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RESEARCH ARTICLE

THYROID HORMONE RELATED METABOLIC CHANGES IN NORMAL PREGNANCY

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ABSTRACT

Objective: Pregnancy is a physiological state accompanied by a high-energy demand and an increased oxygen requirement which leads to altered metabolic and hormonal status of the body. The present study was conducted to find out thyroid hormone status in various trimesters of normal pregnancy and its relation (if any exists) to various metabolic changes during pregnancy such as in lipedimic and glycemic index and oxidative stress levels. **Design and Methods:** Study designed with 40 healthy non pregnant females (group 1) who served as control and 60 normal pregnant females divided as group 2 (1st trimester), group 3 (2nd trimester), group 4 (3rd trimester). Thyroid function tests carried out by measuring total (T3, T4) and TSH levels investigated their association with changes in Lipedimic status (Total cholesterol, Triglycerides, High density lipoprotein, Low density lipoprotein, Very low density lipoprotein), Glycemic status (fasting serum glucose, insulin, c-peptide, HOMA-IR) and oxidative stress level (Superoxide dismutase, Malondialdehyde) in all groups. **Results:** Thyroid hormones (T3,T4) and TSH increased (at $p<0.05$) in pregnant females in all three trimesters as compared to non-pregnant females . Total cholesterol, triglyceride levels (at $p<0.001$) and levels of insulin and Insulin resistance (at $p<0.05$) increased significantly during pregnancy especially in 2nd and 3rd trimesters when compared with non pregnant females. Oxidative stress level also increased during pregnancy in terms of significant increased levels of MDA (at $p<0.05$) from 2.50±0.73nmol/ml in non pregnant females to 2.65±0.57nmol/ml in 1st trimester, 3.67±0.73nmol/ml (at $p<0.05$) in 2nd trimester, 5.35±0.57nmol/ml (at $p<0.05$) in 3rd trimester respectively. Insulin was found to be directly related to thyroid hormone increase. **Conclusions:** Thyroid hormone changes during pregnancy is associated with alterations in Lipedimic and glycemic status along with increase in oxidative stress.

Keywords : Pregnancy, thyroid hormones, oxidative stress, glycemic index, lipedimic index

INTRODUCTION

Pregnancy a normal physiological condition is interplay of various metabolic and hormonal parameters to meet the demands of growing fetus. During the growth of fetus in pregnant mothers all these changes are reflected as increased metabolic demand of the mother (Kumar *et al*., 2005). Thyroid hormones have been known to play a significant role in the process of embryogenesis and fetal development. The metabolic effects of thyroid hormones have been directly shown to be linked to the production of ROS and thus oxidative stress in various ways (Aurousseau *et al*., 2006). The present study was aimed at 1) Finding out the variations if any exists between the thyroid hormone status of pregnant mothers in various trimesters of pregnancy and normal healthy non pregnant age matched females. 2) To correlate the variations if any in the levels of thyroid hormones to the metabolic changes and their effects like production of oxidative stress and the consequent impact on glycemic index and lipedimic index between normal healthy females and pregnant mothers and also during various trimesters of pregnancy.

MATERIAL AND METHODS

The present study comprised of pregnant women (sixty) who reported for various biochemical investigations in Department of Biochemistry, Govt Medical College, Amritsar, India. The control group involved forty apparently healthy non pregnant volunteer females. An informed consent was taken from every patient. The project was approved by ethical committee of the institute. A Performa was designed to collect the data on socioeconomic status, previous obstetric history, height, weight and history of any metabolic disease.

Exclusion Criteria : Any female having history of DM, hypertension, obesity, anemia and any other systemic disorder were excluded from the study.

Inclusion Criteria : Healthy non pregnant and pregnant women ranging in age from 19-35 years, divided into following groups:

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Group 1 (n=40) – Non pregnant
Group 2 (n=10) – 1st trimester (average 9.2±2.0 weeks)
Group 3 (n=23) – 2nd trimester (average 19.6±2.2 weeks)
Group 4 (n=27) – 3rd trimester (average 32.0±2.8 weeks)

