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Original Article

Anatomical Studies on the Thyroid Gland of One-Humped Camel (Camelus Dromedarius) found in Nigeria-Niger Boarder Region

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Abstract *- Thirty-one-humped camels, with an equal number of males and females, were grouped into three: juvenile (Group A), adult (Group B), and old ages (Group C), and were used for the study. The gross anatomical studies showed that the one-humped camel's thyroid gland was a paired-lobe, lengthy organ with blood supply from superior, middle, and inferior thyroid arteries. The morphometric measurements, especially the weight of the thyroid gland in a one-humped camel, were directly proportional to the age of the camels. In contrast, the concentration of the thyroid hormones (T3 and T4) was inversely proportional to the age and weight of the thyroid gland in the indigenous one-humped camel. The Histochemical studies revealed the presence of follicular and parafollicular cells in all the groups, appearances of numerous vacuolated colloids in the follicles, and the follicular cells showed apoptosis in the older camels. The results demonstrated the anatomical features of the thyroid gland in one-humped camel and its correlations with its follicular cells and T3 and T4 hormones concentrations, and with age, were mostly closer to humans than most domestic animals and rodents, which suggests that one-humped camel will be a good experimental research animal model for human thyroid gland experiments in understanding the etiology and possible solution to human hyper and hypothyroidism and other related diseases. Similarly, this study will enhance the provision of basic and clinical information on endocrinology and related research fields, such as animal breeding and biotechnology, and in supporting United Nations sustainable development goals advocated by Food and Agricultural Organization (FAO, 2007) for preventing further desert encroachment in the sub-Saharan African countries like Nigeria, due to dromedary's suitability in mitigating drought, and its unique endurance traits and economic importance. The study can be improved by including a stereological study to determine cell density and population between age groups and sex and by employing transmission electron microscopy study to observe the cell organelles further and find age and sex differences.*

Keywords *- Thyroid Gland, Follicular Cells, Parafollicular cells, Thyroid Morphometry, Thyroid Histochemistry, Camel Triiodothyronine, Camel Tetraiodothyronine, One-humped Camel.*

1. Introduction

The dromedary camel is a specie that can survive in an incredibly harsh environment without food and water for days, which can be fatal to other animals (Ouajd, S. and Kamel, 2009). The performance of the camel is a result of the vital link between its anatomical features and ecological adaptations. The camel is known to be well adapted to their respective environments in the harsh and hot deserts. They have well-developed remarkable characteristics which help them survive in unfriendly environments to many animals (HJ 1999; M, 2007; Ouajd, S. and Kamel, 2009). The United Nations Food and Agriculture Organization (FAO) reported that there are approximately 18 million camels in the world, which maintain the life of millions of people in arid and semi-arid areas, such as India, Iran, all Arabian states, and all arid and semi-arid zones including Nigeria, which made information and scientific investigations around the biology of the camel at the forefront of science (FOA, 2007).

The thyroid gland is one of the largest endocrine glands in all mammals. It receives 2% of the cardiac output and takes up iodide from the blood to produce the hormones thyroxin and triiodothyronine (Abdullah S. I. *et al., 2010.*; Igwenagu E. *et al.* (2016)). Thyroid gland hormones are implicated in growth, general metabolism, regulation of energy, thermoregulatory, and specific organ functions, among many, yet numerous morphological variations of these glands in camels, are not well explored which contribute to camels in mechanisms of adaptation to unfavorable weather conditions (Taurog, 1986; Kausar and Shahid, 2006; Ouajd, S. and Kamel, 2009; L; Kleerekoper M. H., 2009)

Hence, there is a paucity of information on the thyroid gland morphology, histology, and hormones indigenous to northwestern Nigeria. It will lead to a poor understanding of this gland's role behind the biology of the dromedary's many

peculiar characteristics, including adaptation mechanisms to the hostile environment. The study is pertinent in providing baseline information, as no research was reported on thyroid gland anatomy in one-humped camels found in Northwestern Nigeria around the Nigeria-Niger border axis, where the one-humped camel population are most concentrated in Nigeria (Abdussamad *et al.,* 2011). The study could be useful in comparative anatomy and evolutionary studies with other species of the *Artiodactyla* order and higher orders like humans. It could also enhance and provide basic and clinical investigative information in endocrinology, and related research fields, such as animal breeding and biotechnology.

Therefore, the aims of this study were to:

- Study the gross morphology and morphometry of the thyroid gland of the one-humped camel.
- Study the histology of the thyroid gland of the one-humped camel using routine stain (H & E) for light microscopy.
- Study the histochemistry of the thyroid gland using Periodic Acid Schiff (PAS) for demonstration of glycogen and Azure A Acid hydrolysis technique for demonstration of parafollicular cells.
- Determine the serum levels of Thyroid Stimulating Hormone (TSH), Triiodothyronine (T3) and Tetraiodothyronine (T4) using ELISA auto-analyzer kits.
- And determine the sexual dimorphism in the one-humped camel's morphometric variables of the thyroid gland.

2. Materials and Methods

2.1. Study Animals

Thirty (30) one-humped camels, comprising fifteen (15) males and fifteen (15) females, between the ages 1-5, 6-10 and above 10 years, representing juvenile, adult, and old ages, respectively, were used for the study. The camel gland's tissues studied were obtained from the Kano Abattoir. An introduction letter was issued from the Department of Human Anatomy, Ahmadu Bello University Zaria, to the Veterinary Department of Kano State Ministry of Agriculture and Mineral Resources for permission and ethical conduct of the procurements of the tissues. Standard protocols and procedures were followed during the procurement with the guidance of a qualified Veterinary practitioner. Only apparently healthy camels were used for this study. The age of the animals was determined according to their dentition, as described by Faye (1997)

2.2. Dissection, gross and morphometry of the thyroid gland

The camels were slaughtered by skilled veterinary butchers according to the halal method of animal slaughter as described by WHO, 1997 and Pozzi *et al.*, 2015, and according to the World Organization of Animal Health (OIE) by an incision through the trachea, esophagus and both the Carotid arteries and Jugular veins (Plate 1). After the camel died, the skin was reflected, the neck detached, the upper part of the respiratory tract and the associated organs were separated and removed intact from the heavy muscles of the posterior neck (Plate 1). An incision was made sagitally through the anterior neck muscles, specifically sterno-thyroid muscles. It exposed the thyroid cartilage, cricoid cartilage, tracheal rings and the thyroid gland. The glands were observed grossly to determine the shape, color, surfaces, borders and angles. Pictures of the thyroid glands and related structures were taken in situ (Plate 2). The thyroid glands were removed. The weights of the glands were obtained using a digital weighing scale immediately after dissection, and linear measurements of their width, thickness and length were taken using a digital vernier caliper. The length was measured from the top pole to the lowest pole of the gland. The width was measured from the medial to the lateral borders, whereas the thickness was measured from the anterior to the posterior surfaces of the gland's gland, halfway the length of the gland (Plate 3).

2.3. Collection of blood

Blood samples were collected at the point of slaughter via the jugular vein at the neck into bottles containing ethylene diamine tetra-acetic (EDTA) and placed on ice until laboratory arrival (< 2h) and centrifuged at 10,000 rpm for 10 min; the resulting blood serum was used for hormonal assays for the quantitative determination of TSH, T3, and T4 concentrations in One-humped camel serum by a MicroPlate Immunoenzymetric assay.

