ORIGINAL ARTICLE

A Genomic Analysis of Subclinical Hypothyroidism in the Developing Rat Brain

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ABSTRACT

Background: The effects of thyroid hormone on brain development and function are largely mediated by the binding of T3 to its nuclear receptors (TR) to regulate gene expression positively or negatively. Hypothyroidism during the early period of pregnancy and has severe consequences for the developing rat brain.
Aim: To examine effects of maternal hypothyroidism on gene expression in the developing rat brain.
Methods: Dose-dependent thyroid hormone insufficiency was induced by the delivery of methimazole (MMI) to pregnant rats via drinking water from gestation days 3 (GD 3) until the sacrifice of pups at PN20.
Results: Maternal blood collected for thyroid hormone analysis. qRT-PCR was used to compare *BDNF, NT3, NGF, Bcl2* relative gene expressions in the brain of postnatal day (PN) 20 pups experiencing thyroid hormone insufficiency induced by delivery of 0, 50, 75 and 100 ppm MMI to the dam. Daily body weight in dams and pups and BDNF expression in brain extract were determined in the preweaning rats as a function of MMI exposure. The obtained data indicate that genes driving important developmental processes in developing rodent CNS are sensitive to perturbations of the thyroid axis and the level of gene expression is related to the degree of hormone insufficiently. Altered patterns of gene expression in developing rat brain indicate that thyroid disease induces structural and functional abnormalities in the developing central nervous system.
Conclusion: The study suggests a continuum of thyroid disease with neurological disorders. Alteration in BDNF levels in early life could contribute to the adverse neurodevelopmental effects that occur after prenatal hypothyroidism.
Keyword: Methimazole, gene expression, CNS, subclinical hypothyroidism

INTRODUCTION

The action of thyroid hormones on brain development and function is gaining renewed interest. It has been known for many years that thyroid hormones are very important in mammalian brain development, maturation, influencing many aspects related to neural cell migration, differentiation, glial cell differentiation and signaling[1]. Maternal T_4 insufficiency has serious adverse consequences for the neurodevelopment of the offspring, including irreversible cognitive deficits[2-4]. Even modest reductions in thyroid hormones during the critical period of brain development produce morphological alterations, synaptic dysfunction, and behavioral impairments[5]. Early maternal hypothyroidism alters cerebral cortex[6] hippocampal[7] and cerebellar[8] cytoarchitecture of the pups. The general picture that emerges is that T4 and T3 may enter the brain through monocarboxylate transporter 8 (MCT8) and MCT 10 (*SLC16A2* and *SLC16A10* genes), The organic anion transporter polypeptides (OATP, especially *OATP1C1* and *SLCO1C1* gene), the large neutral amino acid transporters (LAT-1 and LAT-2, products of *SLC7A5* and *SLC7A8* genes), and sodium/ taurocholate co-transporting polypeptide (*SLC10A1*, or *NTCP*genes)[9]. Thyroid hormone action in CNS development is mediated through nuclear receptors (TRs) and integrin $\alpha_v\beta_{3[10]}$ and activation of nuclear receptors modulate the timing of expression of genes controlling process critical for brain development[11]. T4 is converted to the active hormone, T3, in glial cells, astrocytes, and tanycytes, although the main target cells are neurons and mature oligodendrocytes.

T3, acting through the nuclear receptors, exert transcriptional control over a number of target genes. Although many genes have been reported to be altered by perinatal hypothyroidism[12-19], the subset of TR-regulated genes that is critical for normal brain development has not been elucidated. In vivo T3 regulates gene expression during development from fetal stages, and in adult animals. A large number of genes are under direct and indirect regulation by thyroid hormone. In neural cells, T3 may control around 5% of all expressed genes, and as much as one-third of them may be regulated directly at the transcriptional level [20]. The purposes of this study are to identify alteration of many critical genes in developing rat brain and the goals were to determine whether dose-dependent changes in total patterns of multiple brain regions could be detected. To date, limited data available for thyroid hormone reduction on *BDNF,NT3, NGF, Bcl2* gene transcript alteration in different brain regions. In the present study qRT-PCR was utilized to identify some critical gene transcripts alteration in the developing rat brain. Our findings suggest that maternal thyroid hormone insufficiency could affect early CNS development via dysregulation of critical genes.

