Original Article

Study the Effect of Exogenous Iodine on the Thyroid Transcription Factors during Pregnancy in Rat Model

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Abstract

Background: This study was designed to evaluate whether a mother's iodide supplement would be correlated with embryonic thyroid gland development. **Objective:** Thyroid transcription factors were be measured by an enzyme-linked immunosorbent assay technique in all studied samples. **Materials and Methods:** The study included three groups as follows: group 1: involved 20 adult female rats, and the animals in this group were administered distilled water and they served as control, group 2: involved 15 adult female rats, and the animals in this group were treated with a low dose of iodine (0.06 mg), and group 3: involved 15 adult female rats, and the animals in this group were treated with a medium dose of iodine (0.18 mg). After 18 days of pregnancy. The animals were sacrificed and the serum was collected to determine rat paired box protein (PAX8), rat Forkhead box protein E1 (FOXE1), rat Homeobox protein (Nkx-2.1) using enzyme-linked immunosorbent assay assay for the mother whereas the thyroid factors data were analyzed using SPSS (version 15.0). Statistical tests were conducted using SPSS version 26. The descriptive analysis (Mean ± S.E) and the significance were done using Duncan at $P \leq 0.01$ (the significance level was 0.01 and the *P*-value was 0.05), respectively. *P*-value and significance level were (0.05) and (0.01), respectively. **Results:** Results reported the highest value in FOX1, NK2.1, and PAX8 concentration was in 1.8 gm followed by a concentration of 0.06 gm, whereas the lowest value recorded in control group was $224.49^{a} \pm 7.61$ with significant differences at ($P \leq 0.05$) between the studied groups in both mother and embryo, however. **Conclusions:** Depending on the obtained results, the current study found the thyroid transcription factors were involved directly with excess iodine taken.

Keywords: Iodine, rat Forkhead box protein E1 (FOXE1), rat Homeobox protein (Nkx-2.1), rat paired box protein (PAX8)

Introduction

Iodine is a microelement that can be found as a dietary supplement, added to some foods, and naturally in others. Before being absorbed by the gastrointestinal tract, iodine from the meal is transformed into the iodide ion.[1] Iodide entering the blood is concentrated by the thyroid gland in the precise levels needed for the manufacture of thyroid hormone, and the majority of the residual quantity is eliminated in the urine.[2] Iodine requirements may be inferred using median urinary iodine concentrations of 100 g/L, which equate to around 150 g of iodine consumption per day.[3] Iodine intakes are adequate as evidenced by median urinary iodine concentrations of 100–199 g/L in children and adults, 150–249 g/L in pregnant women, and >100 g/L in lactating women.[4] In thyroid metabolism of iodine as previously stated, the thyroid gland's production of thyroid hormones is the primary physiological function of iodine.[5] Na+ actively transports bloodstream iodide through the plasma membrane into the cytoplasm of thyrocytes by using the Na^{+}/K^{+}-ATPase transporter's produced concentration gradient of Na+ as a propulsion source.[6] Several transporters, such as PENDRIN, ANO1, and CFTR, then carry iodide to the lumen of thyroid follicles.[7]

The quantity of iodine that our bodies require depends on a variety of conditions, including physiological changes

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such as pregnancy. The need for iodine rises dramatically during pregnancy due to three factors: (i) a 50% increase in maternal thyroxine (T4) production required to maintain maternal euthyroidism and meet the fetus's thyroid hormone needs; (ii) the need to transfer iodine to the fetus for fetal production of thyroid hormone, particularly in later gestational stages; and (iii) a likely increase in renal. To ensure good fetal neurodevelopment throughout pregnancy, enough iodine consumption is necessary for both the mother's and the fetus' generation of thyroid hormones.[8]

Differentiated thyroid epithelial cells form follicular structures surrounding a lumen, with the resulting follicle were considered as the functional unit of the thyroid gland. Recent studies, however, have revealed that the elements that comprise the functional unit of the thyroid are more complex than originally thought. In this regard, the concept of an angiofollicular unit has been proposed, which also includes endothelial cells of the blood vessels that sheath the thyroid follicles, and which is formed during embryonic development.[9]

Differentiated follicular cells express four genes—Nkx-2.1 (previously known as thyroid transcription factor 1, TTF-1 or T/ebp), FOXE1 (formerly known as thyroid transcription factor 2, TTF2), Pax8 and Hhex (hematopoietically expressed homeobox protein)—are expressed by differentiated follicular cells.[10]

The aim of this study was to investigate whether there is a relation between the effect of high concentration exogenous iodine on the thyroid transcription factors in pregnant mother rat and embryos.