2.4. Hormonal Assay

The hormones were assayed using three different ELISA Kits; the T3 ELISA kit, T4 ELISA kit as prescribed by INTECO Diagnostic, UK, ISO 13485 accredited company (purchased from NAZO Medical Diagnostic Services No. 13 Winfunke Olowe Crescent, Off Lagos/ Abeokuta Express Way, Lagos state) according to manufacturer's guidelines.

2.5. Histological Studies

The gland tissues were taken to the Histology Laboratory in the Department of Histopathology, Aminu Kano Teaching Hospital (AKTH). The tissues were prepared for standard histological study using haematoxylin and eosin (H and E) for light microscopy following the procedure described by Bancroft and Gamble (2008) and Suvarna *et al.* (2019).

The stained slides were examined using a binocular light microscope at different magnifications (40, 100, 250 and 400). Photomicrographs of the slides were taken using Amscope digital camera attached to a computer system via USB.

2.6. Histochemical Studies

The tissue blocks were fixed using 10% neutral buffered formalin and prepared for H and E slides according to Bancroft and Gamble, 2008; Suvarna et al., 2019 procedures. The thyroid gland tissue blocks were sectioned and stained with Periodic Acid Schiff (PAS) special stain to demonstrate glycogen and glycoprotein. In contrast, some were stained with Acid Hydrolysis Azure A technique special stains specifically to demonstrate C-cells in the thyroid. The hydrochloric acid in the hydrolysis solution precedes staining with Azure A and suppresses basophilia due to nucleic acids and any acid mucins present (Cook, 1974; Bancroft and Gamble, 2008; Suvarna *et al.*, 2019).

Finally, sections were dehydrated, cleared and mounted in DPX. Slides were studied on the binocular light microscope Olympus at different magnifications (40, 100, 250 and 400). Photomicrographs of slides were taken using a microscope armscope digital camera attached to a computer system via USB.

2.7. Statistical Analysis

Data were expressed as mean ± Standard Error of mean. An Independent sample t-test was used to analyze the differences in morphometric variables between the right and left lobes of the thyroid gland within each group, regardless of sex, and sexual dimorphism was determined. Comparisons were also done between all males and females regardless of their (age) group. Pearson's correlation analysis was carried out between morphometric measurements of the glands' weight, length and width with the concentration of the hormones they secret. The level of significance was set as $P< 0.05$. The statistical analysis was done using the student package for IBM SPSS version 20.0

3. Results

3.1. Gross Anatomical studies

The study revealed that the thyroid glands in the one-humped camel were seen as reddish brown organs (Plate 4.1). The gland was a paired-lobe organ located on the lateral surfaces of the trachea (Plate 4.1). The two lobes were joined together at the inferior pole by an isthmus which passes on the ventral surface of the third (3rd) to fifth (5th) tracheal rings. It has lengthy lobes that extend from the lower border of the cricoid cartilage (CC) and up to the fifth (5th) tracheal rings (Plate 4.1). The thyroid gland lobes appeared triangular and like a long shield in shape, with lateral, medial and superior borders. The thyroid lobes also have two broad surfaces; the ventral surface is covered ventrally by sternothyroid muscle, and the dorsal surface is bordered by a ventro-lateral surface of the cricoid cartilage and first to fifth tracheal rings (Plate 4.2). The isthmus is thin and elongated (Plate 4.1). The blood supply is from superior, middle and inferior thyroid arteries, as shown in Plate 4.3, which are direct branches from the external carotid or common carotid artery.

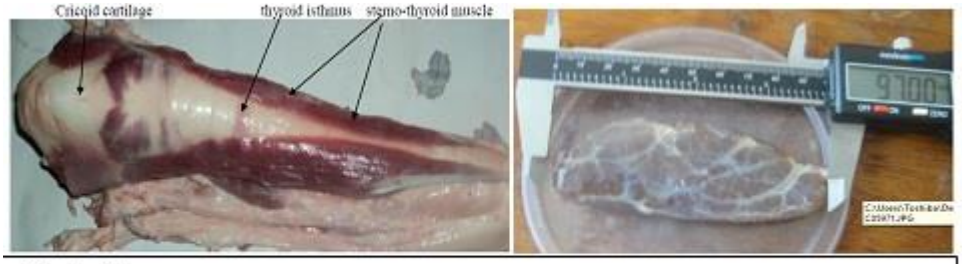

Plate 4.1: **a.** Removal of the ventral organs of the camel by sagital incision, including trachea, esophagus (dorsally), thyroid gland covered by long sterno-thyroid muscle **b.** thyroid lobe measurement from superior to inferior poles by digital vernier caliper

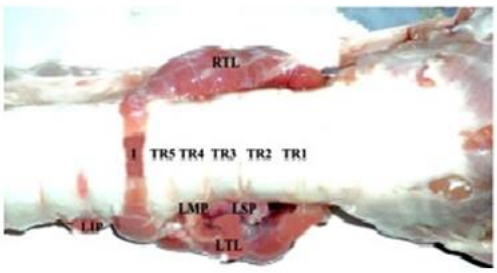

Plate 4.2: The thyroid gland of camel dromedary. Note the Isthmus (I), the right thyroid lobe (RTL), left thyroid lobe (LTL). TR1 denoted the first tracheal ring, TR2 second tracheal ring, TR3 third trach

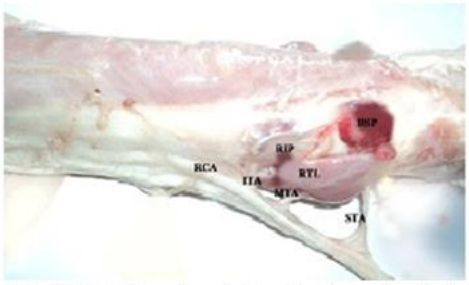

Plate 4.3: Blood supply to the thyroid and parathyroid glands; showing branches of right common carotid artery (RCA), inferior thyroid artery (ITA), middle thyroid artery (MTA), and superior thyroid artery (STA) in one-humped camel.

3.2. Morphometric Studies

The study showed that the morphometric variables expressed as the mean plus /or minus standard Error of mean (SEM) of the weight, length, width and thickness in the female one-humped camel in group A were: 34.48g ±3.64g, 77.76mm ± 2.99mm, 29.03mm ± 1.05mm, and 11.93mm ± 51mm respectively (Table 4.1). The mean plus /or minus SEM of the male's weight, length, width, and thickness in group A were also presented in Table 4.1. The mean plus /or minus SEM of the weight, length, width, and thickness in males of group B were: 45.64g ± 5.01g, 93.10mm ± 7.93mm, 41.30 ± 6.12mm, and 21.10mm ± 5.05mm, respectively (Table 4.1). The females of the same group B were also presented (Table 4.1). The adult female one-humped camel among the group C in the study showed that the mean plus /or minus SEM of the weight, length, width, and thickness were: 44.44g ± 3.91g, 86.64mm ± 5.28 mm, 29.80mm ± 1.24 mm, and 11.40mm ± 0.40mm, respectively (Table 4.1), while those of males in the same group were also shown in Table 4.1.

The thyroid glands have two lobes, and the difference in morphometric variables between the lobes in each group was determined; it was asymmetrical, and the results are shown in Table 4.