MATERIAL AND METHODS

All experimental procedures involving animals were performed following the Pasteur Institute of Iran guidelines. Pregnant Wistar rats (n=60), weighting 200-260 g, were obtained from Pasteur Institute of Iran on gestation day (GD) 3 and housed individually in standard hanging cages under controlled temperature (24±1 °C). The housing

rooms were maintained on a 12:12 light-dark cycle, and animals were permitted free access to standard laboratory chow and tap water. Body weights of dams were monitored from GD0 to GD23 and body weight of pups were determined from PN0 to PN20. Successful delivery day monitored in each treatment group. Beginning on GD3 and continuing until postnatal day (PN) 20,dams were rendered hypothyroid by addition of 0, 50, 75 or 100 ppm of the thyroid hormone synthesis inhibitor MMI (Daroupakhsh, Iran) to the drinking water (n=12/dose group) , and a group of rats received 50 ppm MMI in drinking water and 200 µg L-T4 (Daroupkhash, Iran) by gavage (n=12/dose group). Thyroid hormone (TH), l-thyroxine (Daroupkhash, Iran) was dissolved in 0.9% sodium chloride containing 10 mM KOH at a concentration of 200 µg/ml. Drinking MMI solution was replaced daily with a fresh MMI solution. MMI not only enters the bloodstream, but also it can be passing through the mother's breast milk to the rat pups as well. These doses of MMI could produce a severe state of hypothyroidism (based on serum hormones, body weight gains, and profound developmental delays). In each experiment a pregnant rat was transferred to plastic hanging cages and was permitted free access to food and tap water. All animal treatment began on GD 3 and continuing until weaning of the pups (PN 20). The day of birth was designated PN0. Exposure to MMI terminated when pups were weaned on PN20.

Determination of plasma TSH, T3, T4 by Serological assay: Blood was collected directly from the maternal orbital sinus in PN 20. Blood samples were centrifuged at 3000g for 20 min at room temperature to obtain the plasma. Plasma was used to analyze levels of thyroid hormones TSH, Ts, fT4 and Total T4. Serum levels of total T3 (tT3) and total T4 (tT4) and free T4 (fT4) were measured by ELISA (Padtangostar, Tehran, Iran) and Serum levels of TSH were measured by rat TSH ELISA kit (Life Span Bio Sciences) according to the manufacture's instruction

BDNF expression assay: BDNF expression was determined with Rat Brain-derived neurotrophic Factor (BDNF) ELISA kit (Zellbio, Germany) according to the manufacture's instruction. In each treatment group 10 neonates, and in 100 ppm hypothyroid group 6 neonates were sacrificed for mature BDNF analysis, Brains quickly removed and weighted up and added PBS (pH7.4, 100 mM) and homogenized the sample throught by homogenizer (totally 100 mg tissue/1ml buffer). The PBS contains ananti-protease cocktail (Sigma). Then centrifuged (at 6000 RPM) for approximately 10 minutes. After collecting the supernatant, an experiment conducted immediately. Aliquots of each sample lysate were analyzed in duplicate for total protein by The Lowry protein assay and sample mature BDNF protein expression was determined in duplicate in each experimental set of plates.

Real-time quantitative –PCR assay: The identification of transcripts was changed as a function of MMI treatment in each experimental group was performed using Reverse transcriptase-polymerase chain reaction (RT-PCR). In each treatment group, Six offspring (n=6) were sacrificed at PN20. Brains were quickly removed and cortex, hippocampus, medulla oblongata, and bulbous olfactories dissected. Total RNA was prepared individually from multiple brain regions using RNXTM-Plus (Cinnagen, Iran) according to the manufacture's instruction. The concentration of RNA was determined by measuring the absorbance at 260 nm (A260) in a spectrophotometer (SHIMADZU, UV-1800. Switzerland). The 260/280 ratio was calculated that was greater than 1.6. Then RNA stored at -70°C. First-strand cDNA was synthesized from 5µg total RNA from one animal using first-strand cDNA synthesis (Cinnagen, Iran). Relative expression levels of the neurotrophic genes of *BDNF*, *NGF* and *NT3* and *Bcl* 2 survival gene in cDNA samples were quantified by Step One Real-Time PCR Systems and normalized using SYBER Green dye. Each sample was run in triplicate for the target and reference genes and the reporter dye signal was normalized using the passive reference dye, ROX, to eliminate the volume-handling error. RT-PCR primers were designed in Oligo 7.0 primer analysis software (Molecular Biology Insights) and blasted in https://ncbi.nlm.nih.gov. Sequences of the PCR Primers are listed in Table 1.