Materials and Methods

Preparation of iodine

Using an electronic balance, the required doses of iodine powder were dissolved in distilled water every day over the experiment period. The solution was administered orally by gavage in a dose after conducting an experimental dose to measure the safety of the dose on one animal for 24–48 h before use.

Lab animals

Laboratory rats with weights ranging 250–300 g and 8 weeks of life were used in the experiment. The animals were placed in the Animal House of the Biology Department, College of Science at the University of Babylon, with environmental conditions that include moderate temperature, a 12-hour dark and 12-hour light cycle. The animals were treated with the approval of the ethics committee at the department, where they were kept in meshed plastic cages containing sawdust; the pellets were fed (mix of corn, wheat and milk) and they drank tap water throughout the experiment. The animals were housed in 45 cm × 27 cm × 16 cm polypropylene cages. They had access to food and water as they desired. The animals were left to adapt for 14 days before starting the experiment, male added for 15 day, Checking the female every day for pregnancy by mucus ring, food intake, mothers weight, mortality rate of mothers.

This study included three groups as follows: Group 1: Involved 20 adult female rats; animals in this group were administered distilled water and they served as control. Group 2: Involved 15 adult female rats; animals in this group were treated with low dose of iodine (0.06 mg). Group 3: Involved 15 adult female rats; animals in this group were treated with medium dose of iodine (1.8 mg).

Blood collection

After 18 days of pregnancy, all the groups of animals were sacrificed. Three milliliters of blood were drawn from the antecubital vein using a G23 needle, and the remaining blood was allowed to clot in a gel test tube at room temperature. The serum was aspirated after centrifugation at 2500 cycles per minute for 15 min, divided into aliquots in Eppendorf tubes, and stored at –20°C.

Determination of the thyroid factors in mother and embryo

Rat paired box protein (PAX8), rat Forkhead box protein E1 (FOXE1), and rat Homeobox protein (Nkx-2.1) were estimated using an enzyme-linked immunosorbent assay (Cusabio, USA). The concentrations were obtained according to the standard curves [Figures 1–3]. To investigate the thyroid factors in the embryo, the tissue was disrupted with glass homogenizers for the thyroid gland of the embryo.

Statistical evaluation methods

Data were analyzed using statistical package for the social sciences (SPSS) version 23.0 (SPSS, IBM Company, Chicago, IL, USA). and statistical tests were carried out using a null hypothesis of no difference student *t* test to determine the significance of difference. *P*-value and significance level were (0.05) and (0.01), respectively.

Results

The mother thyroid factors

The results presented in [Table 1] demonstrated a significant increase in FOX1 concentration in 1.8 gm concentration of iodine (770.9c ± 53.2) and in 0.06 gm concentration of iodine (555.3 ± 25.5), while there was a significant increase in NK2.1 concentration in the 1.8 gm concentration of iodine (6.87c ± 2.33) and in 0.06 gm concentration of iodine (5.83b ± 3.166). There was a significant increase in PAX8 concentration in 1.8 gm concentration of iodine (723.05c ± 5.20) and in 0.06 gm concentration of iodine (527.1b ± 27.1) compared to the control group, which recorded lower values with significant differences at ($P \leq 0.05$). Finally the highest PAX8 concentration was in 1.8 gm 723.05^{c} ± 5.20 followed by in concentration 0.06 gm 527.1^{b} ± 27.1, while

