مع بروز الخلايا داخل التجويف وتجمع كثيف للخلايا اليمفاوية وتساقط الخلايا المبطنة. أما فى مجموعة النقص المحدث عن طريق ربط الأوعية الدموية للغدة الدرقية فأظهر ضمورجريبات الغدة الدرقية thyroid follicles مع تضخم الخلايا المبطنة .خلصت هذه النتائج الى الاهمية البيولوجية للغدة الدرقية فى الحفاظ على توازن التمثيل الغذائى للجسم و سلامة أعضائه المختلفة مثل الكبد والكلى والجلد. كما أكدت النتائج أن قياس هرمونات الثيروكسين تساعد على التشخيص المبكر لاضطرابات الغدة الدرقية..