Table1: Real-quantitative PCR primers sequences and characteristics

Gene Symbol	Direction	Sequences of the PCR primers	Function	Gene bank ID
BDNF	Forward	GATTAGGTGGCTTCATAGGAGAC	Neurotrophic growth factors-Differentiation	NM_001031616.1
	Reverse	AGAACAGAACAGAACAGAACAGG		
NGF	Forward	ACCTCTTCGGACACTCTG	Neurotrophic growth factors-Differentiation	NM_001277055.1
	Reverse	GTGGCTGTGGTCTTATCTC		
NT3	Forward	CAACAGACACAGAACTACTAC	Neurotrophic growth factors-Differentiation	NM_001270868.1
	Reverse	CCTCGGTGACTCTTATGC		
Bcl2	Forward	CTGGTGGACAACATCGCTCTG	Cell survival protein	NM_016993.1
	Reverse	GGTCTGCTGACCTCACTTGTG		
HPRT	Forward	CCAGCGTCGTGATTAGTG	Housekeeping Gene	S79292.1
	Reverse	CGAGCAAGTCTTTCAGTCC		

qRT-PCR data analysis: RT-PCR data analysis was performed using the comparative Ct method ($2^{-\Delta\Delta CT}$). The ΔCt value was calculated as the Ct_{target}- $Ct_{reference}$, using the *HPRT* gene as the reference gene to calculate relative mRNA levels. *HPRT* was chosen as it is a high expression that constitutesa gene whose expression does not change as a function of hypothyroidism[21] .The ΔΔCt value was calculated as the ΔCt test sample -ΔCt calibrator sample., where the mean of the 0 ppm sample ΔCt values were used for the calibrator. Fold changes were calculated as $2^{-\Delta\Delta Ct}$

Statistical analysis: Statistical significance was determined by one-way ANOVA followed by TUKEY'spost hoc test. All numerical data were expressed as mean± standard error of the mean (SEM). Pairwise comparisons were performed by Mann-Whitney *U*-test between groups (

Graph Pad PRISM version 7.0 Software, San Diego, CA, USA). If ρ-values were less than 0.05, differences were considered statically significant.

RESULTS

Determination of plasma TSH, T3, T4 by Serological assay: As a typical thyroid hormone synthesis antagonist, Methimazole induces hypothyroidism by inhibiting thyroid hormone iodination. MMI significantly reduced in a dose-dependent manner reduced T3 and T4, but TSH levels were increased. Thyroid hormones were reduced in a dose-dependent manner in dams in response to 0, 50, 75, 100 ppm, of MMI. fT4 was dose-dependently reduced at 50 ppm, 75 ppm, 100 ppm MMI dose groups on PN20 [F(4,55)=34.18, P<0.0001)](Fig. 1A). tT4 was dose-dependently reduced at 50 ppm, 75 ppm, 100 ppm MMI dose groups on PN20 [f(4, 55)=146.5, P<0.0001)](Fig.1B). T3 was also reduced on PN20[F(4,55), P<0.0001] (Fig.1C). TSH was dose-dependently increased on PN20 [F(4,55)=1187, P<0.0001](Fig.1D).

Body weight in dams and pups: Significant alternation in dam weights were detected in response to MMI in treated groups relative to control (F=85.891 *P*value<0.0001). At GD15 significant reduction in body weight were observed in dams In comparison to control group [Dose×Age interaction, F=5.976 ρvalue<0.0001] and extending to the early postnatal period. Offspring maintained on MMI displayed a slower growth rate (F=146.230 Pvalue<0.0001), and significant reductions in body weight were evident by PN3 and became more profound with age [Dose×Age interaction, F=70.072, *P*value<0.007] and extending to the PN20.

Table2. Maternal plasma fT4, tT4, T3 and TSH levels at PN 20

Treatment Groups	T3(ng/dl)	freeT4(ng/ml)	totalT4(ng/ml)	TSH(ng/ml)
Control(n=12)	1.158±0.137	0.883±0.083	45.425±3.494	1.95±0.288
50PPM(n=12)	0.9500±0.206	0.5458±0.307	22.775±3.721	7.1667±0.314
75ppm(n=12)	0.858±0.131	0.575±0.2701	22.775±3.721	15.00±0.2936
100ppm(n=12)	0.683±0.133	0.375±0.0965	17.65±1.405	22.20±0.385
50PPM MMI+T4(n=12)	0.7917±0.156	1.313±0.238	71.175±12.81	1.4667±0.5551