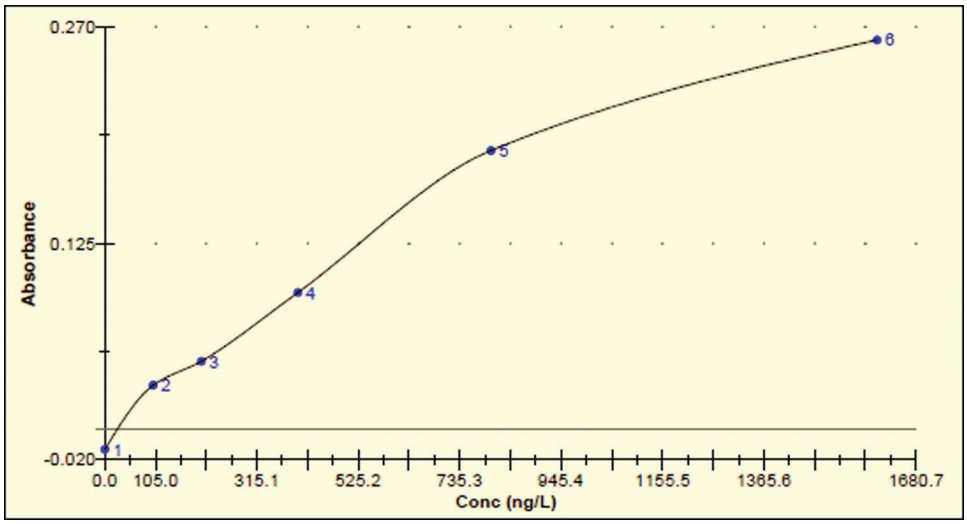

Figure 1: The standard curve of rat Forkhead box protein E1 (FOXE1)

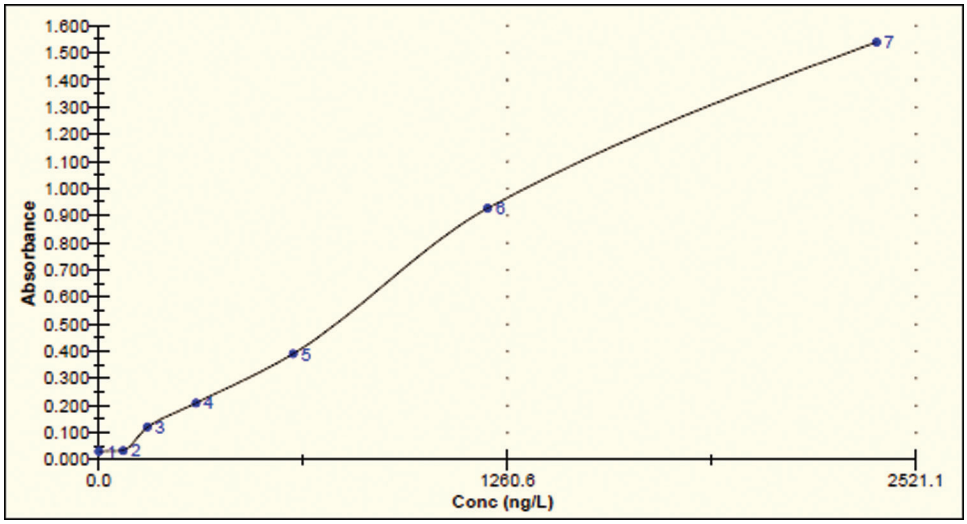

Figure 2: The standard curve of rat paired box protein (PAX8)

