Research Article

Effects of Prenatal Stress Exposure on Stress Neurohormonal Axis in Rat Pups of Thyroid Disturbed Mothers

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Abstract

Thyroid dysfunction during pregnancy is common. Maternal thyroid dysfunction is associated with an increased risk of various adverse maternal and child outcomes, including miscarriage, intrauterine growth retardation, hypertensive disorders, preterm delivery, and a decreased child IQ. During pregnancy, profound changes in thyroid physiology occur to provide sufficient thyroid hormone (TH) to both the mother and fetus. There is growing evidence demonstrating that thyroid hormone affects the various components of the hypothalamic–pituitary–adrenal (HPA) axis- Therefore, this study was designed to investigate that maternal Thyroid dysfunction during pregnancy impaired feedback mechanisms of the HPA- axis and associated with thyroid disorders and neurobiological alterations in off sprigs. Therefore, 42 female wistar rats (200-250gm) after induction of pregnancy and hyperthyroxinemia and hypothyroxinemia. Their pups were divided into twelve groups (control non-stressed group, control stressed group hyperthyroid non-stressed group, treated non-stressed group, hyperthyroid stressed group, treated stressed group) and (control non-stressed group, control stressed group, hypothyroid non-stressed group, treated non-stressed group, control stressed group, hypothyroid stressed group, treated stressed group). Measurement of some hormones and histological analyses were done and result the induction of hyperthyroidism in mothers was significantly increased of plasma T_4 and increased of plasma TSH levels in all pups group compared with control group. ACTH and Corticosterone increased in stressed group compared to other groups. In addition, hyperthyroid group showed small amount of colloid with irregular structure and architecture of the thyroid gland compared with treated and control group. Also induction of hypothyroidism in mothers was significantly decreased of plasma T_4 and decreased of plasma TSH levels in all pups group compared with control group. ACTH and Corticosterone increased in stressed group compared to other groups. In addition, hyperthyroid group showed small amount of colloid with irregular structure and architecture of the thyroid gland compared with treated and control group, While that in the hypothyroid group; the amount of colloid increased and the height and activity of the follicular epithelium decreased, where as in the treated group the amount of colloid increased (abundant basophilic colloid) and the height and activity of the follicular epithelium increased. In conclusion, the effects of maternal stress exposure during pregnancy on HPA-axis regulation and anxiety-like behavior can be transferred via the maternal line to its offspring.

Keywords: Hyperthyroidism, Hypothyroidism, pregnancy, stress, HPA axis.

Introduction

The thyroid hormones act on nearly every cell in the body. They act to increase the basal metabolic rate, affect protein synthesis, help regulate long bone growth (synergy with growth hormone) and neural maturation and increase the body's sensitivity to catecholamines (such as adrenaline) by permissiveness.

The thyroid hormones are essential to proper development and differentiation of all cells of the human body. These hormones also regulate protein, fat, and carbohydrate metabolism, affecting how human cells use energetic compounds. They also stimulate vitamin metabolism. Numerous physiological and pathological stimuli influence thyroid hormone synthesis (Irizarry and Lisandro, 2014).

Thyroid dysfunctions such as hypothyroidism, thyrotoxicosis and thyroid nodules may develop during pregnancy leading to abortion, placental abruptions, preeclampsia, preterm delivery and reduced intellectual function in the offspring. Epidemiological data have shown the significant role of maternal thyroid hormone in fetal neurologic development and maternal health. It has been suggested that the deleterious effects of thyroid dysfunction can also extend beyond pregnancy and delivery to affect neuro-intellectual development in the early life of the child (Aynadis, 2016).

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There is growing evidence demonstrating that thyroid hormone affects the various components of the hypothalamic–pituitary–adrenal (HPA) axis−Experimentally induced hypothyroidism has been reported to reduce adrenal weight as well as alter concentrations of corticosterone Although thyroidectomy decreases plasma adrenocorticotropic hormone (ACTH) levels, Also pituitary content of ACTH has been reported to increase pregnancy with hyperthyroidism produced hyperactivity of the hypothalamic- pituitary – adrenal (HPA) axis. Hyperthyroid patients need higher levels of cortisol due to catabolic transformations leading to inactivation of cortisol and an increase in its elimination (van Bodegom et al., 2017).