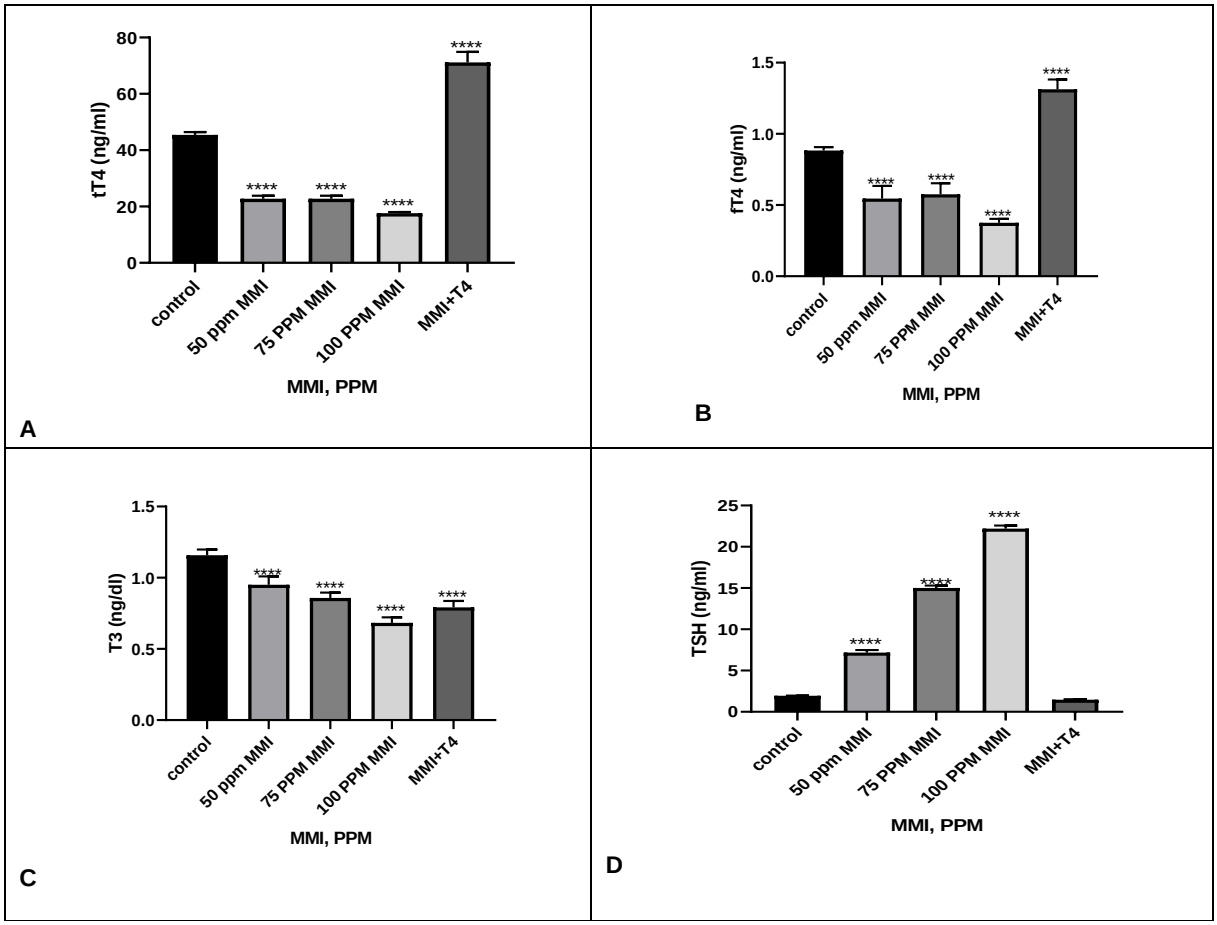


Fig1. Maternal thyroid hormones and thyroid stimulating hormone are dose-dependently changed by MMI in dams. (A) Maternal serum tT4 was reduced significantly as a function of MMI dose in dams (n=12/group) **[F(4,55)= 146.5 P value<0.0001]** (B) Maternal serum fT4 was also reduced significantly as a function of MMI dose in dams[**F(4,55)= 34.18 P value<0.0001] .**(C) Maternal serum T3 levels were reduced

significantly in dams treated with MMI and its concentration was recovered in 50 ppm MMI groups treated with 200 µg L-T4. (D) Thyroid stimulating hormone levels in treated groups with MMI increase significantly, relative to control group. Serum levels of TSH in treated group with thyroid hormone and MMI decreased not statistically significant [**F(4,55)= 1187 P value<0.0001].** All data are shown as mean±SEM.

