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Behavioral Psychomotor Performance, Thyroid Functional and Structural Changes with Glucagon-Like Peptide-1 Receptor Agonists in Rats

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Abstract

Background: **Liraglutide, a glucagon-like peptide-1(GLP-1) receptor agonist, is used to treat patients with type-2 diabetes mellitus. It may have several adverse effects on thyroid glands as GLP-1 receptors are highly expressed in thyroid glands.** ***Aim of Study:*** **To investigate the effect of (liraglutide) on structure and function of thyroid gland in rats and its effect on behavioral and psychomotor function.** ***Material and Methods*****: This study was carried out in the animal house, Faculty of Medicine, Suez Canal University. Rats were divided randomly into 3 groups, 6 rats/group. Control group: rats received normal saline once daily, GLP -short term and GLP -long term groups: rats received (0.075 mg/kg/day) liraglutide intraperitoneally (i.p.) once daily for 11 days and 12 weeks respectively. Anxiety was assessed using open field test and forced swimming test for depressive-like behavior. Estimation of TSH, FT3, FT4 and calcitonin in addition to histopathological and immunohistochemical changes for calcitonin expression in thyroid tissue as a clinical biomarker for C-cell diseases were done.** ***Results:*** **Anxiety-like behaviors were significantly increased in GLP -long term group. Forced swimming showed longer immobility time in the GLP -long term group. Reduced TSH, elevated calcitonin in GLP -long term group. FT4 and FT3 were insignificantly elevated. Histopathological changes were noticed and immunohistochemical expression of calcitonin significantly increased in thyroid tissue of GLP -long term group.** ***Conclusion:*** **There is a probable high risk of thyroid hyperplasia and cancer in GLP -long term treatment.**

Keywords: Glucagon-Like Peptide, thyroid hyperplasia, calcitonin.

Introduction

Liraglutide, a GLP-1 receptor agonist, is an analog of human glucagon-like peptide-1 (1). GLP-1 is an essential hormone released by L-cells in the ileum after meals (2). It plays a role in increasing insulin secretion from the pancreas while suppressing glucagon secretion, which opposes insulin action (3). Liraglutide and exenatide are GLP-1 drugs that are approved by FDA for clinical use in treating type 2 diabetes mellitus (4,5). On 2010, the FDA declared liraglutide can be used once daily as a glycemic control agent in type 2 diabetes mellitus (4,5).

While GLP-1 analogs like liraglutide effectively lower blood glucose levels in type 2 diabetic patients (4,5), GLP-1 receptors are present in human thyroid glands, so they may have adverse effects on the thyroid glands (6) in rodents (7). GLP-1 receptors are G-protein coupled receptors and exhibit a 93% similarity between rats and humans (8,9).

Calcitonin is secreted by thyroid C-cells and considered as a biomarker for C-cell diseases and hereditary C-cell hyperplasia (10,11).

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Numerous studies have shown that activation of GLP-1 receptor leads to secretion of calcitonin, and this effect is diminished by the GLP-1 receptor antagonist [12]. Calcitonin induces a rapid but temporary decrease in blood calcium levels and is employed as a diagnostic marker for various C cell-derived lesions to confirm C-cell hyperplasia. It is also used to distinguish Medullary thyroid carcinoma (MTC) from other follicular cell-derived carcinomas [13].

Knudsen et al. Studied the effect of GLP-1 receptor agonists on rat C-cell lines [11]. They found that GLP-1 receptor agonists could stimulate the release of calcitonin and expression of calcitonin gene in rodent C cells . GLP-1 receptor agonists, include liraglutide and exenatide.

A minimum dose of liraglutide (0.075 mg/kg/day) is required to increase C-cell tumor formation in rats which is equivalent to the recommended dose for treating type 2 diabetes in humans [10,11].Two-year studies on mice, found that doses that raised calcitonin levels lead to C-cell hyperplasia and neoplasia [11]. As a general, vivo and vitro studies found that long-term GLP-1 receptor activation lead to increased calcitonin gene expression and C-cell proliferation.

Thyroid dysfunctions can impact mental health, potentially leading to mood abnormalities. These dysfunctions may result in emotional and cognitive disturbances [15]. Anxiety and depression are more prevalent in cases of hyperthyroidism [16,17] and self-rating scales have indicated elevated scores for depression and anxiety [18].

Material and Methods

This experimental study was conducted at the animal house and tissue culture unit located in the Center of Excellence, Faculty of Medicine, Suez Canal University. The study involved three distinct groups and the details of each group are as follows:

1. Control Group: This group consisted of 6 adult male albino rats with an average weight ranging from 175 to 187 grams. These rats were obtained from The Ophthalmology Research Institute in Giza, Egypt. In the control group, rats were administered 0.075 mg/kg/day of normal saline once daily.

2. GLP - Short Term Group: Another 6 adult male albino rats, also with an average weight of 175-187 grams and obtained from The Ophthalmology Research Institute, were part of this group. These rats were subjected to a short-term treatment with liraglutide. They were administered liraglutide (0.075 mg/kg/day) intra peritoneal (i.p.) once daily for a total of 11 days.[11,12].