The HPA axis plays a pivotal role in the regulation of metabolic function, and this effect constitutes an important component in stress response. Many studies suggest that the HPA activity plays a key role in perinatal programming of metabolic disease. Stress activates the hypothalamic–pituitary–adrenal (HPA) axis, which then modulates the degree of adaptation and response to a later stressor. It is known that early-life stress can impact on later health but less is known about how early-life stress impairs HPA axis activity, contributing to maladaptation of the stress–response system. Early-life stress exposure (either prenatally or in the early postnatal period) can impact developmental pathways resulting in lasting structural and regulatory changes that predispose to adulthood disease. Epidemiological, clinical, and experimental studies have demonstrated that early-life stress produces long term hyper-responsiveness to stress with exaggerated circulating glucocorticoids, and enhanced anxiety and depression-like behaviors (Maniam et al., 2014).

Material and Methods

Animals

Fourty-eight albino rats were chosen to be the model of this work. Thirty-six mature adult time-dated pregnant female albino rats of Westar strain weighting (200-250 g), and twelve mature adult time-dated male albino rats weighting (200-250 g) were used in this study. Purchased from animal house of the Faculty of Medicine Assuit University, Assuit Egypt The experimental procedures were conducted accordance to the Ethical Guidelines for investigations of experimental pain in conscious animals (Zimmermann, 1983).

At room temperature and maintained under light/dark cycles consisting of 12 h of light and 12h of dark cycle. They were fed a standard commercial pellet diet and water throughout the experimental period. All rats were kept isolated for two weeks in the laboratory room at comfortable room temperature for adaptation before any experimental interference. On the day 8 before mating rats will be randomized into three groups; control euthyroid, hypothyroid and hyperthyroid groups (Bruno et al., 2005). Rats were housed in stainless steel cage, each cage was 25 x 25 x 30 cm. for four rats (one male and three female).

Procedures

1- Induction of Pregnancy

The reproductive cycle in rats lasts for 4-5 days. The cycle is divided into 4 phases and vaginal smear will show that:

1- proestrous phase (8-12 hr.): in which there are nucleated cuboidal epithelial cells due to increase level of follicle stimulating hormone (FSH).
2- estrous phase (24 hr): this is the priod of copulation in which there are keratinized cells (polygonal non-nucleated cells++++) and high estrogen level stimulates mitosis of cells of uterus and vagina.
3- metestrous phase (30-35 hr): in which there are keratinized cells(+) and leukocytes(+++) this the time of ovulation due to increase level of luteinizing hormone(LH) and increase the progesterone level.

4- diestrous phase (60-70 hr): in which there are nucleated epithelial cells, fragment of keratinized cells and leukocytes(+).

The presence of spermatozoa in the vaginal smears the morning after caging with a fertile male from same strain on the night of pro-oestrus will be considered indicative of pregnancy and this day will be counted as day 0 of pregnancy (Kamilaris et al., 1991).

2- Control groups

- Control of hyperthyroidism group: Control euthyroid rats received daily intraperitoneal injections of 1ml saline solution for same 14 days.
- Control of hypothyroidism group: Control euthyroid rats received daily 1ml of saline solution orally for same 14 days.

3- Induction of Hyperthyroxinemia

(as model of hyperthyroidism mother of albino rats)

Hyperthyroxinemia will be induced by daily intraperitoneal injections of L-thyroxin 25 µg/ 100 g body weight for 14 days. L-thyroxin will be dissolved in 0.9% (w/v) solution immediately before injection. Animals will be kept under daily observation to notice any clinical changes; they will be weighed every 3 days until the end of experiment (Bruno et al., 2005).

4- Induction of Hypothyroxinemia

(as model of hypothyroidism mother of albino rats) Hypothyroxinemia will be induced by anti thyroid drugs mathemazole add to drinking water at concentration 0.02% for 14 days. Animals will be kept under daily observation to notice any clinical changes; they will be weighed every 3 days until the end of experiment (Bruno et al., 2005).

5- Induction of Treatment

- The hyperthyroid group is randomly divided into two groups; treated and nontreated groups. Treated group rats with hyperthyroximia will be treated by administration of 20mg/kg/day methimazole orally. The administration of treatment will be continued even during lactation till blood sample of mothers become with normal T4. The fetus will access to the drug by placenta transfer and pups through milk secretion (Mestman, 2004). Nontreated groups' rats will be continuous received L-thyroxin 25µg/100g body weight till the end of the experiment.
- The hypothyroid group is randomly divided into two groups; treated and nontreated groups. Treated group rats with hypothyroximia will be treated by daily intraperitoneal injections of L-thyroxin 25 µg/100 g body weight. The administration of treatment will be continued even during lactation till blood sample of mothers become with normal T4. Nontreated groups rats will be continuous received 20 mg /kg/ day methimazole orally till the end of the experiment.