Fasting blood samples collected were allowed to clot and serum was separated for various investigations. In vitro quantitative determination of hormones- T3 (Ingbar and Beaverman, 1975), T4 (Attwood *et al*., 1978) and TSH (Bristow *et al*., 1982) and homeostatic parameters (Insulin, C-peptide) was carried out by using direct solid phase enzyme immunoassay based ERBA thyrokit & Monobind Inc. ELISA kits respectively, on Erba Mannheim LISA scan. Levels of blood glucose and and lipid profile were measured on autoanalyzer- (Erba XL 300). Glucose was estimated by oxidase- peroxidase method as described by Trinder P (1969)(Trinder, 1969). Insulin and C- peptide were estimated by method based on direct solid phase enzyme immunoassay as described by Boehm and Lebovitz (1979) and Kuzuya *et al*. (1977) respectively; and the tests were performed by using commercially available kits from Dia- metra, (Italy). Estimation of total cholesterol was done by method of Charles CA 1974 (Charles *et al*., 1974) using kits from Biosystems, (SA Costa Brava, 30- Barcelona Spain). HDL-C was estimated by method described by Brustein *et al*. (1970) using kit from Transasia Biomedicals Ltd. Serum Triglycerides (TG) were estimated by Trinder's method as described by Gowan *et al*., (1983) using kits from Transasia Biomedicals (Daman). Serum LDL was determined by using Friedwald's and fredrickson's formula (William *et al*., 1972) i.e. LDL= Total Cholesterol – (HDL + VLDL). VLDL was estimated by using: VLDL = Triglycerides/ 5 based on the average ratio of TG to Cholesterol in VLDL. Insulin resistance (IR), Insulin Sensitivity (%S) and β cell function (%B) were estimated by the HOMA-IR (Matthews *et al*., 1985). Levels of antioxidant enzymes i.e Superoxide dismutase (SOD) and Malondialdehyde (MDA) were measured with methods of Yisun *et al*., (1988) and Satoh (1978) respectively.The data collected was statistically analyzed using computer software SPSS (version 16.0). Student's t- test was applied to study the variation between two groups. Comparison of means of different groups was done using one way ANOVA (SPSS 16.0). Pearson's coefficient of correlation was calculated to study the significance of correlation between different parameters. Level of significance used was $p < 0.05$.

RESULTS

Females of 1st, 2nd & 3rd trimester showed significant increase (at $p<0.05$) in the levels of TSH as compared to non pregnant females. T3 and T4 hormones also showed significant increase in their levels by the progression of pregnancy. T3 levels increased to 1.3±0.5 ng/ml at $p\leq0.05$ in 2nd trimester to 1.4±0.05 ng/ml at $p\leq0.001$ in 3rd trimester with respect to 1st trimester. On the other hand T4 levels in blood showed an insignificant increase from 10.4±5.5 µg/dl in 1st trimester to 15.2±5.3 µg/dl in 2nd trimester but slightly significant increase at $p\leq0.5$ to 16.02±4.4 µg/dl in 3rd trimester. In 2nd and 3rd trimester the levels of T3 and T4 were significantly high at $p\leq0.05$ with respect to control group 1 (Table 1). In context with antioxidant status, the levels of SOD and MDA, both showed significant increase at $p\leq0.05$ in 2nd and 3rd trimester with respect to 1st trimester. Also the levels of both enzymes were high in all three trimesters with respect to group 1 non pregnant females (Table 2). The increase was significant at $p\leq0.05$ in 2nd and 3rd trimesters. Study of Lipedimic index revealed that the total cholesterol and triglycerides slightly increase in the 1st trimester of pregnancy with respect to group 1 whereas the levels of these parameters showed highly significant rise ($p\leq0.001$) in 2nd and 3rd trimesters with respect to non pregnant control group.

Table 1 Thyroid hormones status of the groups in the study

Group	TSH (µIU/ml) (Mean±SD)	T3 (ng/ml) (Mean±SD)	T4 (µg/dl) (Mean±SD)
Group 1 (Non Pregnant)	1.8±0.7	0.73±0.5	6.6±3.2
Group 2 (1st Trimester)	2.9±0.8 ǂ	0.82±0.4	10.4±5.5
Group 3 (2nd Trimester)	3.8±1.0 ǂ	1.3±0.5* ǂ	15.2±5.3 ǂ
Group 4 (3rd Trimester)	3.3±1.0 ǂ	1.4±0.5*** ǂ	16.02±4.4* ǂ

ǂǂǂ $p < 0.001$ w.r.t group1, ǂǂ $p < 0.01$ w.r.t group 1, ǂ $p < 0.05$ w.r.t group 1
*** $p < 0.001$ w.r.t group 2, ** $p < 0.01$ w.r.t group 2, * $p < 0.05$ w.r.t group 2

Moreover the total cholesterol also increased significantly (at $p\leq0.001$) from 207.0±52.0 mg/dl in 2nd trimester to 269.0±74.0 mg/dl in 3rd trimester with respect to 1st trimester. Similarly, triglyceride levels showed significant increase (at $p\leq0.001$) from 172.0±37.0 mg/dl to 255.0±43.0 mg/dl (Table 3). The lipoproteins i.e LDL and VLDL except HDL, showed constant increase throughout the various trimesters of pregnancy, however the increase was highly significant (at $p\leq0.001$) through 2nd and 3rd trimesters with respect to non pregnant and group 2 first trimester females.