3. GLP - Long Term Group: Similar to the previous two groups, this group comprised 6 adult male albino rats with an average weight in the specified range. They were obtained from The Ophthalmology Research Institute. The rats in this group received liraglutide intra peritoneal (i.p.) (0.075 mg/kg/day) once daily, but the dosing regimen was extended over a longer period of 12 weeks, representing a long-term treatment approach.[11,12]

These three groups of rats were used in the study to investigate the effects of liraglutide on them under both short-term and long-term treatment conditions.

Behavioral assessment:

I The open-field test for anxiety-like behavior evaluation

An open-field area consisting of 25 squares, each measuring 20x20 cm, was employed to assess anxiety-like behavior in the study. During a 5-minute**s**, a rat was initially positioned in the center . The time spent in the nine central squares, which is an indicator of lower anxiety levels, and the time spent in the peripheral 16 squares, which typically signifies higher anxiety, were both measured using video recordings.[14]

II Forced swimming test for depressive-like behavioral

A cylindrical container measuring 50x20 cm was used. This container was filled with water at 22°C to a height of 30 cm. The primary parameter measured in this test was the immobility time, which represented the time the rat spent passively floating in the water. This immobility time served as an

index to assess depressive-like behavior in the study [19].

III Biochemical analysis:

Blood samples were obtained from the retro-orbital plexus of the rats after administering light anesthesia with sodium pentobarbital at a dosage of 50 mg/kg via intraperitoneal (i.p.) injection, each rat had a 2 mL blood sample taken from its retro-orbital plexus for biochemical analysis. Immediate centrifugation at 2000× g for 15 min was performed on the blood samples following collection. The serum was chilled to −80 ◦C after separation until it was needed for various tests.This centrifugation process separated the serum, which was subsequently used for the estimation of TSH (Thyroid-Stimulating Hormone), Free T3 (Free Triiodothyronine),T4 (Free Thyroxine), and calcitonin levels by ELISA using Rat calcitonin ELISA kits from Origene company, Cat # SKU S-1197 and TSH ELISA kits from CucaBio company, Catalog No. CSB-E05115r FT3 from ABBKine Catalog no(KTE100169) , FT4 from ABBKine , cat no:KTE100170

IV Histological examination:

The rats were humanely euthanized through cervical dislocation, and their thyroid glands were extracted and weighed. The extracted thyroid glands were then fixed in a solution of 4% buffered formalin. Subsequently, 4-µm sections of these glands were embedded in paraffin and prepared for hematoxylin-eosin (H&E) staining.

For image capture and analysis, the following equipment and software were used:

- A calibrated standard digital microscope camera, TuopCam® XCAM1080PHA, from Hangzhou, Zhejiang, China. This camera has a resolution of 10 megapixels, resulting in images with dimensions of 3656 x 2740 pixels.
- "ToupView" software will be employed for capturing images and enhancing their quality.
- All slides stained with H&E will be captured at their original magnifications of 100x and 400x, using objectives of 10x and 40x, respectively, with the UIS optical system (Universal Infinity System) from Olympus® in Japan.

V Detection of calcitonin by immunohistochemistry:

The detection system employed in the study is the Polymer HRP system. Here are the fixation requirements and interpretation results:

FIXATION REQUIREMENTS:

A. The volume of formalin fixative used at least 10 times the specimen volume.

B. Strong acid-based decalcification solutions should not be utilized.

C. Specimens need to be immersed in the fixative within 1 hour of the biopsy or resection procedure. It's important to note and record both the time of specimen removal and the time of immersion in the fixative.

D. For all resection (large) specimens, the tumor must be bisected (cut in half) prior to immersion in the fixative.

INTERPRETATION RESULTS:

The interpretation results for calcitonin staining are as follows:

- Non Immunoreactive: This indicates that the specimen is negative for calcitonin, meaning that there is no immunoreactivity or presence of calcitonin in the sample.
- Immunoreactive: This indicates that the specimen is positive for calcitonin, meaning that there is immunoreactivity and the presence of calcitonin in the sample.

Statistical analysis:

All quantitative data collected during the study were subjected to statistical analysis using SPSS statistical software version 20. The results were reported in the format of (mean ± SD). To compare the means of a specific variable across the three groups, the study employed a one-way analysis of variance (ANOVA). If the ANOVA analysis revealed a statistically significant difference among the groups, post hoc tests, specifically Tukey tests, were subsequently employed to conduct a more detailed and in-depth analysis of the results. This approach allowed for the identification of specific

group differences following the initial ANOVA analysis.

Ethical consideration:

The study emphasized the importance of ethical considerations in the context of experimental animal research. It adhered to mandatory ethical guidelines.

The study protocol was approved by the official approval of the Ethics Committee at the Faculty of Medicine, Suez Canal University in Egypt. The ethical approval for the study was granted, and it was assigned the unique ethical approval number 4849. This approval demonstrates the commitment to upholding ethical standards and ensuring the welfare and ethical treatment of animals used in the research.

Ethical consideration for the animal experiment:

In this experimental study, the utilization of rats was justified based on valid scientific objectives, and there were no viable alternatives available at the current stage of knowledge that could replace their use. The experiment held a high probability of achieving the specified research goals, which were deemed to have a reasonable potential for contributing to human welfare. These considerations support the ethical basis for using rats as experimental subjects in this study.

Results:

I. The open-field test for anxiety-like behavior evaluation

Anxiety-like behavior was assessed in open-field area, distinguishing between the central and peripheral zones. The results of ANOVA showed significant differences among groups in both time spent in the central zone and grooming frequency ($P < 0.001$).