6- Stress Procedure

Rats of stressed group will be subjected to restraint by fixing the four limbs to a wooden board and placed in a refrigerator at 4°C for three hours, and the process will be repeated every day for 8 days (Bhatnagar et al., 1998).

7- Testing Groups

Group 1: the control non stressed of hyperthyroidism group: Animals of this group were left in animal house without exposure to any form of stress with normal mother.

Group 2: the control stressed of hyperthyroidism group: Rat pups were subjected to restraint stress by fixing the four limbs to a wooden board and placed in a refri-gerator at 4°C for three hours and this process were repeated every day for 8 days.

Group 3: the control non stressed of hypothyroidism group: Animals of this group were left in animal house without exposure to any form of stress with normal mother.

Group 4: the control stressed of hypothyroidism group: Rat pups were subjected to restraint stress by fixing the four limbs to a wooden board and placed in a refrigerator at 4°C for three hours and this process were repeated every day for 8 days.

Group 5: the hyperthyroidism treated non stressed of group: Rat pups of this group from treated hyperthyroid mother without exposure to stress.

Group 6: the hyperthyroid treated stressed group: Rat pups of this group from treated hyperthyroid mother with exposure to stress.

Group 7: the hyperthyroid non stressed non treated group: Rat pups of this group from

hyperthyroid mother without treatment or exposure to stress.

Group 8: the hyperthyroid stressed group: Rat pups of this group from hyperthyroid mother with exposure to stress.

Group 9: the hypothyroid treated non stressed group: Rat pups of this group from treated hypothyroid mother without exposure to stress.

Group10: the hypothyroid treated stressed group: Rat pups of this group from treated hypothyroid mother with exposure to stress.

Group 11: the hypothyroid non stressed non treated group: Rat pups of this group from hypothyroid mother without treatment or exposure to stress.

Group 12: the hypothyroid stressed group: Rat pups of this group from hypothyroid mother with exposure to stress.

8- Labour and Pups Experimental Groups

At day 3 before delivery the rats will be caged individually. The maternal animals will be allowed to deliver pups naturally. The date of birth will be designated day 0 (= postnatal day 0= pnd 0). On pnd 1, litters will be culled to 10 pups (5 males and 5 females) and housed with their mothers in separate cage. There will be no significant effect of sex on HPA axis response so individual pups will be used as the unit of analysis (Mastorakos et al., 2007).

N.B: Mortality Rates

The rat pups didn't die in the normal control groups 0%, whereas three rat pups died in group with hyperthyroid group (stress) with mortality rate 30%. Two rat pups died in the treated group (stress) with mortality rate 20%. Also tow rat pups died in group with hypothyroid group (stress) with mortality rate20%. one rat pups died in the treated group (stress) with mortality rate 10%. One mother died in hyperthyroid group.

Weight

the weight of mothers of hyperthyroidism and hypothyroidism groups were measured every three days to detect changes in weight.

Collection of samples

The experiment was terminated at the end of 7 weeks and after overnight fast), neonates were anesthetized using light ether anesthesia. Blood samples of the neonates (1ml the whole blood was withdrawn by retro-orbital puncture from each rat and allowed to clot in their glass tube. The obtained samples were collected in clean dry centrifuge tubes then were centrifuged at 3000 rpm for about fifteen minutes using pasture pipette then sera, which were collected using an automatic micropipette were separated, then stored at -20^{o}C in a special clean dry serology tubes until assayed.

For the histophathological investigation a pieces of thyroid tissues of pups were quickly removed and washed in 0.9% sodium chloride then fixed immediately in 10% neutral buffered formalin.

For the subsequent biochemical assays

1- thyroxine (T4)
2- thyrotropin (TSH)
3- Adrenocorticotropic hormone (ACTH)
4- Cortisol

Thyroxine, Thyrotropin, Adrenocorticotropic hormone and cortisol hormone in serum were determined using the electrochemiluminescence immunoassay (ECLIA) (Surks., 1990), (Nelson and Wilcox., 1996), (Derme, 2006) and (Gatti et al., 2009) respectively.

Histopathological investigation

Specimen preparation for Haematoxylin and Eosin (HE) staining for light microscopic study, the thyroid gland were fixed in 10% neutral buffered formaldehyde, dehydrated in ascending grades of alcohol, cleared, embedded in paraffin and serially sectioned (8μm). Sections were stained with haematoxylin and eosin.

Statistical Analyses

The data were submitted to one-way analysis of variance (ANOVA) followed by Duncan's post hoc test considering $P<0.05$ using SPSS 11,0 software (SPSS Inc, Chicago, IL, 2001), results were expressed as mean ±S.E.