Table 2 Enzymatic antioxidants status of the groups in the study

Group	SOD (U/ml) (Mean±SD)	MDA (nmol/L) (Mean±SD)
Group 1 (Non Pregnant)	2.61±0.87	2.50±0.73
Group 2 (1st Trimester)	3.18 ± 0.47	2.65 ± 0.57
Group 3 (2nd Trimester)	3.72 ± 0.47*ǂ	3.67 ± 0.73* ǂ
Group 4 (3rd Trimester)	6.14 ± 0.93*#ǂ	5.35 ± 0.57*#ǂ

ǂǂǂ $p < 0.001$ w.r.t group 1, ǂǂ $p < 0.01$ w.r.t group 1, ǂ $p < 0.05$ w.r.t group 1
*** $p < 0.001$ w.r.t group 2, ** $p < 0.01$ w.r.t group 2, * $p < 0.05$ w.r.t group 2
$p < 0.001$ w.r.t group 3, ## $p < 0.01$ w.r.t group 3, # $p < 0.05$ w.r.t group 3

Levels of fasting glucose almost remain unchanged with advancing pregnancy and also with respect to control non pregnant females. The levels of insulin showed insignificant increase in 2nd and 3rd trimesters with respect to 1st trimester, but significant increase (at $p\leq0.05$) with respect to control non pregnant females (Table 4). C-peptide showed no variation while comparing different trimesters of pregnancy, but the levels were significantly high ($p\leq0.05$) with respect to non pregnant women. Although the β-cell function increased with decreased sensitivity to insulin yet the variations were insignificant. As compared to normal females, pregnant females showed significantly increased IR (at $p\leq0.05$), however the levels remained constant throughout the pregnancy. The statistically analysed data of pearson's co-efficient of correlation, showed variable trends in 1st trimester with more clarity in 2nd and 3rd trimesters. Thyroid hormones, specifically T4 showed non significant positive correlation with SOD and MDA (at r=0.102, r=0.098 & r=0.120 , r=0.246) and negative correlation with IR (r=-0.251, r=-0.99) in 2nd and 3rd trimesters of pregnancy whereas TSH was

negatively correlated with SOD and MDA. T4 also showed negative correlation with total cholesterol and LDL in these trimesters with highly significant positive correlation of TC and LDL with each other throughout the pregnancy. Interestingly, Insulin and C-peptide were positively correlated with SOD and MDA in all three trimesters while %S was negatively correlated (non significantly) with Insulin, C-peptide, SOD and MDA.

Table 3 Lipedimic index of the groups in the study

Group	TC (mg/dl) (Mean±SD)	TG (mg/dl) (Mean±SD)	HDL(mg/dl) (Mean±SD)	LDL (mg/dl) (Mean±SD)	VLDL (mg/dl) (Mean±SD)
Group 1 (Non Pregnant)	145.51±18.10	78.11±9.20	40.12±3.10	73.71±5.33	18.11±3.43
Group 2 (1st Trimester)	158.5±21.0	116.0±38.4	43.1±4.1	92.1±21.1	23.2±7.6
Group 3 (2nd Trimester)	207.0±52.0*** $^{\text{iii}}$	172.0±37.0** $^{\text{iii}}$	43.0±2.5	132.0±38.0*** $^{\text{iii}}$	34.4±8.4** $^{\text{iii}}$
Group 4 (3rd Trimester)	269.0±74.0*** $^{\text{iii}}$	255.0±43$^{***, ###}$ $^{\text{iii}}$	45.4±7.7$^{\#}$	174.0±62.0$^{***, ###}$ $^{\text{iii}}$	51.0±11.0$^{***, ###}$ $^{\text{iii}}$

$^{\text{iii}}$ p < 0.001 w.r.t group 1, $^{\text{ii}}$ p < 0.01 w.r.t group 1, $^{\text{i}}$ p < 0.05 w.r.t group 1
*** p < 0.001 w.r.t group 2, ** p < 0.01 w.r.t group 2, * p < 0.05 w.r.t group 2
p < 0.001 w.r.t group 3, ## p < 0.01 w.r.t group 3, # p < 0.05 w.r.t group 3

Table 4 Glycemic index of the groups in the study

Group	Fasting Glucose (mg/dl) (Mean±SD)	Insulin µIU/ml) (Mean±SD)	C-peptide (ng/ml) (Mean±SD)	β cell Function (% B) (Mean±SD)	Sensitivity to insulin (% S) (Mean±SD)	Insulin Resistance (IR) (Mean±SD)
Group 1 (Non Pregnant)	72.0 ±5.21	6.4 ±1.3	1.2 ±0.02	98.4 ±20.1	94.9 ±15.3	0.3 ±0.1
Group 2 (1st Trimester)	82.6 ± 19.0	9.5 ± 2.8	5.7 ± 1.8 $^{\text{i}}$	140 ± 42.0	68.2 ± 19.0	1.2 ± 0.9 $^{\text{i}}$
Group 3 (2nd Trimester)	89.7 ± 24.8	11.5 ± 5.0 $^{\text{i}}$	4.7 ± 1.7 $^{\text{i}}$	160 ± 36.0	62.7 ± 13.0	1.5 ± 0.9 $^{\text{i}}$
Group 4 (3rd Trimester)	92.8 ± 34.6	15.1 ± 3.8 $^{\text{i}}$	4.3 ± 2.8 $^{\text{i}}$	154 ± 55.0	55.4 ± 12.0$^{\text{i}}$	1.8 ± 0.6 $^{\text{i}}$