Specifically, both anxiety-like behaviors were notably increased in the GLP -long term group. This group spent significantly less time in the central areas during the 5-minute period in comparison to the control group (87.21 ± 6.41 seconds vs. 133.12 ± 11.32 seconds, $P<0.05$). Additionally, the GLP -long term group exhibited a significantly lower grooming frequency (3.02 ± 1.37 seconds vs. 13.01 ± 2.31 seconds, $P<0.05$). In contrast, reduced both anxiety-like behaviors as indicated by increasing center ambulation time(133.12 ± 11.32 (control group), 125.22 ± 11.39 (GLP-short term) vs 87.21 ± 6.41(GLP-long term) ,$p<0.05$ for both) and increased grooming frequency(13.01 ± 2.31 (control group), 9.21 ± 3.01 vs 3.02(GLP-short term) ± 1.37 (GLP-long term),$p<0.05$), in the control and GLP -short term groups when compared to the GLP -long term group (table 1).

Table (1): The open-field test for anxiety-like behavior evaluation. center ambulation time and grooming frequency

Groups	center ambulation time/s	grooming frequency/s
Control	133.12 ± 11.32	13.01 ± 2.31
GLP -short term	125.22 ± 11.39	9.21 ± 3.01
GLP -long term	87.21 ± 6.41[ab]	3.02 ± 1.37 [ab]

Results were expressed as mean ± SD and analyzed using one-way ANOVA followed by Bonferroni's *post-hoc* test at *P*<0.05. control, GLP -short term and GLP -long term Superscripts [a, b] Represents a statistically significant decrease when compared to control and GLP -short term respectively.

II. Forced swimming test for depressive-like behavioral

The results of a one-way ANOVA revealed significant differences among the study groups ($P < 0.001$). In terms of immobility time, it was significantly longer in the GLP -long term group when compared to the control group (14.25 ± 1.09 seconds vs. 2.02 ± 1.03 seconds). In contrast, both the control and GLP -short term groups displayed significantly shorter immobility times compared to GLP-long term (2.02 ± 1.03 and 4.21 ± 1.27 respectively vs 14.25 ± 1.09, $P<0,05$) (table 2).

Table (2): Forced swimming test for depressive-like behavioral	
Groups	Immobility time /s
Control	2.02 ± 1.03
GLP -short term	4.21 ± 1.27
GLP -long term	14.25 ± 1.09 [ab]

Results were expressed as mean ± SD and analyzed using one-way ANOVA followed by Bonferroni's *post-hoc* test at *P*<0.05. control, GLP -short term and GLP -long term, Superscripts[a, b] Represents a statistically significant longer when compared to control and GLP -short term respectively.

III. Biochemical analysis

1. Calcitonin Marker:

Serum calcitonin level was increased significantly in the GLP -long term group ($p < 0.05$) when compared to the control and GLP -short term group (0.176±0.033 (GLP-long term vs 0.024±0.008 (control), 0.024±0.009 (GLP-short term) $p<0.05$)
(table3, figure 1).

2. Serum TSH (Thyroid-Stimulating Hormone):

Serum TSH level was decreased significantly in the GLP -long term group when compared to the control and GLP -short term group (0.02±0.008 (GLP long term) vs 0.278±0.15 (control), 0.25±0.102(GLP-short term), $p < 0.05$). These differences are illustrated in figure 2 and were confirmed through one-way ANOVA and post hoc testing.

Serum FT3 was significantly increased in the GLP -long term group compared to the control and in the GLP -short term group compared to the control (3.96±0.578 (GLP long term) vs 3.01±0.435 (control), 3.91±0.365(GLP-short term),$p < 0.05$). These findings are depicted in figure 3 and were established through one-way ANOVA and post hoc analysis.

4. Serum FT4 (Free Thyroxine):

Serum FT4 did not exhibit a significant difference in the GLP -long term group when compared to the control or in the GLP -short term group compared to the control
(1.96±0.332 (GLP long term) vs 1.78±0.370 (control), 1.94±0.178 (GLP-short term), $p > 0.05$). . This is demonstrated in table 3, figure 4 and was determined through one-way ANOVA and post hoc testing.

These results provide insight into the impact of long-term GLP-1 treatment on the levels of calcitonin, TSH, FT3, and FT4 in the study subjects

3. Serum FT3 (Free Triiodothyronine):

Table (3): Biochemical analysis				
Groups	Calcitonin(ng/ml)	TSH (mIU/L)	FT3(pmol/L)	FT4(pmol/L)
Control	0.024 ±0.008	0.278±0.150	3.018±0.435	1.78±0.370
GLP -short term	0.024 ±0.009	0.251±0.102	3.91±0.365[a]	1.94±0.178
GLP -long term	0.176 ± 0.003 [ab]	0.021±0.008[ab]	3.96±0.578[a]	1.96±0.332

Results were expressed as mean ± SD and analyzed using one-way ANOVA followed by Bonferroni's *post-hoc* test at *P*<0.05. control, GLP -short term and GLP -long term, Superscripts [a, b] Represents a statistical significance when compared to control and GLP -short term respectively.

