ORIGINAL ARTICLE

Maternal Hypothyroidism-Induced Impaired Development of Neurons in Rat Offspring: Insights for Antenatal Care

Tayyaba Fahad*, Shabana Ali, Tayyaba Qureshi, Noor Fatima Khan

ABSTRACT

Objective: To determine the effect of maternal hypothyroidism on the development of neurons in the motor cortex of rat pups by using light microscopy.

Study Design: Laboratory-based experimental study.

Place and Duration of Study: The study was carried out in collaboration with the National Institute of Health (NIH) Islamabad and the Department of Anatomy, Islamic International Medical College, Riphah International University, Islamabad, Pakistan from March 2023 to May 2023.

Methods: In this study, 24 females and 12 male Sprague dawley rats were sorted into control and hypothyroid groups. The control group received a standard diet and plain drinking water throughout the study. In contrast, the hypothyroid group had 4.5mg of propylthiouracil administered per rat per day, mixed into their drinking water. This treatment started a week before mating and continued until three weeks after delivery. Once successful mating was confirmed via vaginal plugs, gestation proceeded, and newborn pups were born after three weeks, having full access to maternal lactation for 21 days. On the 22nd day after birth, dissection took place, and the brains of the pups were carefully removed. Coronal sections of the motor cortex were then obtained for subsequent examination.

Results: The control group displayed a typical pattern of neurogenesis, characterized by the normal structure of cortical neurons. On the other hand, intracellular vacuolation, glial cell necrosis, and reduced Nissl rim thickness were statistically significant in hypothyroid group which collectively pointed to atypical neurogenesis.

Conclusion: This study highlights the adverse effects of maternal hypothyroidism on the structural integrity of cortical neurons. It focuses on increasing awareness and implementing thorough prenatal healthcare strategies.

Keywords: *Hypothyroidism, Motor Cortex, Neurogenesis.*

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Introduction

Maternal hypothyroidism, a medical condition that can arise during pregnancy, is marked by elevated levels of thyroid-stimulating hormone in the blood, impacting fetal development.[1] During human development, the initiation of fetal thyroid hormone activation typically occurs between the 10th and 16th week of gestation, which closely corresponds with the second trimester of pregnancy. However, it's essential to acknowledge that the primary phase of neurogenesis predominantly unfolds in the first trimester. Similarly, in the case of rats, thyroid hormone activation generally commences after the 18th day of embryonic life, aligning with the critical period for normal brain development that spans from the 12th to the 18th embryonic days.[2] As a result, during this early developmental phase, the

Department of Anatomy
Islamic International Medical College, Al-Mizan Campus, Rawalpindi, Pakistan

Correspondence:
Dr. Tayyaba Fahad
Assistant Professor, Anatomy
Islamic International Medical College
Riphah International University, Islamabad, Pakistan
E-mail: tayyaba.fahad@riphah.edu.pk

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developing fetus relies on maternal thyroid hormones for neurogenesis, influencing neuronal proliferation, migration, differentiation, and myelination.[3-5]

The neocortex, a prominent cortex area, plays a central role in coordinating the planning, initiation, and execution of voluntary movements in rodents. Additionally, it acts as a central hub for receiving input from various brain regions and effectively integrating sensory information with motor commands. Rats can acquire and improve motor skills through repetitive training, with the neocortex taking on a crucial role in storing and refining motor representations.[4]

Propylthiouracil (PTU) is an antithyroid medication because it inhibits the conversion of inactive thyroid hormones into their active forms, ultimately affecting the storage of thyroid hormones within the thyroid gland.[5] It is employed to establish animal models of maternal hypothyroidism in rats.[6,7]

Inadequate thyroid hormone levels inducing oxidative stress,[8] cause increased DNA fragmentation, leading to the dispersal of the rough endoplasmic reticulum and resulting in neuronal injury. This manifests as neuronal necrosis, pyknotic nuclei, and diminished Nissl rim thickness,[9] hampering the process of neuronal development. Numerous rat studies have illuminated the consequences of maternal hypothyroidism on cognitive functions and coordinated motor skills. However, most of these investigations have predominantly prioritized the examination of biochemical markers and gene expressions, with limited attention given to histological parameters.[10,11]

The objective of this study is to provide histological evidence of the influence of maternal hypothyroidism on neurogenesis in the motor cortex of rat offspring. This study suggests the adoption of comprehensive and well-organized screening programs aimed at promoting optimal fetal brain development. Additionally, it highlights the importance of integrating serum TSH biochemical testing as a valuable tool for identifying maternal hypothyroidism during prenatal examinations.