Result

3.1 Histopathological Changes:

The present study revealed that in the hyperthyroidism- like group the amount of colloid decreased (sparse pale colloid) and the height and activity of the follicular epithelium increased, whereas in the treated group the amount of colloid increased (abundant acidophilic colloid) and the height and activity of the follicular epithetlium decreased. While that in the hypothyroid group; the amount of colloid

increased and the height and activity of the follicular epithelium decreased, whereas in the treated group; the amount of colloid increased (abundant basophilic colloid) and the height and activity of the follicular epithelium increased.

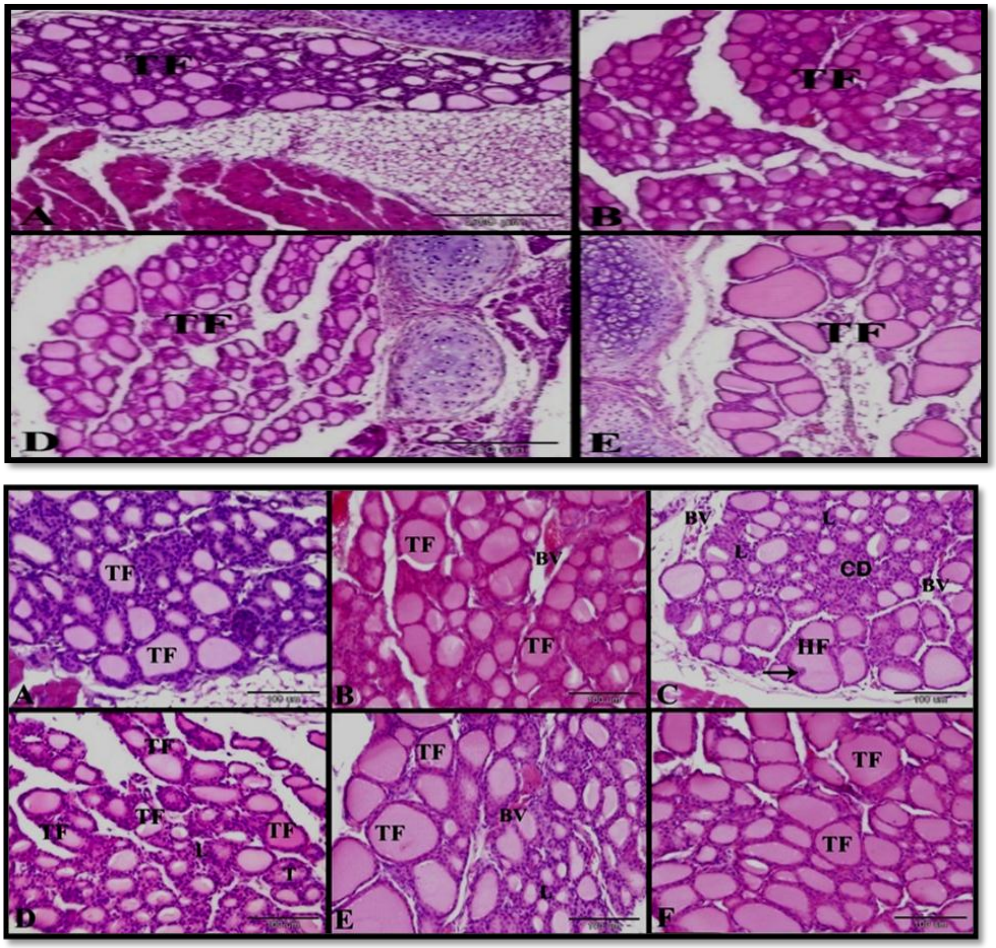

Fig. (1): Photomicrograph of paraffin sections in thyroid gland in rat pups; A; control non-stressed group (pups of normal mothers), B; control stressed group (pups of stressednon-treated hyperthyroidal mothers), C; hyperthyroidism non-stressed group (pups of non-stressed hyperthyroidal mothers), D; hyperthyroidism stressed group (pups of stressedhyperthyroidal model mothers), E; treated non-stressed group (pups of treated non-stressedhyperthyroidal mothers) and F; treatedstressed group (pups of treated stressed hyperthyroidal mothers). **Fig. A;** showing normal structure of the thyroid gland; Lobulated parenchyma formed of regular shaped (rounded or oval) and size thyroid follicles (TF). **Fig. B**, showing Lobulated parenchyma formed of irregular shapeand size thyroid follicles(TF). **Fig. C,** showing enlarged hypertrophied parenchyma formed of irregular large sized thyroid follicles (TF). **Fig. D,** showing enlarged parenchyma formed of irregular large sized thyroid follicles (TF). Fig. E & F showing slightly enlarged parenchyma formed of large sized thyroid follicles (TF). Original magnification, 100X, scale bar = 200µm, Hematoxylin and Eosin stain.