Figure 1

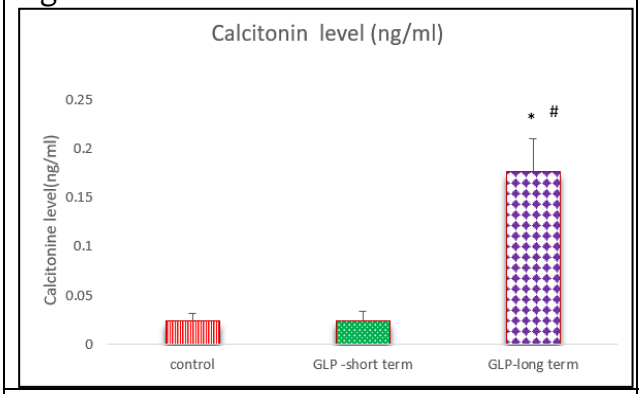


Figure 2

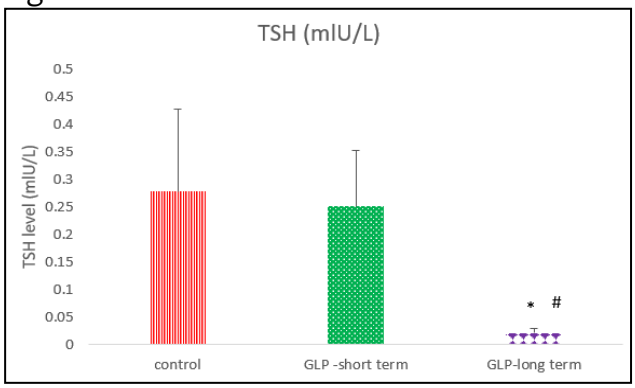


Figure 3

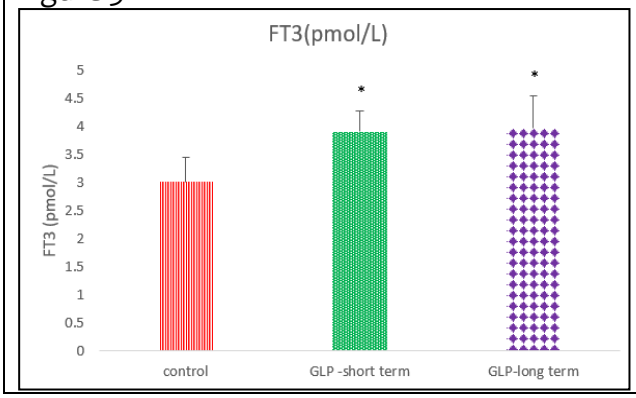


Figure 4

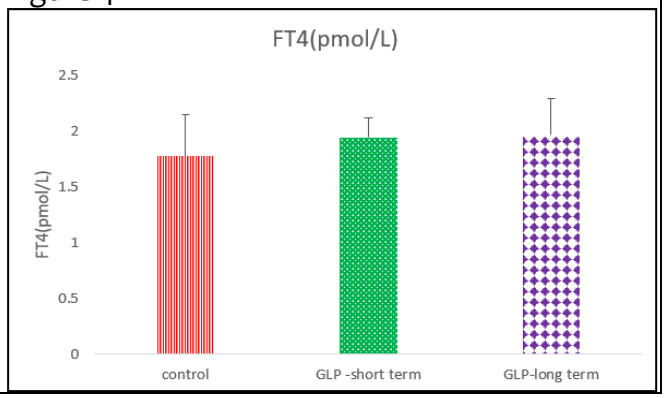


Figure 1: Calcitonin level (ng/ml) in all study groups: Results of calcitonin were expressed as mean ± SD and analyzed using one-way ANOVA followed by Bonferroni's post-hoc test at $P < 0.05$. GLP -long term group show increased significantly compared to control and GLP -short term groups, respectively ($n = 6$). Superscripts *****, # Represents a statistically significant difference when compared to control and GLP -short term respectively

Figure 2: TSH level (mIU/L) in all study groups: Results of TSH were expressed as mean ± SD and analyzed using one-way ANOVA followed by Bonferroni's post-hoc test at $P < 0.05$. GLP -long term group show decreased significantly compared to control and GLP -short term groups, respectively ($n = 6$).
Superscripts *,# Represents a statistically significant difference when compared to control and GLP -short term respectively

Figure 3: FT3 level (pmol/L) in all study groups: Results of FT3 were expressed as mean ± SD and analyzed using one-way ANOVA followed by Bonferroni's post-hoc test at $P < 0.05$. Serum FT3 significantly increased in GLP -long term group compared to the control and in GLP -short term group compared to the control.

Figure 4: FT4 level (pmol/L) in all study groups: Results of FT4 were expressed as mean ± SD and analyzed using one-way ANOVA followed by Bonferroni's post-hoc test. Serum FT3 show No significant difference in GLP -long term group compared to the control and in GLP -short term group compared to the control.

IV. Histological examination:

In the GLP -short term group, no significant difference was observed when compared to the control group. Furthermore, the thyroid tissue in the GLP -short term group displayed no evidence of neoplastic growth.

Conversely, in the GLP -long term group, a significant difference was detected in comparison to the control group. The thyroid tissue in the GLP -long term group exhibited the presence of follicles containing colloid material, along with a nodule of tumor encased within a fibrous capsule. This nodule was comprised of epithelial cells characterized by oval vesicular uniform nuclei and eosinophilic cytoplasm. The observed nodule is indicative of an adenoma, representing abnormal growth in the thyroid tissue.