The differences within each group in the morphometric values of weight, length, width, and thickness of the thyroid gland in one-humped camel between males and females were determined. The results showed sexual dimorphism in the length of thyroid gland of the one-humped camel within group A, where female juvenile camels have longer thyroid lobes than the male (Table 4.1) significantly. There was sexual dimorphism in the thickness of the thyroid gland between males and females in group C (old adults), with the male thyroid lobes thicker than the female's (Table 4.1).

Hormonal Assay results were: In group A; the mean plus/ minus SEM concentrations of the TSH, T4, and T3 hormones were 9.69 ± 8.43µlu/ml, 7.11 ± 0.36µg/dl, and 1.92 ± 0.17ng/ml, respectively in female (Table 4.5), and that of male in the same group A was shown (Table 4.5). In group B, males were: 7.62 + 4.45 µlu/ml, 8.21+0.57 µg/dl, and 1.76+0.24 ng/ml, respectively (Table 4.5), while that of the female was also shown in Table 4.5. And in Group C female have 15.40 ± 8.12µlu/ml, 6.25 ± 0.74µg/dl, and 1.22+0.14ng/ml respectively (Table 4.5). The male hormonal concentrations in group C are shown in Table 4.5. The differences in the concentrations of TSH, T4, and T3 were analyzed by Pearson's correlation between all the groups (A, B, and C) and found a strong positive correlation between T4 and T3 regardless of their sex (Table 4.4, Figures 4.3 and 4.4)

Correlation analyses were also conducted between all the morphometric variables (weight, length, width, and thickness) and the hormonal concentrations of TSH, T4, and T3. The results showed a strong positive correlation (at $P<0.01$) specifically between weight and length, weight and width, length and width, width and thickness, weight and T3 hormone, and between the Tetraiodothyronine (T4) and Triiodothyronine (T3) (Table 4.4, Figure 4.1, 4.3 and 4.4).

Table 4.1 Morphometric Mean values (± SEM) of the thyroid gland of the one-humped camel within the groups A, B, and C.

	GROUP A		**GROUP B**		**GROUP C**	
	Mean ± SEM		**Mean ± SEM**		**Mean ± SEM**	
VARIABLES	**Female (n=5)**	**Male (n=5)**	**Female (n=5)**	**Male (n=5)**	**Female (n=5)**	**Male (n=5)**
Weight (g)	34.48 ± 3.64	27.04 ±4.39	49.09 ± 6.97	45.64 ± 5.01	44.44 ±3.91	53.30±7.02
Length (mm)	77.76 ± 2.99**	65.63 ± 4.51	89.55±3.50	93.10±7.93	86.64±5.28	86.90±6.26
Width (mm)	29.03 ± 1.05	25.06 ± 2.62	37.71 ± 1.66	41.30±6.12	29.80±1.24*	35.20±1.39*
Thickness (mm)	11.93 ± 0.51	11.14 ± 0.95	14.27 ± 1.14	21.10 ±5.05	11.40 ±0.40	10.80 ± 0.97

Analysis by Students T-test between Female and Male One-humped Camel among the age groups. Data were expressed in mean ± standard Error of mean (SEM) values collected.

*=P< 0.05 (the difference is significant between males and females)

**= P < 0.01(difference significant between Male and Female)

Table 4.2 Morphometric mean values (±SEM) of the mean of the thyroid gland of the one-humped camel differences between males and females in all the groups (A, B, and C) regardless of age

Mean ± SEM		
Variables	**Female (n=15)**	**Male (n=15)**
Weight (g)	42.67±3.16	41.99±4.20
Length (mm)	84.65±2.54	81.89±4.64
Width (mm)	32.18±1.27	33.85±2.76
Thickness (mm)	12.54±.52	14.35±2.06

Students T-test analysis between Female and Male One-humped Camel in all the age groups. Data is expressed in mean ± standard Error of mean (SEM) values collected.

Table 4.3 Comparison between right and left lobes of the thyroid gland in indigenous one-humped camel within each of the groups (A, B, and C)

	Mean ± SEM					
	Group A		**Group B**		**Group C**	
Variables	**Right Lobe (n=10)**	**Left Lobe (n= 10)**	**Right Lobe (n=10)**	**Left Lobe (n= 10)**	**Right Lobe (n=10)**	**Left Lobe (n= 10)**
Weight (g)	14.43±1.41	15.37±1.54	24.06 ± 2.91	22.99 ± 1.57	24.17 ± 1.96	24.66 ± 2.28
Length (mm)	70.82±3.26	72.58±3.76	92.59 ± 4.85	90.08 ±3.95	85.30 ± 3.74	88.10 ± 4.67
Width (mm)	28.66±1.95	25.42±1.43	33.56 ± 1.51	45.21 ±6.01	33.10 ± 1.54	32.40 ± 1.485
Thickness (mm)	11.88±0.68	11.20±0.53	13.94 ± 0.96	21.49 ± 5.60	11.00 ± 0.683	11.00 ± 0.730

Analysis by students T-test between right and left lobes of the thyroid gland within each age group. Data is expressed in mean ± standard Error of mean (SEM) values collected.

Table 4.4 Correlation of thyroid gland morphometric variables and hormone concentrations of TSH, T4, and T3

Variables	**Length (mm)**	**Width (mm)**	**Thickness (mm)**	**TSH (µlu/ml)**	**T4 (µg/dl)**	**T3 (ng/ml)**
Weight (g)	0.655**	0.528**	0.211	-0.353	-0.193	**-0.494****
Length (mm)		0.812**	0.665**	-0.295	0.085	**-0.251**
Width (mm)			0.847**	-0.289	0.212	**-0.142**
Thickness(mm)				-0.211	0.378*	**0.022**
TSH (µlu/ml)					-0.291	**0.130**
T4 (µg/dl)						**0.558****
T3 (ng/ml)						

Pearson Correlation
*= correlation is significant at the P<0.05 level (2-tailed).
** = correlation is significant at the P<0.01 level (2-tailed).
TSH = Thyroid Stimulating Hormone
T4= Tetraiodothyronine
T3= Triiodothyronine

Table 4.5 Mean values (± SEM) of hormonal concentrations of thyroid hormones (thyroid function test) of the one-humped camel within Groups A, B, and C to determine sexual dimorphism

			Mean ± SEM			
	GROUP A		GROUP B		GROUP C	
VARIABLES	Female (n=5)	Male (n=5)	Female (n=5)	Male (n=5)	Female (n=5)	Male (n=5)
TSH (µlu/ml)	9.69 ±8.43	9.07 ±6.86	-1.45 ± 0.25	7.62 ± 4.45	15.40 ±8.12	-1.00± 0.69
T4 (µg/dl)	7.11 ± 0.36	7.71 ± 0.57	7.80±0.63	8.21±0.57	6.25±0.74	6.41±0.53
T3 (µg/dl)	1.92 ± 0.17	8.21 ± 0.57	2.34 ± 0.59	1.76±0.24	1.22±0.14	1.04±0.09

Analysis by t-test to determine sexual dimorphism in One-humped Camel among the age groups (A, B, and C). Data are expressed in mean ± standard Error of mean (SEM) values collected.