Fig. (2): Photomicrograph of paraffin sections in thyroid gland in rat pups; A; control non-stressed group showing normal rounded or oval thyroid follicles (TF). B; control stressed group showing acidophilic thyroid follicles and congested blood vessels (BV). C; hyperthyroidism non-stress group showing hyperplastic thyroid follicles (HF)with papillary infolding's (arrow),congested blood vessels (BV), increased cellular density (CD) and lymphocytic infiltration (L). D; hyperthyroidism stressed group showing irregular thyroid follicles (TF) some with tall columnar cells (T) and lymphocytic infiltration (L). E; treated non-stressed group showing enlarged hypertrophied thyroid follicles (TF) and congested blood vessels (BV). F; treated stressed group showing slightly hypertrophied thyroid follicles (TF). Original magnification,200X,scale bar=100µm(H&E)stain.

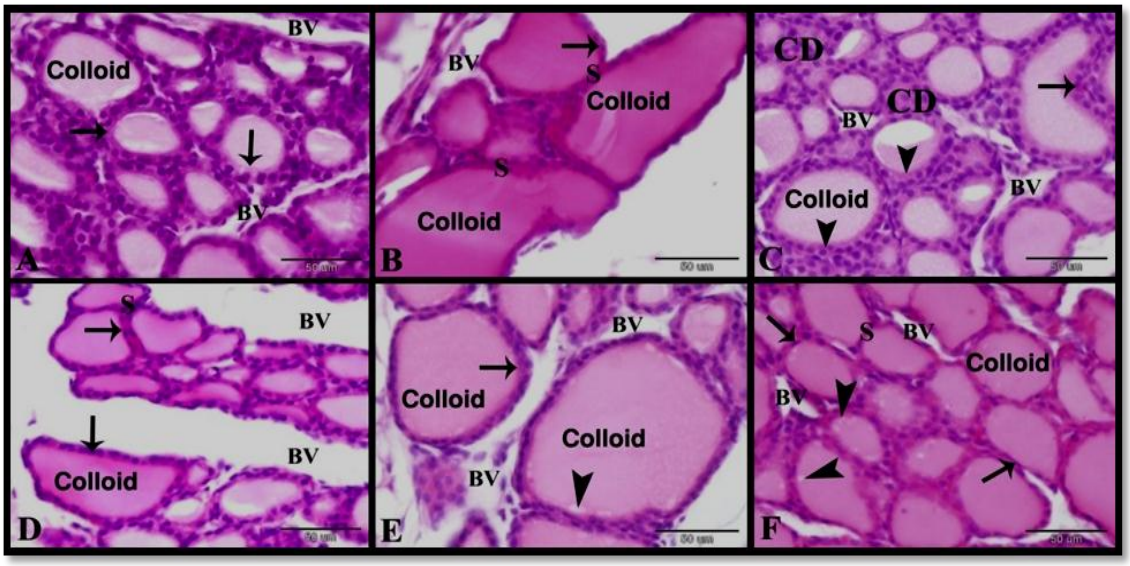

Fig. (3): Photomicrograph of paraffin sections in thyroid gland in rat pups; A; control non-stressed group showing normal thyroid follicles with lightly stained acidophilic colloid and cuboidal follicular cells (thyrocytes) (arrow), the follicles separated by blood vessels (BV).B; control stressed group showing hypertrophied thyroid follicles with flattened follicular cells (arrow), abundant deeply stained acidophilic colloid and thin interfollicular septum (S), the follicles separated by wide blood vessels (BV). C; hyperthyroidism non-stress group showing hyperplastic thyroid follicles with papillary in folding's (arrow)and lined by cuboidal to columnar follicular cells (arrow head), foamy vacuolated lightly stained acidophilic colloid, increased cellular density (CD) and blood vessels (BV). Note, thepapillary infolding's had no fibrovascular cores .D; hyperthyroidism stressed group showing irregular thyroid follicles lined by flattened follicular cells (arrow) and contained acidophilic colloid, the follicles separated by wide blood vessels (BV) and thin intermolecular septum (S), E; treated non-stress group showing enlarged hypertrophied thyroid follicles with low cuboidal follicular cells (arrow) andcontained moderate amount of acidophilic colloid with peripheral scalloping (arrow head), the follicles separated by numerous blood vessels (BV). F; treatedstressed group showing slightly hypertrophied thyroid follicles with flattened follicular cells (arrow) and contained abundant acidophilic colloid with peripheral scalloping (arrow head), the follicles separated by numerous blood vessels (BV) and had thin intrafollicular septum (S). Original magnification, 400X, scale bar = 50 µm, Hematoxylin and Eosin stain.