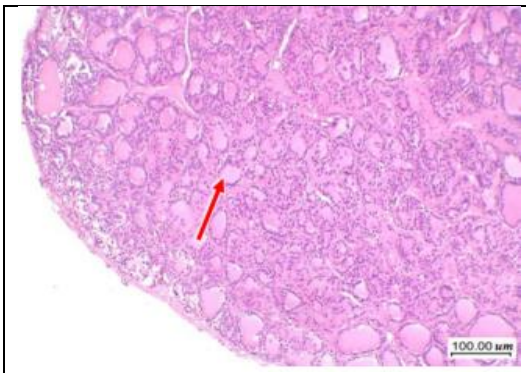
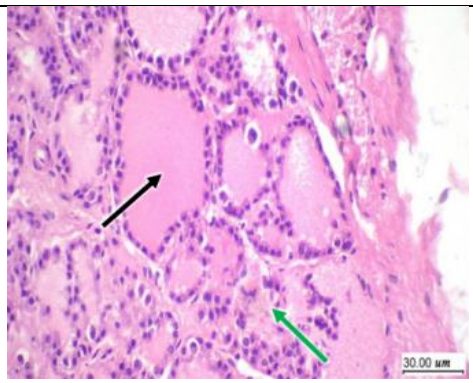
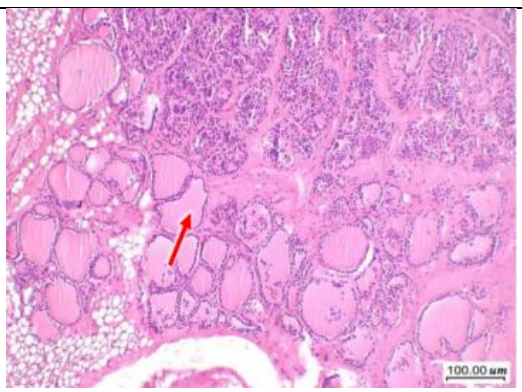
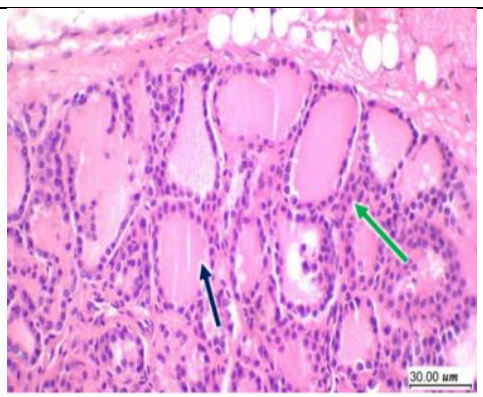
V. Immunohistochemistry:

Calcitonin expression in the GLP -short term group, there was no significant difference compared to the control group. Furthermore, the thyroid tissue in the GLP -

short term group exhibited no specific staining for calcitonin, and the epithelial cells in both the normal and GLP -short term groups were negative for calcitonin. These findings indicate a non-immunoreactive, negative status for calcitonin in both the normal and GLP -short term groups.

Conversely, in the GLP -long term group, a significant difference was observed in comparison to the control group. The thyroid tissue in the GLP -long term group revealed a tumor nodule that exhibited positive cytoplasmic staining for calcitonin, signifying an immunoreactive, positive status for calcitonin. This suggests that the long-term GLP-1 treatment was associated with the presence of calcitonin in the tumor nodule within the thyroid tissue.

Figure 5: H&E findings in Thyroid gland tissue

<table>
<tr><td>  </td><td>  </td></tr>
<tr><td colspan="2">Normal thyroid tissue formed of small to moderate sized follicles (red arrow) lined by epithelial cells showing regular nuclei (green arrow) and containing colloid material (black arrow).</td></tr>
<tr><td colspan="2">GLP-1 Short term</td></tr>
<tr><td>100X</td><td>400X</td></tr>
<tr><td>  </td><td>  </td></tr>
<tr><td colspan="2">Thyroid tissue showing no evidence of neoplastic growth, formed of small to moderate sized follicles (red arrow) lined by epithelial cells showing regular nuclei (green arrow) and containing colloid material (black arrow).</td></tr>
</table>