*= P < 0.05(Significant between Male and Female)

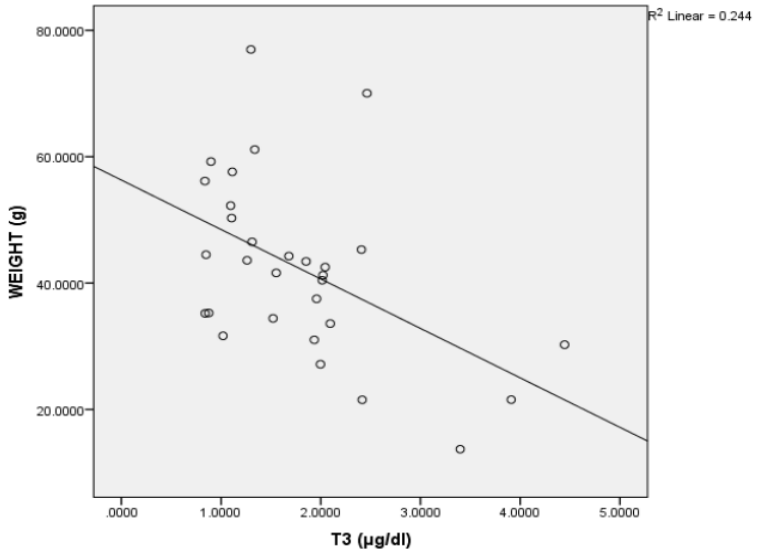

Figure 4.1 Scattergram graph showing strong correlation between the Weight of thyroid gland against the concentration of Triodothyronine (T3). Note the relationship is inverse.

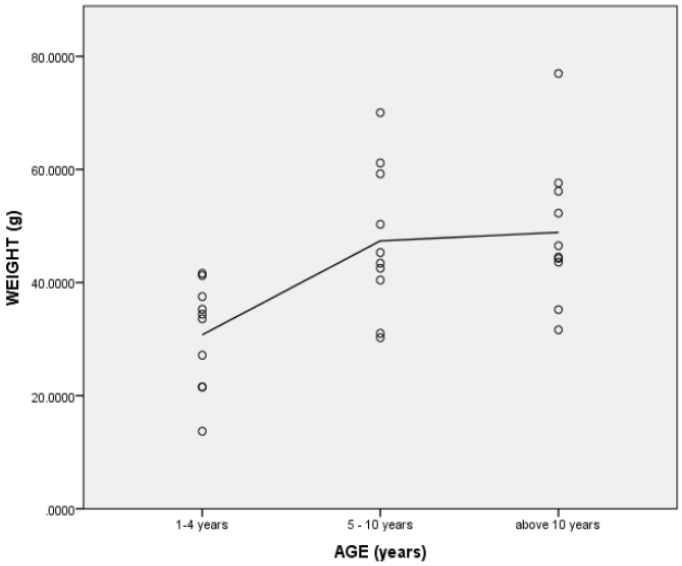

Figure 4.2 Scatter gram graph showing strong correlation between the weights of thyroid gland against the respective age in groups of the animals. Note the line is straight upward (directly proportional) then it becomes constant

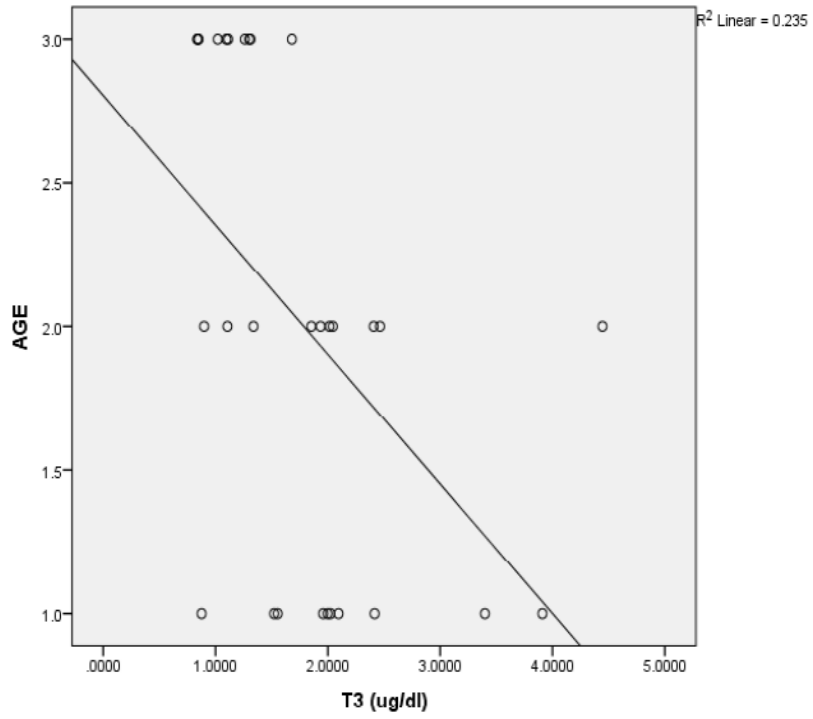

Figure 4.3: Scatter plot graph showing correlation of age with T3 concentrations.
Note the correlation between the **Triiodothyronine (T3)** and the Age are inversely proportional

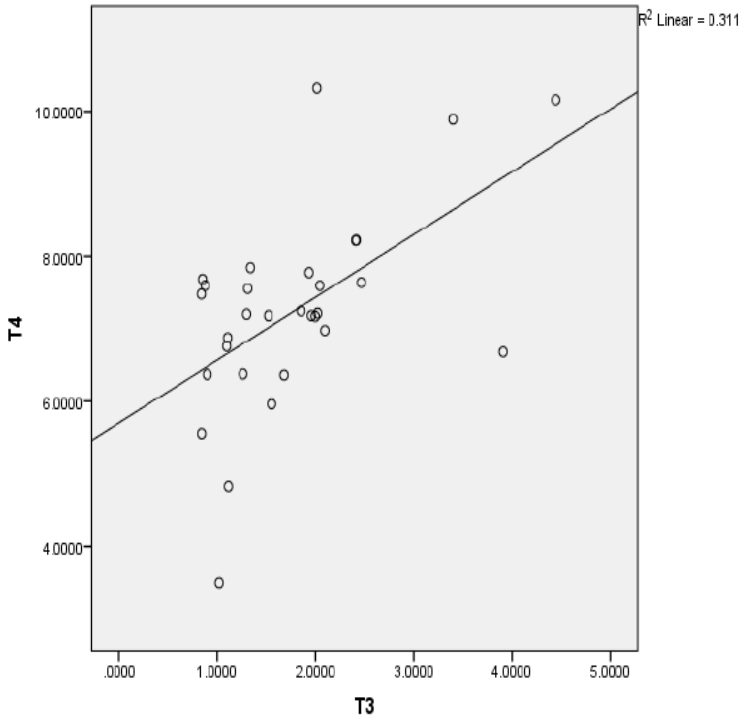

Figure 4.4: Scatter plot graph showing correlation of T4 concentration to T3 concentration. Note the correlation between the **Tetraiodothyronine (T4)**, and **Triiodothyronine (T3)** is directly proportional.