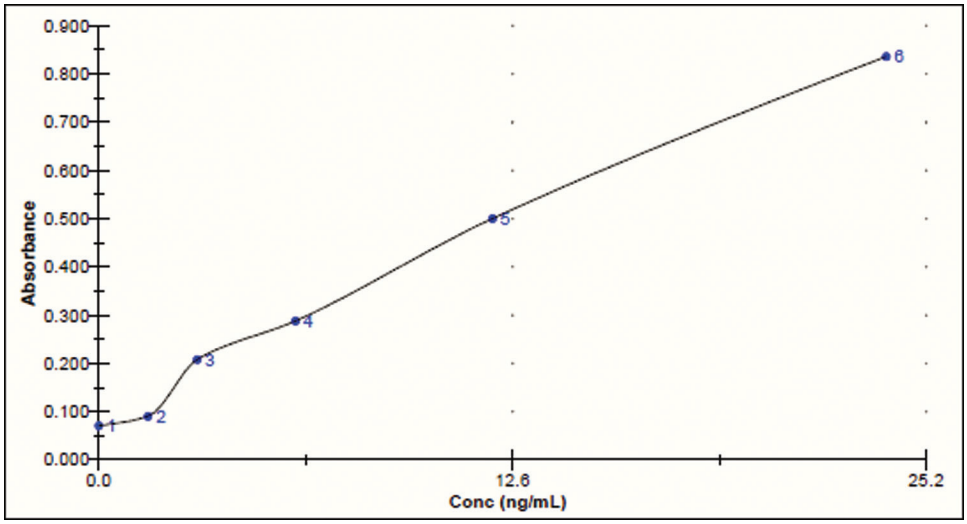

Figure 3: The standard curve of rat Homeobox protein (Nkx-2.1)

Table 1: The effect of different concentrations of exogenous iodine on the mother thyroid factors

Doses	Doses of Iodine (Mean ± SE)				
Biomarker	**Control**	**0.06 gm**	**1.8 gm**	**F-test**	**P-value**
FOX1	390.6[a] ± 56.9	555.3[b] ± 25.5	770.9[c] ± 53.2	237.73	0.0001
NK2.1	4.27[a] ± 9.104	5.83[b] ± 3.166	6.87[c] ± 2.33	643.18	0.0001
PAX8	414.49[a] ± 9.71	527.1[b] ± 27.1	723.05[c] ± 5.20	912.39	0.0001

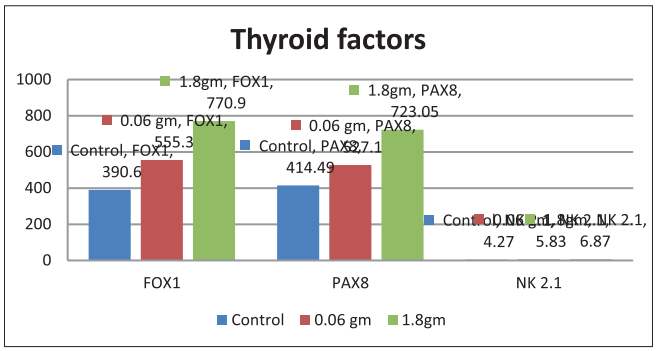

Figure 4: Effect of exogenous iodine on the mother thyroid factors

the lowest value recorded in control group 414.49[a] ± 9.71 with significant differences at ($P \leq 0.05$) between the studied groupsPAX8, FOXE1, and Nkx-2.1 [Table 1, Figure 4].

Discussion

However, if the adaptation to high iodine intake fails, various diseases occur. Chronic excessive iodine supply can also lead to goiter[11] and may accelerate the development of subclinical thyroid disorders to overt hypothyroidism or hyperthyroidism, increase the incidence of autoimmune thyroiditis, and increase the risk of thyroid cancer.[12] Our findings showed agreement with mentioned reports, The role of FOXE1 in development and differentiation is well understood,[13,14] yet little is known about its potential role in thyroid carcinogenesis, or how different allelic variants in or near FOXE1 are associated with thyroid cancer risk. The characterized FOXE1 expression and FOXE1 expression levels are unaltered or are upregulated in human tumors, suggesting that it might be important in the initiation and progression of these tumors.[15]

Loss of thyroid-specific proteins and differentiation markers is common in thyroid carcinogenesis. Indeed, several studies have reported the abnormal expression of thyroid-specific transcription factors in some thyroid carcinomas and propose that their deregulation is a pivotal event for the initiation and progression of thyroid neoplasms. This agreed with Fan *et al.*[16] who revealed the expression of FOXE1 was significantly increased in papillary thyroid cancer (PTC) tumor tissues and correlated with clinical prognosis. Therefore, FOXE1 is a potential biomarker for PTC prognosis as well as a new therapeutic target. Also, it agreed well with Morillo-Bernal *et al.*[17] who found FOXE1 expression in thyroid cancer cell lines to be higher.