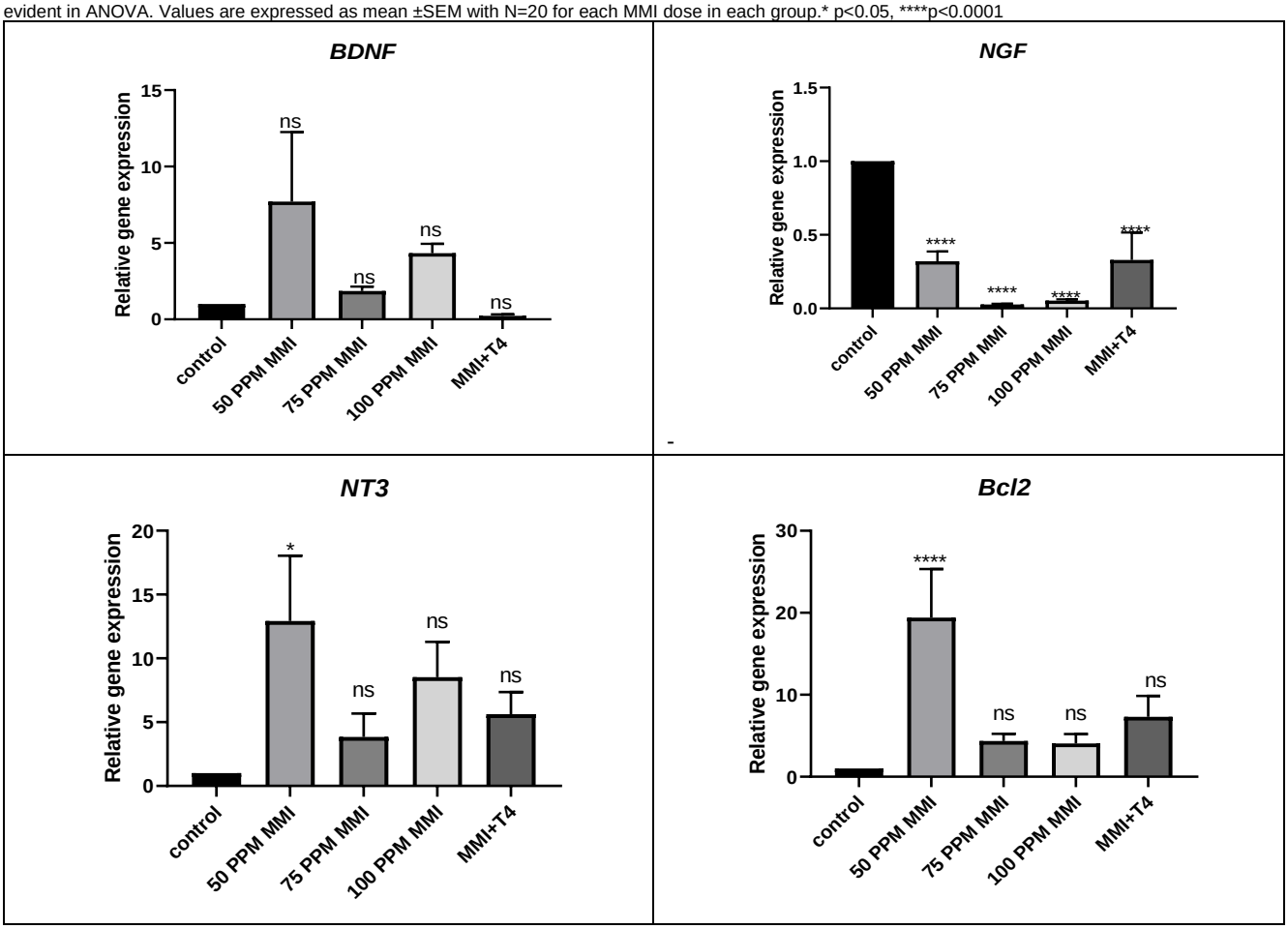
Table3. Mean±SEM body weight of dams. Significant alternation in dam weights were detected in response to MMI in treated groups relative to control (F=85.891 Pvalue<0.0001). Significant main effects of Dose and Age in repeated measures ANOVA were further evaluated by TUKEY's post-hoc test.