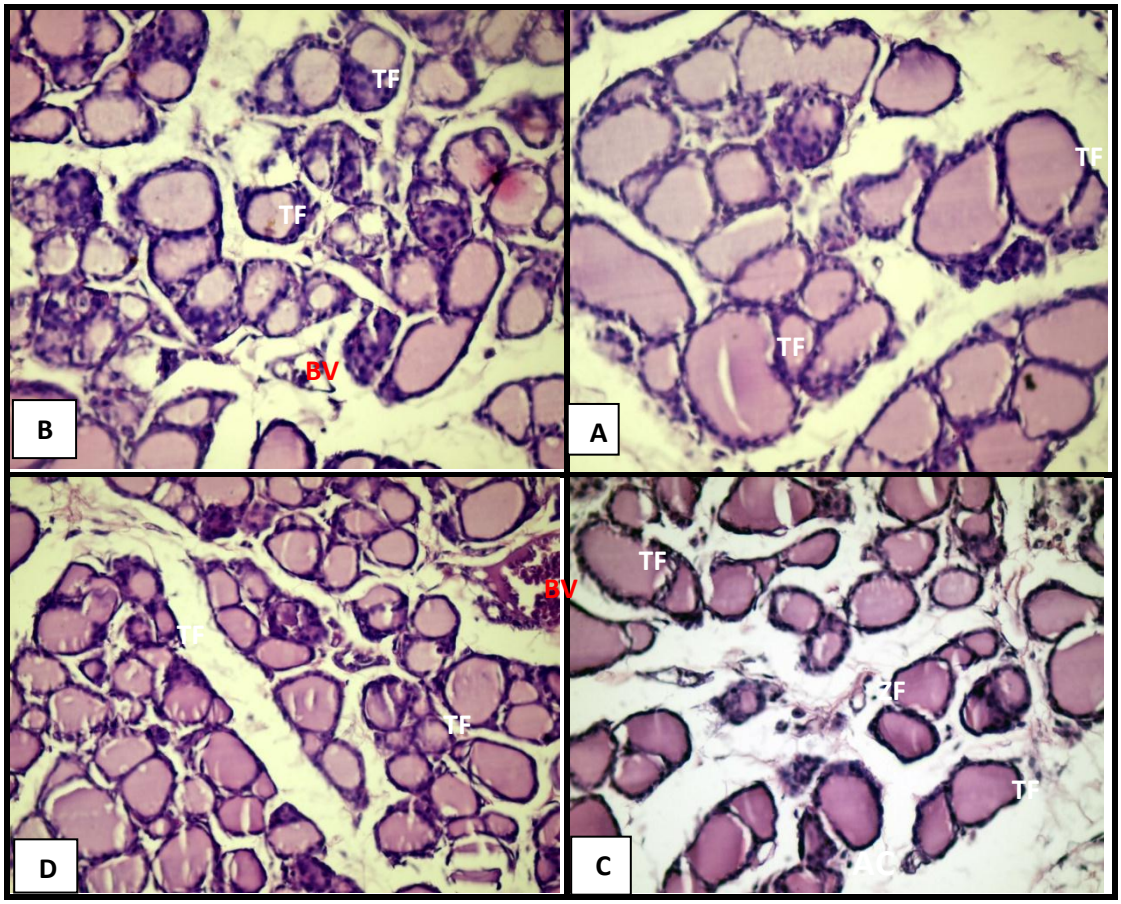

Fig (4): Histological sections of thyroid glands obtained from rat pups. A; Hypothyroid non-stressed group showed thyroid follicles of variable size and shape lined by a single layer of flat cells and contain esinophilic colloid material. B; Treated hypothyroid non-stressed group showed proliferated small to medium size follicles lined by cuboidal thyrocytes and focal peripheral vacuolation of colloid contents. The stroma showed congested vascular spaces. C; Hypothyroid stressed group showed thyroid follicles of slightly variable size and shape; lined by a single layer of flat or low cuboidal thyrocytes and contain pale esinophilic colloid material with occasional peripheral vacuolation. The stroma is hypovascular. D; Treated hypothyroid stressed group showed follicles of small to medium size; lined by a single layer of cuboidal thyrocytes and the lumen showed pale esinophilic colloid material with peripheral vacuolation. The stroma showed dilated congested vascular spaces. TF; thyroid follicles, BV; blood vessel. H&E sections; magnification is X400 for all.