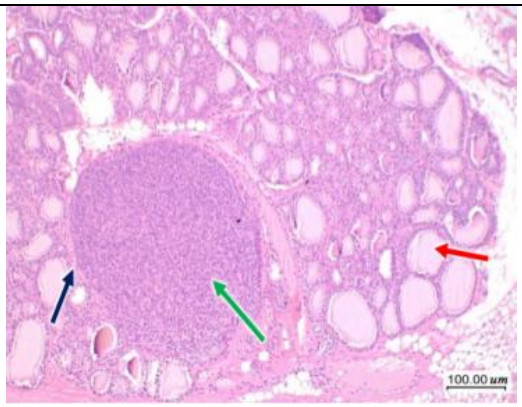
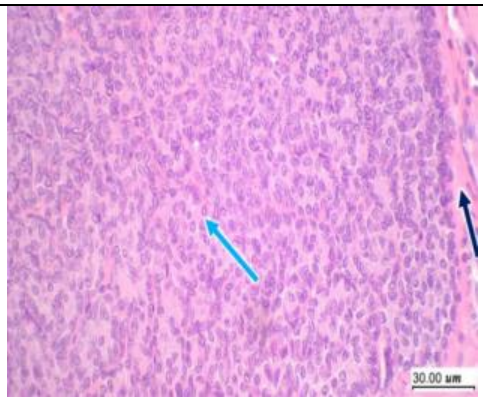
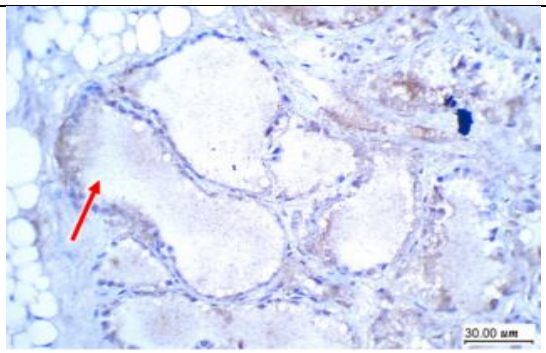
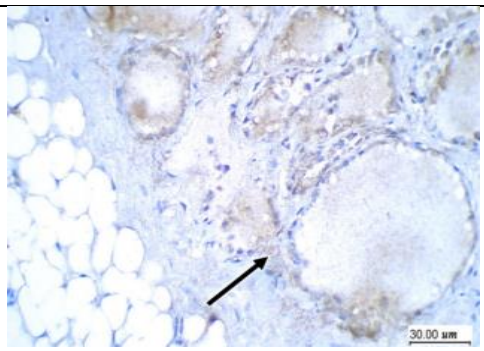
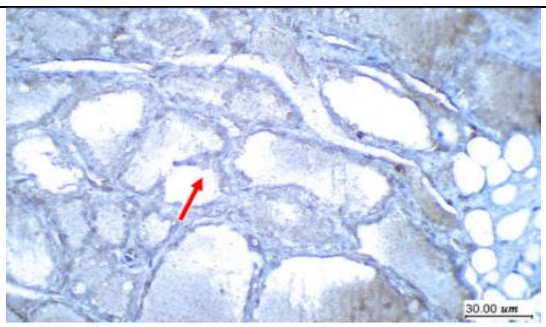
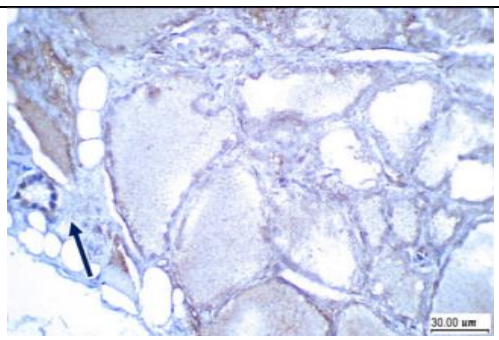
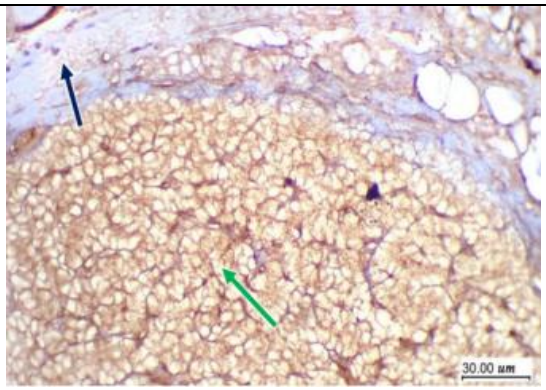
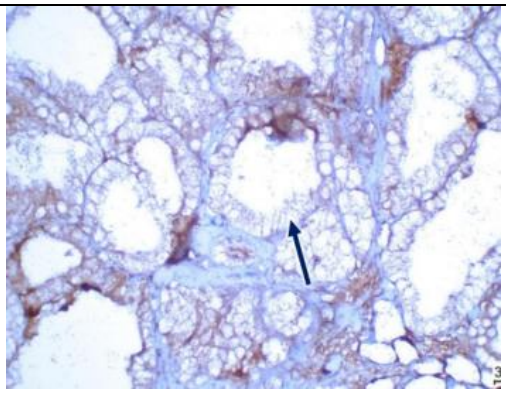
Long term	
100X	400X
	
Sections in thyroid tissue show follicles containing colloid material (red arrow) with a nodule of tumor (green arrow) surrounded by fibrous capsule (black arrow). Nodule is formed of epithelial cells having oval vesicular uniform nuclei with eosinophilic cytoplasm (blue arrow). The nodule represents an adenoma.	

Figure 6: Calcitonin expression by (Immunohistochemstry)

Normal	
400X	400X
	
Thyroid tissue showing no specific staining for calcitonin (colloid background, red arrow) with epithelial cells are negative for calcitonin (black arrow).	
Short term	
400X	400X
	
Thyroid tissue showing no specific staining for calcitonin (colloid background, red arrow) with epithelial cells are negative for calcitonin (black arrow).	

Long term	
400X	400X
	
Sections in thyroid tissue show tumor nodule positive for cytoplasmic staining of calcitonin (green arrow), while surrounding thyroid tissue (black arrow) was negative for calcitonin.	

Discussion

Comprehensive information outlines several important findings and observations from our study, especially the effects of GLP-1 receptor agonists on the thyroid and related consequences.

The study observed that the risk of thyroid disorders are increased especially with GLP-1 receptor agonists administration [20], with particular attention to liraglutide and dulaglutide, suggesting an elevated risk compared to both placebo and other antidiabetic medications [21].