Methods

This experimental study was conducted in collaboration with the National Institute of Health (NIH) Islamabad and the Anatomy Department of Islamic International Medical College, Riphah International University, Islamabad, Pakistan from March 2023 to May 2023 following the approval of the study synopsis by the ethics review committee held on 17th January 2023 as indicated under Reference Number Riphah/IIMC/IRC/22/2077. A total of twenty-four females and twelve male Sprague Dawley rats, aged between 12 to 16 weeks and weighing in the range of 250 to 300 grams, were selected by a convenient sampling method. These rats were housed in a controlled environment, with conditions that included maintaining a consistent temperature of 22 ± 0.5°C, adhering to a 12-hour light-dark cycle, and sustaining a humidity level of 50%.

Twenty four female and 12 male Sprague Dawley rats aged between 12 to 16 weeks, weighing within the range of 250 to 300 grams were divided into two separate groups, with dietary and water regimens commencing one week before the mating and remaining consistent throughout the entire gestation, weaning, and up to the 22nd day following delivery.[12]

Control Group

6 females and 3 male Sprague Dawley rats were fed a standard chow diet and received water via the oral route.

Hypothyroid Group

18 female and 9 male rats were administered a daily oral dose of 4.5mg of propylthiouracil/rat.

Before the mating process, the thyroid hormone levels of all female rats were examined to verify the absence of natural hypothyroidism. Mating occurred within 12 separate cages, comprising 3 control cages and 9 hypothyroid cages. Each of these cages housed 2 female rats and 1 male rat. Pregnancy confirmation was established by observing the presence of a vaginal plug on the first day of gestation.[12]

On the 10th day of gestation, the thyroid hormone levels of maternal rats were re-evaluated to verify the presence of hypothyroidism. The pregnancy progressed for 3 weeks, during which the pups were delivered, and for the subsequent 3 weeks, they had unrestricted access to their mother's feed.[13]

12 full-term rat pups,[14] comprising both males and females without any congenital abnormalities, were

incorporated into the study while premature pups or those displaying evident congenital abnormalities, were not included in this study.

On the 22nd day, 12 pups from each group were subjected to anesthesia and subsequently sacrificed. The skulls of each animal were then carefully opened to provide access to the cerebrum, which was dissected with precision. A portion of the specimens was preserved in neutral buffered formalin (10%) for a 24-hour duration, after which a dehydration process involving increasing concentrations of alcohol was employed. Following this, the specimens underwent clearing and were embedded in paraffin wax. Subsequently, tissue sections with a thickness of 5 microns were obtained using a microtome, and these sections were subjected to staining. Hematoxylin and Eosin staining was employed to examine the cytoarchitecture of cortical neurons, while Toluidine staining was used to assess the thickness of the Nissl rim.[15]

Histological Analysis

High-resolution images of the slides were captured using an Olympus BX53 microscope at magnifications of 4x, 10x, and 40x. Subsequently, these images were transferred to a laptop for in-depth analysis, utilizing Image J software. A systematic evaluation of microscopic parameters was conducted on sections obtained from both cerebral hemispheres. This assessment encompassed four specific regions on each hemisphere, commencing from the midline and extending towards both the right and left sides.

Statistical analysis

The data obtained from the adjusted images was recorded and subsequently analyzed using SPSS version 26. An independent samples t-test was used to assess potential differences in means for quantitative variables and the outcomes were reported as Mean ± Standard Deviation (SD). To examine variances between groups for qualitative factors, the Chi-square test was applied. Significance was determined at the conventional threshold of $p < 0.05$, which is commonly recognized as the criterion for statistical significance.

In this study, the Imperial system was used units of measurements.

Results

Granule Cell Necrosis

In the control group, 91.7% of the rats displayed no granule cell necrosis (Grade 0), while 8.3% exhibited minimal necrosis (Grade 1). Conversely, in the hypothyroid group (as illustrated in Figure 1).