	GD0	GD3	GD7	GD10	GD15	GD17	GD19	GD20	GD21	GD22
Control	222.500±10.193	225.500±10.942	233.333±11.523	234.000±25.161	258.833±12.698	272.333±18.097	292.917±23.761	302.167±25.222	311.417±26.071	224.000±14.863
50 PPM MMI	215.250±14.623	218.000±14.844	221.250±15.621	224.083±16.903	233.417±19.523	236.417±19.126	238.000±20.676	243.043±20.676	226.583±11.547	226.583±11.547
75 PPM MMI	197.583±8.543	198.583±8.027	202.667±8.616	210.417±10.415	218.250±10.575	222.417±13.898	222.417±15.096	226.500±17.901	229.167±20.910	235.083±27.830
100 PPM MMI	216.667±9.490	214.833±9.897	219.250±12.447	228.333±17.839	244.417±27.828	247.250±30.427	255.083±37.826	258.583±42.621	262.500±46.802	218.500±7.452
MMI+T4	196.000±10.788	198.000±11.189	202.455±16.305	214.727±18.363	229.818±29.047	240.636±37.524	246.364±46.128	200.727±8.521	200.727±8.521	200.727±8.521

Table4. Mean±SEM body weight of pups. Significant reductions in body weight were seen in pups in response to 50 ppm, 75 ppm and 100 ppm MMI. Body deficits (mean±SEM) were evident by PN 3 in the offspring that became more apparent with age. Significant main effects of Dose and Age in repeated measures ANOVA were evaluated by TUKEY's post-hoc test. [Dose×Age interaction, F=70.072, *P*value<0.007] **P*<0.05.

Experiment					
	PN0	PN3	PN7	PN10	PN20
Control (n=88)	9.9943± 0.05330	10.9886±0.10660	12.8068±0.98094	18.7614±2.0850	40.8409±6.35879
50 ppm MMI (n=37)	9.9459±0.22924	10.9189±0.34387	12.5946±0.86472	13.7432±1.26722	31.9189±10.03709
75 ppm MMI (n-28)	9.6964±0.34263	10.6071±0.61399	11.5357±0.79266	12.3750±1.11076	21.1071±5.13044
100 ppm MMI (n=14)	9.5000±0.27735	9.8929±0.48748	10.3571±0.71867	11.5357±1.59885	19.8571±3.48308
MMI + L-T4 (n=64)	10.0154±0.12403	11.0000±0.0000	13.1719±0.88290	17.3906±3.35470	27.6562±3.24267

Fig.2. Effect of Dose-dependent T3 insufficiency on gene expression in developing rat brain relative to control at PN=20. Significant main effects of Dose were evident in ANOVA. Values are expressed as mean ±SEM with N=20 for each MMI dose in each group.* p<0.05, ****p<0.0001



No significant changes in the *BDNF* mRNA expression observed in comparison to control[F(4, 95)= 2.171 *P*value=0.0781]. Relative expression of mRNA *NT3* relative to the control group has increased significantly[F(4, 95)=2.590 *P*value<0.05] and the expression of gene NGF has reduced significantly[F(4,95) *P*value ≤0.0001]. Changes in *Bcl₂* have been shown a significant increase in the group 50ppm but in other treated groups, no significant increase was shown[F(4, 95)= 2.171 Pvalue<0.0001].

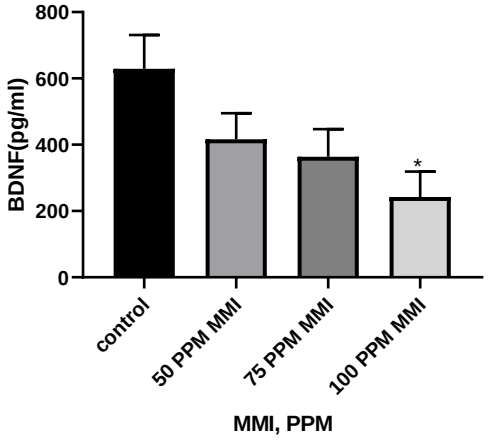


Fig-3.Effect of MMI on mature BDNF levels in the Wistar rat's pups at PN20. As shown in this diagram BDNF (pg / ml) in the total brain of the test groups relative to the control were decreased and in the groups that exposed to 100ppm MMI its decreasing was significant.($F(3,32)=3.144$ $\rho_{value}=0.0386$ n=36) .* p<0.05