3.3. Histological and Histochemical Studies

Group A results of the histology and histochemical studies were as follows:

The results of H&E stained thyroid gland tissue slides revealed that the tissue was surrounded by a thin capsule of connective tissue, which invades the parenchyma and provides a passage, by thin trabeculae**,** for the rich capillary plexus/ sinusoids (Plate 4.5), and divided it into lobules (Plates 4.4, 4.5, and 4.6)**.** The sinusoids were seen as more prominent and numerous in females of group A than in males of the same group (Plates 4.5 and 4.6). This intralobular connective tissue consists of numerous follicles of various sizes (Plate 4.4) that was filled with colloid (Plates 4.4, 4.5, and 4.6), with bigger follicles situated towards the periphery more in female of group A than in the male (Plate 4.4). The shapes of the follicles were round, oval and irregular (Plate 4.4). Each follicle comprised simple follicular epithelial cells encircled it (Plates 4.4, 4.5, and 4.6) and consisted of parafollicular cells (Plate 2). The follicular and parafollicular cells rest on a thin basal lamina but were separated from the thin trabeculae surrounding the follicles/ lobules (Plate 4.5)**.** Follicular cells of neighboring follicles were seen, in some tissue slides, came into contact with each other and disrupted the continuity of the basal lamina and were seen within the follicles/ lobules, and more prominent in males than in females of group A (compare Plates 4.4 and 4.5)

The follicles were mostly inactive and filled with colloids and had the smoother peripheral surface of follicles in both males and females of group A (Plates 4.4, 4.5, and 4.6). The follicular cells were low cuboidal in shape (Plates 4.5 and 4.6) and with very few vacuolated follicles seen only at the periphery in females (Plate 4.4). Parafollicular cells or C cells occurred among the follicular cells and between them (Plates 4.5 and 4.6). They were larger, had a paler cytoplasm than the follicular cells, and occurred singly and in groups (Plates 4.5 and 4.6). It was virtually the same in both females and males.

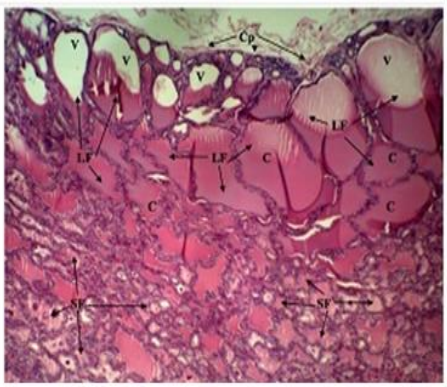

Plate 4.4: Transverse Section of female in group A. Note the Capsule (Cp), Follicles of different sizes, Large follicles (LF), and small follicles (SF) surrounding the Colloids (C). The vacuoles (V) in some colloids, vacuolization of the colloids (H&E × 40).

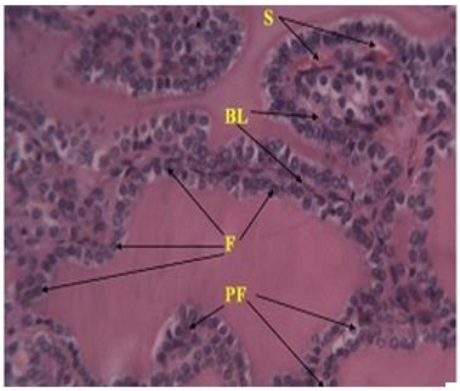

Plate 4.5: Transverse Section of female of group A. Note the Follicular cells (F) laying on basal lamina (BL), and the Parafollicular cells (PF). There are lots of sinusoids (S) around the basal lamina (BL) (H&E × 250).

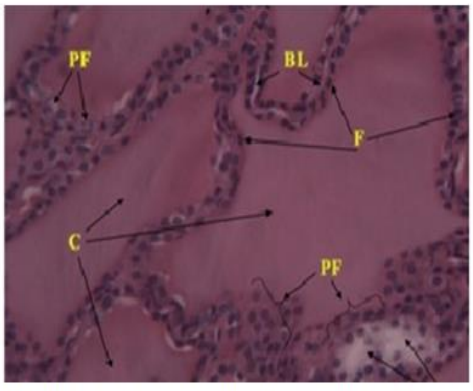

Plate 4.6: Transverse Section of **male** one-humped camel in group A. Note the foll surrounded by follicular cells (F), filled with colloids (C). The parafollicular cells (PF) appeared in groups and sometimes in between the follicular cells. The basal lamina (BL) which the follicular cells rest also separated the follicles (H&E × 250).

A special histochemical stain revealed that the colloid in the follicles was negative to Periodic Acid Schiff (PAS)**.** The PAS slides did not stain different from the H&E (Plate 4.8). No difference between the sexes of the same group.

A special histochemical technique was used to differentiate parafollicular cells from the follicular with some degree of certainty, and Azure A Acid Hydrolysis was utilized, which deeply stained the parafollicular cells purple blue (Plate 4.8)

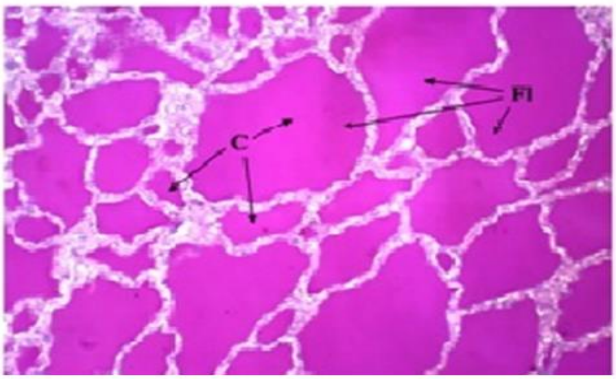

Plate 4.7: Photomicrograph, Longitudinal Section of one-humped camel in group A. Note the colloid (C) within the follicles (Fl) reacted negative to the Periodic Acid Schiff (PAS) reagent (PAS × 100).

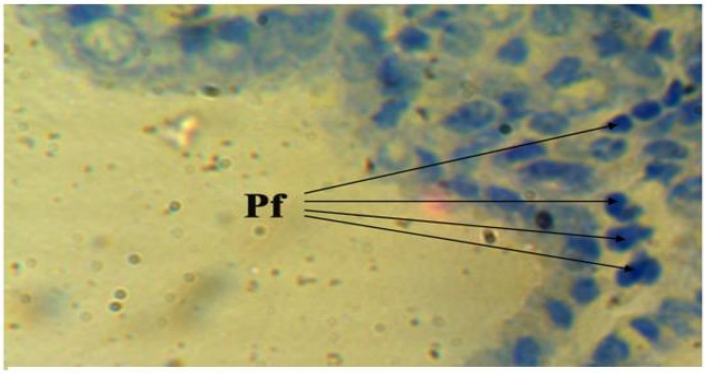

Plate 4.8: Photomicrograph, Longitudinal Section, of camel in group A, demonstrating the parafollicular cells (Pf) that stained purple blue well with Azure A Acid Hydrolysis technique (Azure × 450)

Group B of the study consists of ten (10) equal numbers of female and male indigenous one-humped camels between the age of 5- 10 years (young adults).