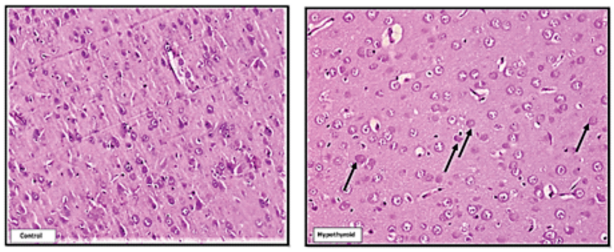

Fig 1: Granule Cell Necrosis (H & E stain) represented by black arrows in Hypothyroid group as compared to Control group. The photomicrograph was taken at 400X

16.7% of the rats exhibited mild necrosis (Grade 2), 16.7% demonstrated moderate necrosis (Grade 3), and 66.7% showed severe necrosis (Grade 4). The results of the chi-square analysis revealed a highly significant difference between the two groups, with a *p*-value less than 0.001 **(**Figure 2, Table 1), underscoring the notable contrast in the occurrence of granule cell necrosis.

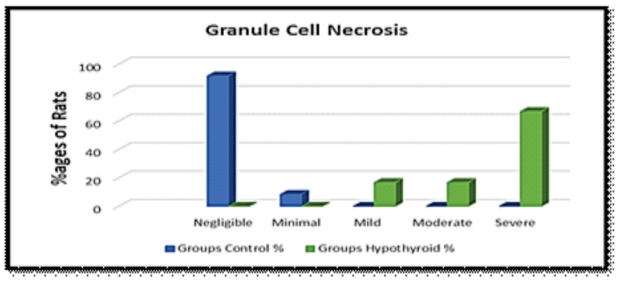

Fig 2: Bar graph showing grades of granule cell necrosis in Control and Hypothyroid groups

Table 1: Statistical comparison of means of granule cell necrosis in both groups

Histological Parameter	Groups	
Granule Cell Necrosis	**Control %**	**Hypothyroid %**
Negligible	91.70	0.00
Minimal	8.30	0.00
Mild	0.00	16.70
Moderate	0.00	16.70
Severe	0.00	66.70

In the control group, 58.3% of the rats showed no intracellular vacuolation (Grade 0), whereas 41.7% exhibited minimal vacuolation (Grade 1). Conversely, in the hypothyroid group (depicted in Figure 3), 58.3% of the rats displayed moderate vacuolation (Grade 3), and a substantial majority of 41.7% exhibited severe vacuolation. The statistical analysis conducted using the chi-square test unveiled a highly significant difference (*P*-value < 0.001) between the two groups concerning the severity of vacuolation (as depicted in Figure 4 and detailed in Table 2). This underscores a pronounced contrast in the extent of intracellular vacuolation between the control and hypothyroid groups.

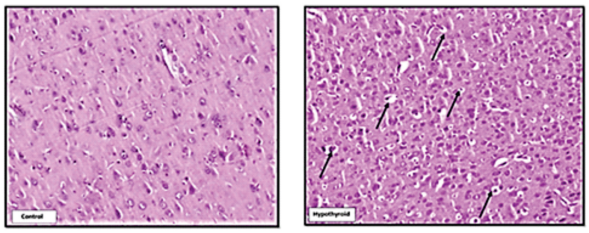

Fig 3: Intracellular vacuolation (H & E stain) represented by black arrows in Hypothyroid group as compared to Control group. Photomicrograph was taken at 400X.

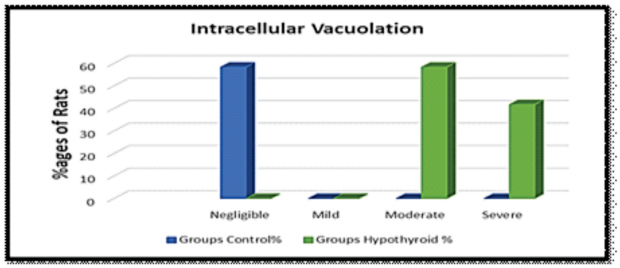

Fig 4: Bar graph showing grades of intracellular vacuolation in Control and Hypothyroid groups

Nissl Rim Thickness

The Nissl granules, situated at the periphery of neurons, demonstrated the development of a circular rim. The evaluation of the thickness of this rim within the cells was conducted. The analysis of thickness, as depicted in Figure 5, revealed a mean value of 1.44 ± 0.17 in the control group. In contrast, the hypothyroid group exhibited a notably lower mean value of 0.89 ± 0.22. The calculated *p*-value, as outlined in Table 3, substantiated the presence of a highly significant disparity between the two groups. This statistical finding underscores a substantial difference in the thickness of Nissl granule rims between the control and hypothyroid groups.