3.2 Hormonal Changes

3.2.1 Measurement of T4 and TSH Hormones in Rats Pups with Hyper thyroidal Model Mothers.

The obtained data (table1) revealed that the induction of hyperthyroidism in rat's mothers significantly ($p<0.05$) increased the plasma T_4 and decreased plasma TSH levels compared to control group. Plasma T_4 levels showed marked increase in hyperthyroidal mother's stressed and non-stressed group compared to other animal's groups. Treated stressed animal's groups revealed significant ($p<0.05$) decline compared to the hyperthyroid (stressed and non-stressed). Regarding to the TSH, animals with hyperthyroidism (stressed and non-stressed) reported noticeable hormone plasma concentration decreases compared to the control non-stressed and treated non-stressed groups.

3.2.2 Measurement of ACTH and Cortico-sterone Hormones in Rats Pups with Hyper thyroidal Model Mothers.

The study presented here has shown that the ACTH plasma concentration **(table: 1)** reported marked increases ($p<0.05$) in hyperthyroidism

(stress and non-stressed) compared to the control groups, the treated animas groups showed marked decreases in the level of ACTH compared to the hyperthyroidism animal group. Corticosterone plasma concentrations indicated significant ($p<0.05$) increase in the hyper-thyroidism-stressed groups compared to the control groups. Treated non-stress animal groups showed clear reduction in the cortico-sterone plasma concentration.

3.2.3 Measurement of T4 and TSH Hormones in Rats Pups with Hypothyroidal Model Mothers.

The obtained data (table1) revealed that the induction of hypothyroidism in rat's mothers significantly ($p<0.05$) decreased the plasma T_4 and decreased plasma TSH levels compared to control group. Plasma T_4 levels showed marked decrease in hypothyroidal mother's stressed and non-stressed group compared to other animals.

3.2.4 Measurement of ACTH and Cortico-sterone Hormones in Rats Pups with Hypothyroidal Model Mothers

The study presented here has shown that the ACTH plasma concentration (table: 1) reported marked increases ($p<0.05$) in hypothyroidism (stress and non-stressed) compared to the control groups, the treated animas groups showed marked decreases in the level of ACTH compared to the hypothyroidism animal group. Corticosterone plasma concentrations indicated significant ($p<0.05$) increase in the hypo-thyroidism-stressed groups compared to the control groups. Treated non-stress animal groups showed clear reduction in the cortico-sterone plasma concentration.

Table(1): Show effect of maternal model hyperthyroidism and stress on plasma T4, TSH, ACTH and Corticosterone of their pup.

Treated (stressed)	Treated (non-stressed)	Hyperthyroidism (stressed)	Hyperthyroidism (non-stressed)	Control (stress)	Control (non- stress)	Groups / parameter
2.92 ± 0.28^{d}	5.13 ± 0.39^{bc}	6.42 ± 0.46^{b}	10.52 ± 1.43^{a}	3.424 ± 0.06^{cd}	4.16 ± 0.26^{cd}	**T_4(ng%)**
0.10 ± 0.02^{c}	0.35 ± 0.06^{a}	0.003 ± 0.001^{c}	0.037 ± 0.011^{c}	0.005 ± 0.001^{c}	$0.20\pm.055^{b}$	**TSH (ng%)**
11.00 ± 0.69^{b}	5.04 ± 0.96^{c}	14.24 ± 0.82^{a}	8.98 ± 0.72^{b}	10.90 ± 1.18^{b}	4.58 ± 0.78^{c}	**ACTH (pg/ml)**
12.52 ± 1.12^{b}	4.30 ± 0.48^{a}	15.90 ± 1.13^{a}	7.98 ± 0.63^{c}	8.32 ± 0.69^{c}	4.36 ± 0.50^{a}	**Corticosterone (µg/dl)**

Table 2: Show effect of maternal model hypothyroidism and stress on plasma T4, TSH, ACTH and Corticosterone of their pup.