DISCUSSION

Despite extensive work on the actions of thyroid hormones in brain development, there are still many uncertainties such as how thyroid hormones influence the maturation of the fetal brain and to related questions such as the relative roles of the maternal and fetal thyroids, or the timing of action of thyroid hormones. One of the difficulties in approaching these problems is the almost complete lack of knowledge on the molecular targets of thyroid hormones in the developing fetal brain. Neurotrophins are important regulators of neural survival, development, function, and plasticity[22-24]. As the central concept of the neurotrophic factor hypothesis, targets of innervation were postulated to secrete limiting amounts of survival factors that function to ensure a balance between the size of a target organ and the number of innervating neurons[25] Nerve growth factor (NGF), the first such factor to be characterized, was discovered during a search for such survival factors[26]. There are four neurotrophins characterized in mammals. NGF, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4) are derived from a common ancestral gene, are similar in sequence and structure, and are therefore collectively named neurotrophins[27]. In this study, the effects of maternal thyroid hormone insufficiency on the relative abundances of *NGF*, *BDNF* and *NT3* RNA in developing rat brain were determined by RT-PCR. The effects of peripherally administered thyroid hormone (TH; 500 µg/kg; i.p.; q.a.) on the relative abundances of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin-3(NT-3) RNA were determined by RT-PCR in the cortex and hippocampus of young adult rats. They showed that abundance levels of NGF and NT-3 were increased significantly in the hippocampus following TH treatment, Whereas ChAT activity was modestly increased by 28% in the cortex without corresponding changes in *NGF*, *NT*-3 or *BDNF*. The induction levels of RNA can not be directly correlated with the responsivity of the cholinergic system as measured by ChAT activity. In other study, in hypothyroid Wistar rats investigators found that the level of NGF mRNA was 20-50% lower in the cortex, hippocampus, and cerebellum of hypothyroid rats on neonatal hypothyroid rats on P15 and also after adult-onset hypothyroidism. Treatment of neonatally-induced hypothyroid rats with a single injection of triiodothyronine led to the recovery of hippocampal but not cortex *NGF* mRNA levels to that of control animals[25]. On the contrary, no differences in the relatively high expression of the two mRNAs encoding BDNF were observed in any brain area. In contrast to a recent report, they did not find a reduction in brain NT-3 mRNA levels in hypothyroid animals. The effect of thyroid deficiency in the hippocampus and cortex seems to be an early up-regulation of NT-3 expression. Some of the effects of thyroid hormone on differentiation and survival might be mediated through control of neurotrophin expression. Interactions between thyroid hormone and NGF are important for the growth and maintenance of cholinergic neurons in the basal forebrain[20]. The cerebellum thyroid hormone also controls the expression of NT-3 *in vivo* and in cultured cerebellar granule cells and it was suggested that the control of Purkinje cell differentiation by thyroid hormone is mediated through NT-3 produced by granule cells and they observed that BDNF was decreased significantly. In this work we have analyzed the contribution of factors others than T3, that might be involved in the regulation of gene expression and that may play diverse roles *in vivo*. This experimental set up allowed us to confirm that genes which expression in the developing rat brain. A number of novel observations resulted from this investigation of the impact of thyroid hormone insufficiency on neurotrophins expression in the whole developing rat brain. MMI did not affect the expression of neurotrophin genes in the neonate, but surprisingly, altered their expression profiles present in the rat brain development. In the present study, we observed that Severe hypothyroidism caused by the administration of MMI results in retarded growth, poorly co-ordinated creeping and gait defects in the progeny. These effects being more pronounced at P7-P20 and are in accordance with previous studies In other study researchers induced a state of subclinical hypothyroidism by oral ingestion of propylthiouracil (PTU; 3.8+/-0.2 mg/kg/day) for 21 days[28]. They showed that Thyroid hormones affect bone remodeling directly via the concentrations of both nerve growth factor (NGF) and norepinephrine (NE) in particular bones. As discussed in this section neurotrophins are important regulators of neural survival, development,