The results of the histology study are as follows: The slides stained with Haematoxylin & Eosin (H&E) revealed that the tissues were surrounded by a thin capsule of connective tissue, which invades the parenchyma and provides a passage, by thin trabeculae, for the rich capillary plexus/ sinusoids (Plate 4.9), and divided it into lobules, which contain follicles (Plates 4.9 and 4.10). The sinusoids were seen as more prominent and numerous in the female of group B, but it was also present in males of the same group (Plates 4.9 and 4.10). There were follicles of various sizes (Plate 4.9), with some filled with colloid while some vacuolated (Plate 4.9). The follicles with vacuoles were mostly bigger and occupied more than half of the tissue from the periphery in males of group B than in the female (Plates 4.9 and 4.10). There were vacuoles in the follicles scattered more in females than in males. Most of the bigger follicles at the periphery were empty or vacuolated in males of group B (Plate 4.9). The shapes of the follicles were round, oval and irregular (Plate 4.9). Each follicle was surrounded by simple follicular epithelial cells (Plate 4.9), which also consisted of parafollicular cells (Plates 4.9, 4.10 and 4.12). The cells rest on a thin basal lamina but were separated from the thin trabeculae surrounding the follicles/ lobules (Plate 4.9). The follicular cells of the neighboring follicles were seen to come into contact with each other and disrupt the continuity of the basal lamina and, in some, seen within the follicles/ lobules. They were more prominent in females than in males of group B (Plate 4.9).

The follicular cells were more columnar in females while males were more cuboidal in shape (Plate 4.9 and 4.10). Some of the cells showed some level of apoptosis and were more prominent in females than in males (Plate 4.10). Parafollicular cells or C cells occurred among the follicular cells and between them (Plates 4.10 and 4.12). They were larger, had a paler cytoplasm than the follicular cells, and occurred singly and in groups (Plate 4.9 and 4.12).

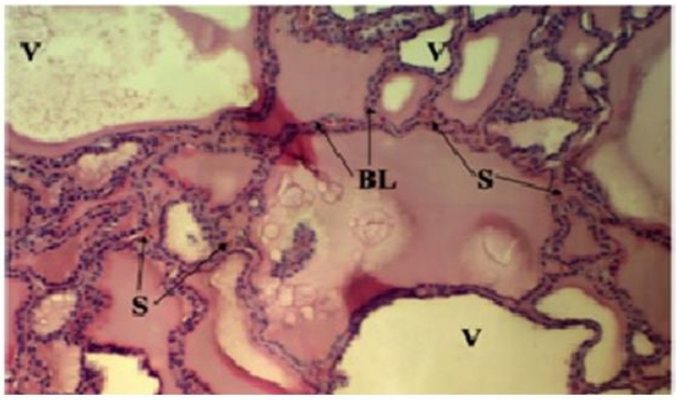

Plate 4.9: Photomicrograph, longitudinal section of the male camel in group B. Note almost half of the tissue was vacuolated, the vacuoles (V) within the follicles, the basal lamina (BL) upon which the cells rested, sinusoid (S) of blood capillaries (H&E × 100).

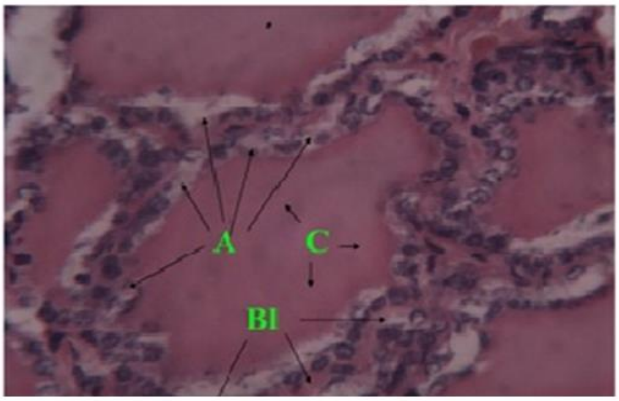

Plate 4.10: Transverse section of female camel in group B. The thyroid cells are showing some level of apoptosis (A). Some of the follicles were filled with colloids while some where vacuolated. BL is the basal lamina upon which the follicular cells lied. (H&E × 400)

Histochemical studies revealed that the colloid in the follicles was negative for Periodic Acid Schiff (PAS) (Plate 4.11). The PAS slides did not stain different from the H&E. No difference was seen between the sexes of the same group. A special histochemical technique, Azure A Acid Hydrolysis, was also used to differentiate parafollicular cells from the follicular cells to achieve certainty, which deeply stained the parafollicular cells purple (Plate 4.12)

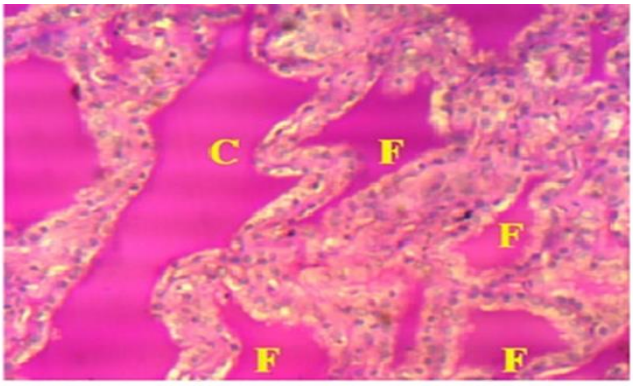

Plate 4.11: Photomicrograph of thyroid gland tissue (Longitudinal section) of male indigenous one- humped camel in group B. Colloid (C) contained within the follicles (F) reacted negative to the Periodic Acid Schiff reagent (PAS × 150).

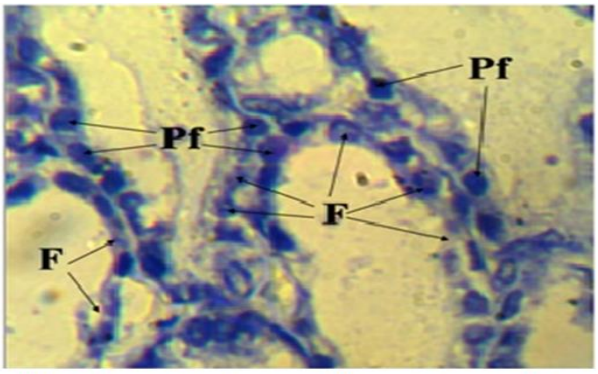

Plate 4.12 Longitudinal section of Male camel in group B. Note how the parafollicular cells (Pf) nuclei were clearly and deeply stained purple blue by the Azure A acid hydrolysis technique, while the follicular cells were irregular and poorly stained (nucleus not clear) (Azure A ×450).

Group C results of the histological study are as follows: The tissue slides stained with Haematoxylin and Eosin (H&E) revealed that the tissues were surrounded by a thin capsule of connective tissue, which invaded the parenchyma and provided a passage, by thin trabeculae, for the rich capillary plexus/ sinusoids (Plate 4.13), and divided it into lobules (Plates 4.13, 4.14, 4.15, and 4.16). Blood vessels, specifically arteries, were seen as more prominent and numerous in males of group C (Plate 4.13). They have follicles of various sizes scattered (Plates 4.13 and 4.14). Most of the follicles were vacuolated (Plates 4.13, 4.14, 4.15, and 4.16). The vacuoles were relatively more male (compare Plates 4.13 and 4.14). The shapes of the follicles were round, oval and irregular (Plates 4.13 and 4.14); they were more irregular in males (Plates 4.13 and 4.14). Each follicle was surrounded by simple follicular epithelial cells (Plates 4.13, 4.14, 4.15, and 4.16) and also consist of parafollicular cells (Plates 4.14 and 4.16). The follicular and parafollicular cells rest on a thin basal lamina but were separated from the thin trabeculae surrounding the follicles/ lobules (Plates 4.13 and 4.14). The follicular cells of the neighboring follicles may come into contact with each other and disrupt the continuity of the basal lamina as seen within the follicles/ lobules (Plates 4.13 and 4.14). Some of the follicular cells look columnar, while some look cuboidal in shape both in females and males (Plates 4.14 and 4.16). Some of the cells underwent apoptosis in both males and females (Plates 4.14 and 4.16).