Table 2: Statistical comparison of means of intracellular vacuolation in both groups

	Groups	
Intracellular Vacuolation	**Control %**	**Hypothyroid %**
Negligible	58.30	0.00
Minimal	41.70	0.00
Moderate	0.00	58.30
Severe	0.00	41.70

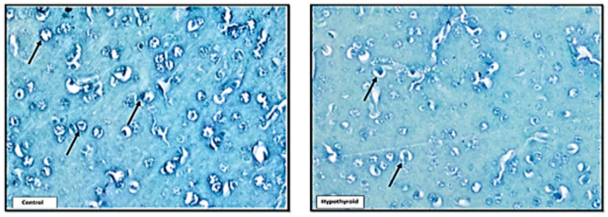

Fig 5: Nissl Rim Thickness (Toluidine Blue stain) represented by black arrows in both groups. The photomicrograph was taken at 400X.

Table 3: Statistical comparison of means of Nissl rim thickness in both groups

Nissl Rim Thickness	**Nissl rim**	
	Thickness in micrometer	**± SD**
Control	1.44	0.17
Hypothyroid	0.89	0.22

Discussion

The thyroid hormones originating from the mother play a crucial role in fetal neurogenesis, particularly during pregnancy when substantial changes occur in the maternal body. The maternal thyroid gland needs to generate a plentiful quantity of thyroid

hormones to satisfy the requirements of both the mother and the developing fetus. Importantly, within the initial 12 weeks of gestation, the fetus is entirely dependent on the maternal thyroid hormones for its sustenance.[16] This study was conducted to investigate the influence of the thyroid hormones on the crucial development of neurons, essential for the systematic development of a strong and fully functioning central nervous system. The findings of this study are consistent with prior research in the same field.[17,18]

Maternal hypothyroidism has been shown to significantly impact neurogenesis in rats, influencing the direct or indirect regulation of approximately 500-1000 genes and disrupting the antioxidant defense system.[19,8] A deficiency of thyroid hormones with oxidative stress leads to increased DNA fragmentation, followed by the dispersal of the rough endoplasmic reticulum. This phenomenon includes central chromatolysis, marked by a decrease in the amount of Nissl bodies and eosinophilic cytoplasm.[20] This research acknowledges the significance of Nissl rim thickness, which was observed to be lower in the hypothyroid group compared to the control group. In a study conducted by Noha in 2023, poorly defined purple Nissl granules were demonstrated in hypothyroid rats.[21] Similarly, in a rat model of carbimazole-induced hypothyroidism, Farag in 2023 made comments regarding the chromatolysis of Nissl granules.[22]

In this study, the hypothyroid group exhibited significant intracellular vacuolation. This observation aligns with the concept of cortical neuron degeneration, which is characterized by the presence of shrunken cells, nuclei displaying pyknosis, and the presence of fluid-filled spaces within the cytoplasm. This phenomenon can be attributed to the impact of free radicals, which have the capacity to selectively affect polyunsaturated fatty acids located in cell membranes. This action triggers the degradation of these fatty acids, leading to a decrease in membrane permeability. Consequently, this sequence of events results in the formation of vacuoles, offering a plausible explanation for the observed edema in neural tissues.[23] Additionally, Mai A. and colleagues in 2023 demonstrated vacuolation in pyramidal cells associated with thyroid-induced psychiatric disorders. El-Kholy's work in 2020 indicated vacuolation during the postnatal development of the cerebellar cortex induced by maternal hypothyroidism,[13] while Marwa and her team in 2021 highlighted pyknotic nuclei and cytoplasmic vacuolation within acinar cells of the parotid gland in hypothyroid rats.[24]

Until recently, there has been limited discussion on how maternal hypothyroidism affects granule cell necrosis in the rat motor cortex. However, in this study, a noticeable increase in granule cell necrosis was observed in the group of rats with hypothyroidism compared to the control group. This discovery is consistent with earlier research that has shown an increase in oxidative stress and the generation of free radicals within cells due to PTU-induced hypothyroidism in rats. These processes are closely linked to alterations in mitochondrial function and the respiratory chain, ultimately leading to a recognized increase in overall oxidative stress. In 2021, Hamidreza and colleagues shed light on the antioxidant defense system of thyroid hormones by studying juvenile rats with hypothyroidism.[25,26]

This study pioneers an examination of the motor cortex, marking the first exploration to unveil the detrimental impacts of inadequate thyroid hormone levels on neuronal development.

The present study did not offer insights into changes at the ultrastructural level, molecular mechanisms, or the dynamics of gene expression, primarily due to resource constraints.