However, also the results showed PAX8 was higher; this agreed with Fan *et al.*,[18] who revealed for the first time that the PAX8-PPARγ protein tyrosine phosphatase expression was significantly higher in thyroid cancer cell lines and thyroid cancer tissues relative to the normal thyroid cell lines and normal and paraneoplastic thyroid tissues. This high expression of PAX8-PPARγ may be involved in the development of thyroid cancer and is closely associated with patient prognosis, TNM staging, as well as lymph node metastasis. The expression level of PAX8-PPARγ was not significantly correlated with patient age and gender ($P > 0.05$). This suggests that high PAX8-PPARγ expression may be involved in the initiation of thyroid malignancy as well as invasion, metastasis, and further tumor evolution.

Accumulating evidence suggests that Na+/I− Symporter (NIS) expression and function are regulated by ROS-dependent mechanisms in cancer and non-cancer contexts, such as during iodide overload. High iodide levels (I−) induce a transient inhibition of thyroid hormone biosynthesis, which is restored around two days after I− administration, a mechanism of thyroid autoregulation known as the Wolff–Chaikoff effect.[19] Thyroid escape from the iodide inhibitory effect is attributed to reduced NIS iodide uptake and increased apical iodide efflux, which reduces concentrations of intracellular iodide and relieves thyroid function inhibition.[20] NIS-related responses to iodide overload are associated with dynamic changes in the cellular redox state.[21]

Leoni and collaborators showed that iodide overload had increased the ROS levels and induced time-dependent decreases in NIS mRNA, protein, and activity *in vitro* and *in vivo*.[22] NIS recovery after I− treatment depended on a compensatory increase in thioredoxin reductase antioxidant activity, showing that ROS levels are directly implicated in NIS regulation. In agreement, subsequent studies also found an ROS-dependent acute decrease in NIS mRNA and NIS inactivation at the plasma membrane in response to excess I−, which was reversed by ROS scavengers.[23], PAX8 is a transcriptional factor required for thyroid development and differentiation that acts as a major regulator of NIS transcription. PAX8 binds to the NIS upstream enhancer (NUE) in human and rat thyrocytes and induces NIS expression.[24] As with other PAX family members, the PAX8 DNA binding activity depends on the redox state of two cysteine residues in its structure: Cys-45 and Cys-57. In thyroid cells, PAX8 binding to NUE and the induction of NIS transcription depend on PAX8 being

converted to a reduced form by apurinic/apyrimidinic endonuclease 1 (APE1) and a reduction cascade involving thioredoxin reductase-1 (TxnRd1).[25]

Finally, numerous studies have shown that antibodies to TTF-1 can be very useful reagents in diagnostic pathology. It is important to realize that only nuclear immunoreactivity to TTF-1 represents "true" reactivity, and for the purposes of diagnosis, any cytoplasmic reactivity should be ignored (similar to certain other nuclear antigens such as ER and PR).[26] In the mature thyroid grand, TTF-1 regulates gene expression of thyroglobulin (Tg), thyroid peroxidase (TPO), sodium-iodide symporter (NIS), as well as thyroid-stimulating hormone receptor (TSH-R) in thyroid cells. TSH regulates thyroid function and growth, and thus various observations are suggesting a potential interaction between TSH and TTF-1. It is supposed that TSH-R activity can elevate TTF-1, the latter being expressed differently in benign and malignant thyroid diseases. TTF-1 mRNA is always detected in papillary carcinomas and it is absent in anaplastic carcinomas.[27-29]

Conclusion

The current study found that the thyroid transcription factors were involved directly when excess iodine is being taken, wherein all the factors were evaluated with iodine doses in both groups' pregnant mother rats.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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