Parafollicular cells or C cells occur among the follicular cells and between them (Plates 4.14 and 4.16). They were larger, had a paler cytoplasm than the follicular cells, and occurred singly and in groups (Plates 4.14 and 4.16).

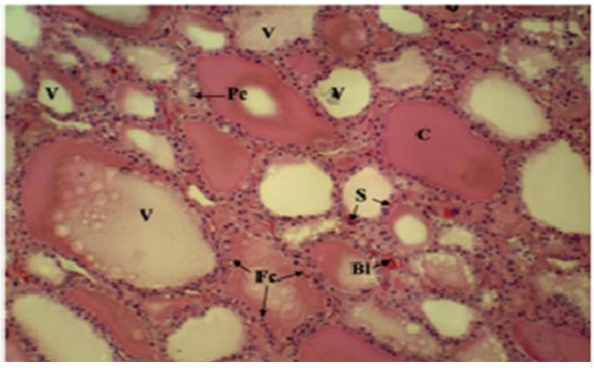

Plate 4.13: Transverse section of male indigenous one-humped camel in group C. Note the spread of vacuoles (V) all over the tissue in the follicles. The follicles were formed by follicular cells (Fc), and parafollicular cells (Pc), and contain colloids (C), mostly vacuolated (V). The follicular cells (Fc) were based on basal lamina (BL) in this tissue were mostly bathed with sinusoids (S) or blood capillaries all over the tissue in these group (H&E × 100).

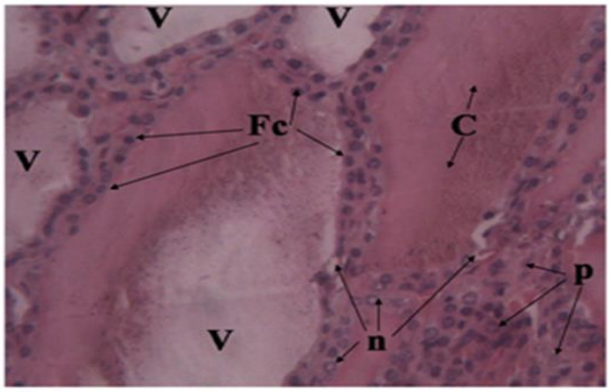

Plate 4.14: Photomicrograph of thyroid gland tissue (Transverse section) of female indigenous one- humped camel in group C. Note the follicles, the follicular cells (Fc) showing a little of apoptosis (n), the parafollicular cells (P). The follicles have vacuoles (V) (H&E × 250)

Histochemical studies revealed that the colloid in the follicles reacted negatively to the Periodic Acid Schiff reagent **(**Plate 4.15**).** The PAS slides did not stain different from the H&E. No difference was seen between the sexes of the same group. A special histochemical technique, Azure A Acid Hydrolysis, was also used, which deeply stained the parafollicular cells purple (Plate 4.16).

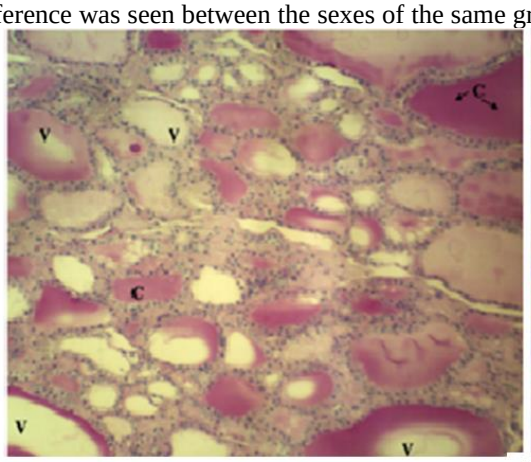

Plate 4.15: Photomicrograph of thyroid gland tissue (Transverse section) of male one- humped camel in group C showing vacuolated (V) follicles. The colloids (C) were negatively stained to Periodic Acid Schiif (PAS × 100).

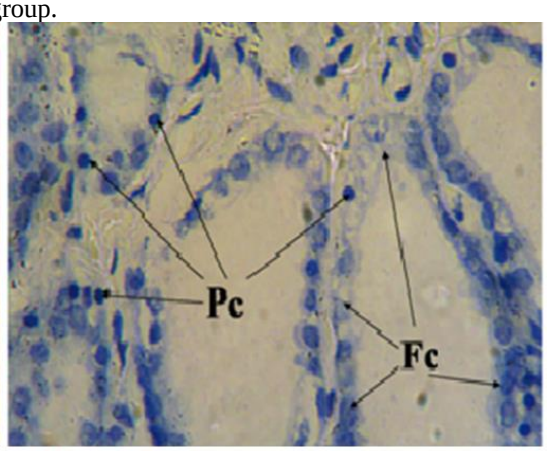

Plate 4.16: Photomicrograph of thyroid gland tissue (Transverse section) of male camel in g Acid hydrolysis stained the parafollicular cells (Pc) deep purple blue. Note the nuclei of the (Pc) how they stand out clearly from the follicular cells (Fc) nuclei (Azure A × 400).

4. Discussion

The gross anatomical study of the thyroid gland of the one-humped camel of our study from its color, shape, and lobes presentation conforms to what was found in the one-humped camel in Maiduguri by igwenagu *et al.* (2016), in Pakistan by Kausar and Shahid (2006) and in Iran reported by Ahmadpanahi and Yousefi (2012). However, Igwenagu *et al.* (2016) reported that the shape of the thyroid gland lobes was irregular, while in our study, we found it triangular, as the gross and morphometric studies showed (Plate 4.1 and Table 4.1). And this is similar to thyroid gland lobes in cattle, sheep and dogs, which were reported as asymmetric in position (McDonald, 2003). The thin isthmus that connects the lobes of the thyroid gland in camels in this study is similar to what was found in the horse (Dyce *et al.*, 2002) and humans. Still, the lobes in humans are positioned anterior to the second, third and fourth tracheal rings, which is not up to the fifth tracheal ring (Singh, 2018; Moore *et al*., 2006; Moore *et al.*, 2018). In rats, mice and African giant rats (AGR), the isthmus is located at the caudal end of the lobes (Ingbar, 1985), similar to the presentation found in the present study. The above thyroid gland morphology in a one-humped camel in the present study showed its closeness to ruminants, humans and even rodents.

The present work revealed that the arterial blood supply to the thyroid gland in all the one-humped camels in the present study was from superior thyroid arteries, middle thyroid arteries and inferior thyroid arteries, which were slightly different from the humans. They were usually from two arteries; superior and inferior thyroid arteries and thyroidea ima artery in 10% of humans (Moore *et al.*, 2018; Singh, 2018). The additional middle thyroid artery was first reported in this study and was all seen in the experimental animals. The extra artery discovered in this study may contribute to the peculiar characteristic of endurance of the camel, which is supported by high metabolic activities, which is one of the important roles of thyroid hormones (Shahid and Kausar, 2006).