Rodent studies indicated that GLP-1 receptor activation may lead to the proliferation of thyroid C-cells and the development of thyroid C-cell tumors [21].

The study suggests a potential connection between the activation of specific signaling pathways by GLP-1 and the development of thyroid cancer, particularly papillary thyroid carcinoma (PTC) [25]. GLP-1 receptors have been identified in papillary thyroid carcinoma tissues, further underscoring their potential role in thyroid-related conditions [23,24].

In our study, the long-term administration of GLP-1 receptor agonists for 12 weeks was associated with changes in thyroid tissue, including the presence of follicles containing colloid material with a nodule of tumor surrounded by fibrous capsule representing an adenoma which was not observed in the short-term group.

Other studies, two-year studies on mice, found that doses that raised calcitonin levels lead to C-cell hyperplasia and neoplasia.

Clinical findings in our study indicated a decrease in serum TSH levels in the long-term GLP-1 group which was in contrast with some previous studies associating elevated TSH levels with thyroid malignancy [26,27]. This study also found changes in serum FT3 and FT4 levels with an increase in FT3 and no significant change in FT4. Other studies have shown associations between thyroid hormones (T4 and T3) and thyroid cancer like Kim et al; found that elevated T3,T4 may be correlated with malignancy as well. Cho et al. found that free T4 (fT4) is increased in patients with thyroid cancer [28]. Inversely, Jonklaas et al. found that it's related to total T3 [29].

The studies confirmed the relationship between GLP-1 receptor activation and calcitonin secretion [12] with calcitonin being an important biomarker for C-cell diseases like thyroid carcinoma [10,11].

In our study, serum calcitonin levels and immunohistochemistry results indicated increased calcitonin levels in the long-term GLP-1 group, whereas the short-term group did not exhibit specific calcitonin staining. Many other studies revealed that thyroid dysfunctions could affect mental health and

can cause abnormalities, anxiety and depression [15,16].

Also, our study linked thyroid dysfunctions to mood abnormalities, including anxiety and depressive-like behavior, which were assessed through behavioral tests and showed significant differences in the long-term GLP-1 group compared to the short term GLP-1group and control.

Overall, the study provides valuable insights into the complex relationship between GLP-1 receptor agonists and thyroid function, and their potential impact on mental health and mood. It highlights the need for further research and clinical attention to these relationships and to discover the underlying mechanisms.

Conclusion

The study highlights the association between GLP-1 receptor agonists and thyroid disorders. While the findings suggest this link. The limited sample size prevented a detailed examination of specific thyroid disorders. To gain a more comprehensive understanding of the relationship between GLP-1 receptor agonists and thyroid disorders, including thyroid cancer, it is essential to conduct large-scale, long-term studies (RCTs). These RCTs should focus on primary or secondary outcomes related to thyroid disorders. Such research would provide more definitive and precise insights into the potential risks associated with the use of GLP-1.

Conflict of Interest:

The authors affirm that they have no relevant affiliations or financial interests with any organization or financial conflicts related to the subject matter or materials discussed in the manuscript. This includes factors such as employment, consultancies, honoraria, stock ownership or options, expert testimony, grants, patents received or pending, or royalties. The study is conducted with impartiality and without external financial influence.

References

1. Bock T, Pakkenberg B, Buschard K. The endocrine pancreas in non-diabetic rats after short-term and long-term treatment with the long-acting GLP-1 derivative NN2211.APMIS.2003 ; 111(12), 1117-1124.
2. Mojsov S, Weir GC, Habener JF. Insulinotropin: glucagon-like peptide I (7-37) co-encoded in the glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas. Journal of Clinical Investigation .1987; 79(2), 616-619.
3. Nauck MA, Heimesaat MM, Orskov C, Holst JJ, Ebert R, Creutzfeldt W. Preserved incretin activity of glucagon-like peptide-1[7-36amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. Journal of Clinical Investigation;1993 , 91(1), 301-307.
4. Buse JB, Rosenstock J, Sesti G, et al. Liraglutide once a day versus exenatide twice a day for type 2 diabetes: a 26-week randomised, parallel-group, multinational, open-label trial (LEAD-6). The Lancet ;2009 , 374(9683), 39-47.
5. Chia CW, Egan JM Incretin-based therapies in type 2 diabetes mellitus. Journal of Clinical Endocrinology and Metabolism; 2008;93(10), 3703-3716
6. Gier B, Butler PC, Lai CK, Kirakossian D, DeNicola MM, Yeh MW. Glucagon-like peptide-1 receptor expression in the human thyroid gland. Journal of Clinical Endocrinology and Metabolism;2012, 97(1), 121-131.
7. Knudsen LB, Madsen LW, Andersen S, et al. Glucagon-like peptide-1 receptor agonists activate rodent thyroid C-cells causing calcitonin release and C-cell proliferation. Endocrinology; 2010 , 151(4), 1473-1486.
8. Mayo KE, Miller LJ, Bataille D, Dalle S, Göke B, Thorens B, et al. International Union of Pharmacology. XXXV. The glucagon receptor family. Pharmacological Reviews.2003; 55(1), 167-194.