Conclusion

This study's findings emphasize the effects of maternal hypothyroidism on neuronal structure in the motor cortex. This observed damage highlights a crucial connection between maternal thyroid dysfunction, especially when under stress, as evidenced by reduced Nissl rim thickness, intracellular vacuolation, granule cell necrosis, and their subsequent impact on the course of nervous system development.

Recommendations

Future research endeavors could explore the incorporation of advanced molecular techniques

and gene expression analyses to further augment the existing discoveries, to elucidate the complete range of mechanisms underlying the intervention, thus facilitating a more holistic comprehension. Furthermore, acknowledging the pivotal significance of thyroid hormones in neural development underscores the necessity for heightened awareness, timely interventions, and comprehensive healthcare approaches.

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REFERENCES

1. Mendes D, Alves C, Silverio N, Marques FB. Prevalence of undiagnosed hypothyroidism in Europe: a systematic review and meta-analysis. European Thyroid Journal. 2019; 8: 130-43. doi: 10.1159/000499751
2. Rashighi M, Harris JE. 乳鼠心肌提取 HHS Public Access. Physiology & Behavior. 2017; 176: 139–48.
3. Moog NK, Entringer S, Heim C, Wadhwa PD, Kathmann N, Buss C. Influence of maternal thyroid hormones during gestation on fetal brain development. Neuroscience. 2017; 342: 68-100. doi: 10.1016/j.neuroscience.2015.09.070
4. Kourosh-Arami M, Hosseini N, Komaki A. Brain is modulated by neuronal plasticity during postnatal development. The Journal of Physiological Sciences. 2021; 71: 134–89. doi: 10.1186/s12576-021-00819-9
5. Shen C, Liang D, Wang X, Shao W, Geng K, Wang X, et al. Predictive performance and verification of physiologically based pharmacokinetic model of propylthiouracil. Frontiers in Pharmacology. 2022; 3: 1013432. doi: 10.3389/fphar. 2022.1013432
6. Ghanbari M, Ghasemi A. Maternal hypothyroidism: An overview of current experimental models. Life Science. 2017; 187: 1–8. doi: 10.1016/j.lfs.2017.08.012
7. Mandel SJ, Cooper DS. The use of antithyroid drugs in pregnancy and lactation. The Journal of Clinical Endocrinology & Metabolism. 2001; 86: 2354-9. doi: 10.1210/jcem.86.6.7573
8. Baghcheghi Y, Salmani H, Beheshti F, Hosseini M. C Contribution of brain tissue oxidative damage in hypothyroidism-associated learning and memory impairments. Advanced Biomedical Research. 2017; 6: 59. doi: 10.4103/2277-9175.206699
9. Zafar M, Ali S, Beenish H, Khursheed T, Hussain N, Zakria I. Curcumin ' s Neuroprotective Efficacy Against Tartrazine-Induced Nissl Rim Alterations in Adult Male Rats ' Motor Cortex. Journal of Islamic International Medical College. 2021; 1: 180–4.
10. Batistuzzo A, Ribeiro MO. Clinical and subclinical maternal hypothyroidism and their effects on neurodevelopment, behavior and cognition. Archives of endocrinology and metabolism. 2020; 64 : 89-95. doi: 10.20945/2359-3997000000201
11. Gui J, Xu W, Zhang J. Association between thyroid dysfunction and perinatal outcomes in women with gestational hypertension: a retrospective study. BMC Pregnancy Childbirth. 2020; 20: 119. doi: 10.1186/s12884-020-2805-6
12. Hidayat M, Chaudhry S, Salman S, Lone KP. Melatonin prevents apoptosis in brains of neonates induced by maternal hypothyroidism. Journal of Ayub Medical College Abbottabad. 2019; 31 :580-5.
13. Elkholy WB, Omar MA, El-Habiby MM, Al-Gholam MA. The effect of induction of maternal hypothyroidism on postnatal cerebellar cortex development in albino rat offspring and the role of thyroxin replacement therapy: histological, immunohistochemical and genetic study. Egyptian Journal of Histology. 2021; 44: 545-62. doi: 10.21608/EJH. 2020.31682.1306
14. Charan J, Kantharia N. How to calculate sample size in animal studies? Journal of Pharmacology and Pharmacotherapeutics. 2013; 4: 303–6. doi: 10.4103/0976-500X.119726
15. Maynard RL, Honorary Professor, University of Birmingham, Birmingham UK, Downes N, Principal Pathologist, Sequani Limited, Ledbury UK. Anatomy and Histology of the Laboratory Rat in Toxicology and Biomedical Research. Maynard RL, editor. Academic Press. UK: Academic press; 2019. 204–8 p.
16. Talat A, Khan AA, Nasreen S, Wass JA. Thyroid Screening During Early Pregnancy and the Need for Trimester Specific Reference Ranges: A Cross-Sectional Study in Lahore, Pakistan. Cureus. 2019; 11: e5661. doi: 10.7759/cureus. 5661
17. Hidayat M, Khaliq S, Khurram A, Lone KP. Protective effects of melatonin on mitochondrial injury and neonatal neuron apoptosis induced by maternal hypothyroidism. Melatonin Research. 2019; 2: 42-60. doi: 10.32794/mr11250040
18. Yang H, Zha X, Cai Y, Wang F, Wu Z, Wu B, et al. Impacts of thyroxine combined with donepezil on hippocampal ultrastructures and expressions of synaptotagmin-1 and