Morphometric findings in the present work revealed that the weight, length, width and thickness of the thyroid gland in adult one-humped camels indigenous to Northern Nigeria agreed with the previous works done on a one-humped camel in Pakistan and Iran by Kausar and Shahid (2006) and Ahmadpanahi and Yousefi (2012) respectively. Similarly, the morphometry of the adult groups was close to the findings of Igwenagu *et al.* (2016) on Northeastern Nigeria's camel. However, Igwenagu did not classify the age of his camel samples. The morphometric findings were double the measurements found in humans as reported by Abdullah *et al.*, 2010 and Moore *et al*., 2018, higher than those of the horses, rats, mice, AGRs, and most of the other domestic ruminants as reported by Ingbar, 1985; Dyce *et al.*, 2002; Capen and Martin, 2003. The similarities of these findings with the camels in Maiduguri confirm the cross-breeding between the camels in Northwest Nigeria and Northeast, as reported by Igwenagu *et al.* (2016) and Abdussamad *et al.* (2011), were exposed to similar environmental semi-arid conditions. The higher morphometric measurements of camel thyroid gland compared to humans and most domestic ruminants were due to peculiar characteristics of endurance of the camels and to bigger body size.

The determination of sexual dimorphism on the morphometric variables of the thyroid gland in the one-humped camel in this study was insignificant but found to be slightly higher in females than in males, especially in weight

and length, while on width and thickness of the thyroid gland, males have slightly higher values than the females. These findings were similar to the findings of Ahmadphanahi and Yousefi (2012) on the thickness's width and invariability. The result of this study corresponds with the report on Wistar rats by Capen and Martin (2003) that the weight of the thyroid gland was greater in females than in males but not statistically significant. These inferred and supported the notion of the traditional breeders that female one-humped camels were more agile than male one-humped camels due to their higher endurance to stressful conditions and exposure to reproductive hazards. Similarly, female dromedary camels reach sexual maturity around three years of age and mate around age four or five. Males begin to rut by age three but do not reach full sexual maturity until six years old (Mukasa-Mugerwa, 1981).

The two lobes of the thyroid gland were tested for the variability of their morphometric variables within each age group, but they were found to be statistically insignificant. Hence they are symmetrical, like humans (Singh, 2018; Moore and Dalley, 2018). However, thyroid gland lobes in cattle, sheep and dogs were reported to be asymmetric (McDonald, 2003). Therefore, one-humped camels were, by this correlation, closer to humans than most domestic animals.

Correlation analysis between the morphometry, hormones and age groups in this study revealed that the weight of the thyroid gland in one-humped camel was directly proportional to the animal's age, especially from the juvenile to the young adult, which showed a sharp increase in Figure 4.1. Amazingly, in the graph plotted in figure 4.3, the concentration of the thyroid hormones (T3 and T4) was inversely proportional to the weight and the age of the thyroid gland in the indigenous one-humped camel. Correspondingly, the histological studies revealed the appearance of numerous vacuolated colloids within the follicles and atrophy of the follicular cells in the young and old adult groups. However, in the juvenile age group, the vacuoles were less or absent, with intact thyroid cells without any sign of atrophy. It signifies that follicular cells, responsible for the synthesis and secretion of the thyroid hormones, which contribute to animal growth and metabolism, underwent programmed cell death. At the same time, some follicles develop vacuoles to reduce the quantity of the hormones as the one-humped camel grows older. These coincide with the findings in humans, where the weight and length of the thyroid gland decreased at an approximately proportional level with age (Abdullah *et al*., 2010). The number of thyroid follicles and parafollicular cells decreased with progressive age increase (Abdullah *et al*., 2010). As well as previous work on the human thyroid gland concerning the weight of the human thyroid gland found that a significant negative correlation existed between age and thyroid weight (Stefano *et al.,* 1995).

The histochemical study in this work revealed the presence of parafollicular cells in the thyroid gland tissues of the one-humped camel. However, the literature debated the presence or absence of the parafollicular cells in the thyroid gland of the one-humped camel between Kausar and Shahid (2006) and Rejeb *et al*. (2011) work. However, none of this work by those great scholars applied the special stain with specific affinity to parafollicular cells like Azure A Acid Hydrolysis, in which the hydrochloric acid in the solution (stain) hydrolysis and suppresses basophilia due to nucleic acids and any acid mucins, and distinctly stained the parafollicular cells with Azure A (Cook, 1974; Bancroft and Gamble, 2008; Suvarna *et al.,* 2019). This result also coincides with Ahmadpanahi and Yousefi (2012), who reported that parafollicular (C) cells were about 5% of the one-humped camel thyroid gland tissue cell population.

The shape of the thyroid gland follicles in the indigenous one-humped camel in this study's groups was round, oval and irregular. Still, it was more irregular in males of group C. Follicular epithelial cells encircled the follicles. Still, the follicular cells of neighboring follicles frequently come into contact with each other and disrupt the continuity of the basal lamina as seen within the follicles/ lobules, and are more prominent in males than in females of group A but more prominent in females than in males of groups B and C. The follicular cells are mostly resting/ inactive and low cuboidal in shape; the follicles were filled with colloids and had a smoother peripheral surface, the follicles with little or no vacuoles in both males and females of group A. While, groups B and C follicular cells were seen in an active secretive stage, more columnar in females than the males who were more cuboidal in shape. Some cells showed atrophy and high occurrence of vacuoles in the follicles, which were more prominent in females than in males. These variations in histology between the juvenile age group and the other two adult groups were indeed related to the hormonal secretions of the thyroid cells, which was discussed above.

In conclusion, the objectives of this study were achieved with the following contributions to the knowledge established:

1. Our gross anatomical study of the thyroid gland in a one-humped camel discovered that the arterial blood supply to the thyroid gland of a one-humped camel was from superior, middle and inferior thyroid arteries. Not from superior and inferior thyroid arteries as reported in other mammals
2. One-humped camels have relatively higher morphometric values of camel thyroid gland compared to humans and most domestic ruminants
3. Sexual dimorphism on the morphometric variables of the thyroid gland in the one-humped camel in this study was insignificant but slightly higher in females than in males, especially in weight and length.

4. The two lobes of the thyroid gland were symmetrical in size and position, like in humans, while they were reported asymmetrical in cattle, sheep and dogs. This made camel thyroid anatomical features closer to humans than most domestic animals.
5. That the weight of the thyroid gland in a one-humped camel was directly proportional to the age of the camel, and the concentrations of the thyroid hormones (T3 and T4) were inversely proportional to the weight and the age of the thyroid gland in the one-humped camel.
6. Histology studies in this study supported the above revelation in one-humped camels, by appearances of numerous vacuolated colloids within the follicles, atrophy of the follicular cells in old adult groups, and absence of that in younger camels.
7. The histochemical study confirm the presence of parafollicular cells in the thyroid gland tissues in a one-humped camel, using Azure A Acid Hydrolysis special stain

5. Limitation and Recommendation

Further studies on ultrastructures, immunohistochemical, genomics, proteomics, metabolomic studies, and characteristics should be carried out to understand these endocrine glands further. The study had a limited number of samples and hence should be increased.

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