9. Knudsen LB, Madsen LW, Andersen S, et al. Glucagon-like peptide-1 receptor agonists activate rodent thyroid C-cells causing calcitonin release and C-cell proliferation. Endocrinology.2010;151(4), 1473-1486.
10. Elisei R, Bottici V, Luchetti F, et al. Impact of routine measurement of serum calcitonin on the diagnosis and outcome of medullary thyroid cancer: experience in 10,864 patients with nodular thyroid disorders. Journal of Clinical Endocrinology and Metabolism.2004; 89(1), 163-168.
11. "Victoza (liraglutide injection): Human relevance of rodent thyroid C-cell tumors,"2009,http://www.fda.gov/downloads/AdvisoryCommittees/Committees%20MeetingMaterials/Drugs/ Endocrinologic and Metabolic Drugs Advisory Committee/UCM151129.pdf
12. Lamari Y, Boissard C, Moukhtar MS, Jullienne A, Rosselin G, Garel JM. Expression of glucagon-like peptide 1 receptor in a murine C cell line: regulation of calcitonin gene by glucagon-like peptide 1. FEBS Letters .1996; 393(2-3), 248-252.
13. Crespel A, De Boisvilliers F, Gros L, Kervran A.Effects of glucagon and glucagon-like peptide-1-(7-36) amide on C cells from rat thyroid and medullary thyroid carcinoma CA-77 cell line. Endocrinology.1996; 137(9), 3674-3680.
14. Zghari O, Rezqaoui A, Ouakki S, Lamtai M, Chaibat J, Mesfioui A, El Hessni A, Rifi EH, Essamri A .Effect of chronic aluminum administration on affective and cognitive behavior in male and female rats. Journal of Behavioral and Brain Sciences. 2018; 8(4), 179-196.
15. Wolkowitz OM, Rothschild AJ. Psychoneuroendocrinology: The Scientific Basis of Clinical Practice. American Psychiatric Publishing, Inc . 2003.
16. Zader SJ, Williams E, Buryk MA.Mental health conditions and hyperthyroidism. Pediatrics.2019; 144(5), e20182874.
17. Bunevicius R, Velickiene D, Prange AJ Jr.Mood and anxiety disorders in women with treated hyperthyroidism and ophthalmopathy caused by Graves' disease. General Hospital Psychiatry.2005; 27(2), 133-139.
18. Demet MM, Özmen B, Deveci A, Boyvada S, Adıgüzel H, Aydemir Ö.Depression and anxiety in hyperthyroidism. Archives of Medical Research.2002; 33(6), 552-556.
19. Rashwan EH, Kamel MM, El-lethey HS, Ciobica A, El Iraqi KG. Caffeine ameliorating effect on anxiety and depression in an aluminum chloride-induced Alzheimer's disease Rat Model. Science. 2018; 7(3).
20. Knudsen LB, Madsen LW, Andersen S, Almholt K, de Boer AS, Drucker DJ, et al. .Glucagon-Like Peptide-1 Receptor Agonists Activate Rodent Thyroid C-Cells Causing Calcitonin Release and C-Cell Proliferation. Endocrinology.2012;151(4), 1473-1486.
21. Brown E, Heerspink HJL, Cuthbertson DJ, Wilding JPH. SGLT2. Inhibitors and GLP-1 Receptor Agonists: Established and Emerging Indications. The Lancet.2021;398(10296), 262-276.
22. Gier B, Butler PC, Lai CK, Kirakossian D, DeNicola MM, Yeh MW. Glucagon Like Peptide-1 Receptor Expression in the Human Thyroid Gland. Journal of Clinical Endocrinology and Metabolism. 2012; 97(1), 121-131
23. Madsen LW, Knauf JA, Gotfredsen C, Pilling A, Sjögren I, Andersen S, et al.GLP-1 Receptor Agonists and the Thyroid: C-Cell Effects in Mice are Mediated via the GLP-1 Receptor and Not Associated With RET Activation. Endocrinology .2012; 153(3), 1538-1547.
.24. van den Brink W, Emerenciana A, Bellanti F, Della Pasqua O, van der Laan JW .Prediction of Thyroid C-Cell Carcinogenicity After Chronic Administration of GLP1-R Agonists in Rodents. *Toxicology and Applied Pharmacology.2017; 320, 51-59.
25. Zhang S, Zhang X, Liu R, Guan H, Zhang H. Effects of Insulin Analogs and Glucagon-Like Peptide-1 Receptor Agonists on Proliferation and Cellular Energy Metabolism in Papillary Thyroid Cancer. Oncology Targets and Therapy.2017; 10, 5621-5631.
26. Sencar ME, Sakiz D, Calapkulu M, Hepsen S, Kizilgul M, Ozturk IU, et al. The Effect of Exenatide on Thyroid-Stimulating Hormone and Thyroid Volume. European Thyroid Journal.2019; 8(6), 307-311.
27. Kim HK, Yoon JH, Kim SJ, Cho JS, Kweon SS, Kang HC. Higher TSH level is a risk factor for differentiated thyroid cancer. Clinical Endocrinology (Oxford.2013; 78(3), 472-477.
28. Cho YA, Kong SY, Shin A, Lee J, Lee EK, Lee YJ, Kim J. Biomarkers of thyroid function and

autoimmunity for predicting high-risk groups of thyroid cancer: a nested case-control study. BMC Cancer.2014; 14(1), 873.
29. Jonklaas J, Nsouli-Maktabi H, Soldin SJ. Endogenous thyrotropin and triiodothyronine concentrations in individuals with thyroid cancer. Thyroid.2008; 18, 943-952