Treated (stressed)	Treated (non-stressed)	Hypothyroidism (stressed)	Hypothyroidism (non-stressed)	Control (stress)	Control (non- stress)	Groups / Parameter
2.712 ± 0.197^{d}	3.520 ± 0.241^{bc}	2.138 ± 0.120^{b}	2.926 ± 0.165^{a}	3.424 ± 0.06^{cd}	4.16 ± 0.26^{cd}	**T_4(ng%)**
0.052 ± 0.014^{c}	0.300 ± 0.071^{a}	0.003 ± 0.0001^{c}	0.024 ± 0.007^{c}	0.005 ± 0.001^{c}	$0.200\pm.055^{b}$	**TSH (ng%)**
11.375 ± 1.008^{b}	4.868 ± 0.52^{c}	9.413 ± 1.077^{a}	6.050 ± 0.444^{b}	12.500 ± 1.849^{b}	7.313 ± 0.334^{c}	**ACTH (pg/ml)**
10.055 ± 0.649^{b}	4.625 ± 0.448^{a}	21.650 ± 0.773^{a}	15.650 ± 1.506^{c}	7.0820 ± 0.618^{c}	4.075 ± 0.534^{a}	**Corticosterone(µg/dl)**

Means label different superscript letter at the same row are significantly ($p<0.05$) different

Discussion

The main findings of the present study indicate that stress applied to pregnant dams during gestation associated with thyroid dysfunction produces adverse effect on an offspring that might be related to HPA axis.

Blood concentration of thyroxin hormones physiologically changes during pregnancy, these variation occurs due to placental hormones like HCG which has TSH like effect that mildly stimulate the thyroid gland for thyroxin production and estrogen which produces higher levels of thyroid- binding globulin that helps in transport of thyroid hormone in blood (Visser, 2018).

Several reports have described the basic role of the thyroid hormones on the development of mammalian and non-mammalian brain (Horn and Heuer, 2010). Other reports have described the harmful effect of hypo- or hyperthyroidism affects the maturation of the CNS system and causes irreversible dysfunction of the brain if not corrected shortly after the birth(Ahmed et al., 2008). Hyperthyroidism in pregnancy is associated with adverse maternal, obstetrical and fetal outcome (Aggarawal et al., 2014).

In the present study L-thyroxin 25 µg /100 g body weight was usedto induce hyper-thyroidism to the ratwith daily intraperitoneal injections for 14 days. This method previously used by Wu et al., (2011), and Yu et al., (2015) In the present work, the induction of hyper-thyroidism in mothers was significantly increased of serum T4 and decreased of serum TSH levels in all pups' groups compared with control groups.

These results are agreed with Karnath (2004), who reported that tissue effects of hyper-thyroidism include accelerated metabolism, low serum cholesterol, increased bone turnover, reduced bone density with an increased risk of osteoporosis and suppressed serum TSH and Leung (2012) who found that hyperthyroidism strongly is associated with lower TSH values and the elevation in total T4 and total T3 in the first trimester of gestation. Thyroid hormone regulation is TSH dependent, the increase in thyroid hormone levels in blood, the decrease in TSH and vice versa, this means that the pituitary gland output more TSH during the decrease in Thyroxine hormone.

While if the plasma level of thyroxine hormone increases the pituitary TSH will decrease (Patil-Sisodia and Mestman, 2010). The obtained data in this study revealed that stress reduced plasma T4, TSH levels compared with non-stressed group. The serum levels ofT4and TSH were significantly increased in hyperthyroid non-stressed group compared with hyperthyroid stressed group. This result consistent with Helmreich et al., (2005).

Who stated that; during stress the level of T3 and T4 were decreased moreover due to the action of glucocorticoids on CNS, besides stress inhibits TSH secretion. Hyperthyroidism has been associated with hyperactivity of the hypothalamic–pituitary–adrenal (HPA) axis (Johnson et al., 2005). These results are in agreement with Maccari and Morley-Fletcher (2007), Rayen et al., (2011), Chen et al., (2012) reported that exposure to stress during early development causes long-lasting alterations in behavior and HPA-axis activity, including increased expression of CRH, these previous findings confirm the current work results that revealed similar pattern regarding to the hypothalamus CRH and plasma corticosterone response to acute restraint stress.

These findings matched the findings of King et al., (2001) who reported that in human pregnancy, regulation of the HPA-axis dramatically changes with the production and release of placental cortisol.

Conclusion

The present study revealed that the maternal stress exposure during pregnancy effects on HPA-axis development and regulation. Anxiety-like behavior can be transferred vertically via the maternal line to their off spring. Stress lead to various changes in the rat's hormones level especially thyroxines, ACTH and corticosterone, these changes may lead to various immunological and endocrine disorders. Of interest Authors declare that there is conflict of interest.

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