function, and plasticity and during development, neurotrophins are expressed in regions being invaded by sensory axons en route to their final targets, so they may provide trophic support to neurons that have not yet contacted their final targets[29,30] many neurons also synthesize neurotrophins. For example, several populations of sensory neurons have been shown to synthesize BDNF[31]. Although some evidence has been presented suggesting that BDNF may act in an autocrine or paracrine fashion to support dorsal root ganglion (DRG) sensory neurons. In other instances it may be transported anterogradely and act *trans*-synaptically on targets of the central afferents of these neurons within the brain [32]. In the next study researchers induced a state of the hyperthyroid animal model by peripheral administration of thyroid hormone (TH; 500 µg/kg; i.p.; q.d.) on the relative abundances of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin-3 (NT-3) RNA in the cortex and hippocampus of young adult rats. Their results demonstrate that TH administration is capable of inducing the accumulation of NT-3, in addition to NGF but that the induction levels of RNA cannot be directly correlated with a responsivity of the cholinergic system as measured by ChAT activity. Altar, C.A., et al.(1997) induced a state of the hyperthyroid animal model by peripherally administration of thyroid hormone daily subcutaneous injections of 0.05 ml sodium-thyroxine (T4;7.5mg) in0.9% saline up to P12.and their results indicated that In the hippocampus of hyperthyroid newborn rats, as compared to controls, was increased higher levels of BDNF and NT-3 mRNA over the total investigation period, whereas in the septum a thyroxine-dependent increase in NT-3 mRNA expression was observed. propylthiouracil (PTU) during development produced dose-dependent reductions in mRNA expression of nerve growth factor (NGF) in the whole hippocampus of neonates[33]. In other study Gilbert *et al* (2013) described an animal model of low-level TH insufficiency and showed that BDNF protein and gene expression have varied as a function of the hypothyroid model[37]. Estrogen has proven to be a potent regulator of BDNF in rat brain[34,35], and administration of exogenous estrogen increases BDNF expression in the hippocampus[36]. Furthermore, hippocampal BDNF expression is more robustly augmented in female rats following traumaticbrain injury than in males[37]. Moreover, estrogen and thyroid receptors share a common consensus site on their hormone response elements (EREs and TREs) and thus interact to regulate gene expression[38-40]. Consequently, the presence of estrogen in adult female offspring may attenuate the effect of developmental thyroid hormone insufficiency on BDNF expression at low PTU doses and underlie the elevation in BDNF observed at the highest PTU dose. Thyroid hormone insufficiency during development did not impact BDNF protein expression during the preweaning period in any of the three brain regions assessed despite significant reductions in circulating thyroid hormones in dams and offspring. In the above studies, the expression BDNF in different concentrations of MMI were examined. Our results show that there were no significant difference in the expression of BDNF in different concentrations of MMI and *BDNF* mRNA alternation were not correlated with BDNF expression in the developing rat brain. No significant reduction in BDNF expression was observed. Li., *et al* (2003)[41] used a rat model to understand the effect of hypothyroidism on apoptosis during the development of rat brain. Their results suggest that hypothyroidism down-regulates the expression of anti-apoptotic genes Bcl_2 and Bcl-$x_L$where's the pro-apoptotic gene Bax is up-regulated throughout the developmental period. Our studies show that Bcl_2 is significantly increased in 50 PPM MMI treated developing rat brain, whereas it is relative but not significantly increased in other MMI treated groups in comparison with controls. We also found that the expression BDNF that regulates Bcl2 is significantly decreased in the developing rat brain .One of the major functions of BDNF is to regulate proteins involved in cell survival. This occurs via the Bcl-2 family of proteins and the cysteine protease caspase family[42]. It is interesting to note that the *BDNF* relative gene expression pattern corresponds with *Bcl2* relative gene expression. The down-regulation of Bcl2 may result from a decreased concentration of brain-derived neurotrophic factor (BDNF), the growth factor that modulates AKT activities. These results suggest that the down-regulation of the BDNF-Akt-Bcl2 anti apoptotic signaling pathway in the hypothyroid developing rat brain could be one of the underlying mechanisms responsible for the Neuronal cell death under the hypothyroid condition.

Thyroid hormones affect bone remodeling directly via receptors in osteoblasts. Euthyroid and hyperthyroid states significantly influence the concentrations of both nerve growth factor (NGF) and norepinephrine (NE) in particular bones. Both NGF and NE directly affect bone metabolism and therefore it is possible that thyroid hormone action on bone may also be indirect via its actions on these two neural-related substances. Previous studies investigate that hypothyroidism influenced NGF and NE concentrations in weight-bearing and non-weight-bearing rat bones. Femoral NGF concentrations were higher in hypothyroid rats in comparison with euthyroid rats. Rib NGF concentrations in hypothyroid rats have increased in comparison to euthyroidribs .Concentrations of both NGF and NE in bone are sensitive to weight-bearing and thyroid hormone status[43].

CONCLUSIONS

The results obtained in the present the study suggests a continuum of thyroid disease with neurological disorders. Alteration in BDNF levels in early life could contribute to the adverse neurodevelopmental effects that occur after prenatal hypothyroidism. The results indicate that genes driving important developmental processes during early brain development are sensitive to perturbations of the thyroid axis function.

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