SNAP-25 in adult rats with hypothyroidism. International Journal of Clinical and Experimental Medicine. 2015; 8: 17922–31.

19. Anifantaki F, Pervanidou P, Lambrinoudaki I, Panoulis K, Vlahos N, Eleftheriades M. Maternal prenatal stress, thyroid function and neurodevelopment of the offspring: A mini review of the literature. Frontiers in neuroscience. 2021; 15: 692446. doi: 10.3389/fnins.2021.692446.
20. Kaufmann W, Bolon B, Bradley A, Butt M, Czasch S, Garman RH, et al. Proliferative and nonproliferative lesions of the rat and mouse central and peripheral nervous systems. Toxicologic pathology. 2012; 40: 87S-157S. doi: 10.1177/0192623312439125
21. Ibrahim NA, Diab M, Hassan A, Anwar H, Ragab W, Morsi E, et al. The Ameliorating Effect of Ashwagandha (Withania Somnifera) Extract on Hippocampus and Growth Plate Changes Associated With Propylthiouracil Induced Hypothyroidism in Juvenile Rats: Ashwagandha on Hippocampus and Growth Plate Changes Associated With hypothyroid rats. Al-Kindy College Medical Journal. 2023; 19: 30-41. doi:10.47723/kcmj.v19i1.920
22. Farag EA, Filobbos SA, Afifi NM, Mahmoud ST, Alghandour SM. Thyroxine restores hippocampal neurogenesis and synaptogenesis in a male rat model of carbimazole-induced hypothyroidism: a histological study. Beni-Suef University Journal of Basic and Applied Sciences. 2023; 12: 1-7. doi: 10.1186/s43088-023-00395-4
23. Elblehi SS, El Euony OI, El-Sayed YS. Apoptosis and astrogliosis perturbations and expression of regulatory inflammatory factors and neurotransmitters in acrylamide-induced neurotoxicity under ω3 fatty acids protection in rats. Neurotoxicology. 2020; 76: 44-57. doi: 10.1016/j.neuro.2019.10.004
24. Hassan NH. Alpha Lipoic Acid Exerts a Therapeutic Effect on Parotid Tissue in a Hypothyroidism Rat Model by LC-3 Suppressing Autophagic Cell Death (Immunohistochemical, Morphometric, Ultra-structural and Biochemical Study). Egyptian Journal of Histology. 2021; 46: 193–205.
25. Rastegar-Moghaddam SH, Hosseini M, Alipour F, Rajabian A, Ebrahimzadeh Bideskan A. The effects of vitamin D on learning and memory of hypothyroid juvenile rats and brain tissue acetylcholinesterase activity and oxidative stress indicators. Naunyn-Schmiedeberg's Archives of Pharmacology. 2022; 395: 337-51. doi: 10.1007/s00210-021-02195-y
26. Salami M, Bandegi AR, Sameni HR, Vafaei AA, Pakdel A. Hippocampal up-regulation of apolipoprotein D in a rat model of maternal hypo-and hyperthyroidism: implication of oxidative stress. Neurochemical research. 2019; 44: 2190-201. doi: 10.1007/s11064-019-02859-5

Authors Contribution

TF: Idea conception, study designing, data collection, data analysis, results and interpretation, manuscript writing and proof reading

SA: Data analysis, results and interpretation, manuscript writing and proof reading

TQ: Data collection, manuscript writing and proof reading

NF: Data collection, data analysis, results and interpretation