$^{\text{iii}}$ p < 0.001 w.r.t control, $^{\text{ii}}$ p < 0.01 w.r.t control, $^{\text{i}}$ p < 0.05 w.r.t control
*** p < 0.001 w.r.t group 2, ** p < 0.01 w.r.t group 2, * p < 0.05 w.r.t group 2
p < 0.001 w.r.t group 3, ## p < 0.01 w.r.t group 3, # p < 0.05 w.r.t group 3

DISCUSSION

In our study it was observed that the levels of TSH raised in pregnant females as is evident from Table 1. The increase was statistically significant (at p<0.01) in TSH levels at 1st , 2nd & 3rd trimesters of pregnancy when compared to that of normal non pregnant females. Although the increase in TSH was significant yet the values were in the normal defined range of method. The levels of thyroid hormones (i.e T3 and T4) also showed significant increase (p<0.01), especially in 2nd and 3rd trimesters of pregnancy, as compared to non pregnant females. This shows that thyroid hormones increase with the progression of pregnancy. This increase attributes to the role of these hormones in embryogenesis and fetal development during pregnancy (Khandakar *et al*., 2002). Along with thyroid hormones the levels of antioxidant enzymes (i.e SOD & MDA) were also increased significantly during 2nd and 3rd trimesters of pregnancy w.r.t control. These observations are in association with the studies which shows that the metabolic effects of thyroid hormones are directly linked to ROS production and oxidative stress in various ways (Villanueva *et al*., 2013). The general metabolic effects of thyroid hormones is a relative acceleration of the basal metabolism that includes an increase in the rate of both catabolic and anabolic reactions (Schwartz and Oppenheimer, 1978). This results in increased energy expenditure, fuel mobilization, fuel oxidation for energy extraction, oxygen consumption, respiratory rate and heat production and release (Dauncey, 1990). The stimulation of the respiratory rate would intuitively lead to greater ROS production. But thyroid hormones do not directly determine the respiratory state of mitochondria (Adamo *et al*., 1989) (Katyare and Rajan , 2005) instead they promote a reduction state in the cell by increasing fuel availability, extramitochondrial production of ATP and NADH to stimulate the synthesis of elements of the respiratory chain and by genomic effects of TH's on UCP genes (considered among non enzymatic antioxidants). Thyroid hormones also affect the cell antioxidant status as some studies showed that the activity of some antioxidant enzymes, such as SOD, increase under Thyroid hormones stimulation along with the rate of ROS production (Qublan *et al*., 2003). The state of pregnancy is usually associated with mild hyperthyroidism and hyperthyroidism implies an increase in oxidative stress (Villanueva *et al*., 2013) which is also reflected in our study in the terms of increased MDA levels which are predictors of oxidative stress. Therefore the general balance that results from the stimulation of both production and elimination of ROS by thyroid hormones implies a net increase in oxidative stress. Another major finding in our study is the state of hyperlipidemia in pregnancy, with significant increase in the levels of TC, TG's, LDL and VLDL especially in 2nd and 3rd trimesters of pregnant women as compared to non pregnant females. The serum levels for TC, LDL, VLDL and TG's increased throughout gestation. Their is negative correlation of T4 with TC and LDL in this study which is in accordance with hyperthyroid state (Kok-Yong Chin *et al*., 2004), shows that in state of pregnancy the increase of thyroid hormones have no direct positive relationship with lipid profile. This increase in TC, TG & LDL is majorly attributed to the estrogen as pregnancy is associated with hyperestrogenaemia. Estrogen induces hepatic biosynthesis of endogenous TG, which is carried by VLDL (Festus *et al*., 2011). This process may be modulated by hyperinsulinism found in pregnancy (Adegke *et al*., 2003) which is also shown by our study. Along with the changes in

parameters reported above, a significant increase in insulin, C-peptide and IR was also observed in pregnant females and insulin was found to be directly related to thyroid hormone increase. This is in accordance with our previous study which also showed that mild increase in thyroid hormones is related to increase in insulin and IR (Navreet *et al*., 2014). The unchecked status of increased insulin resistance may in turn pose a threat to normal pregnant females to become diabetic during gestation. Therefore we can say that during the progression of normal pregnancy there is increase in the levels of thyroid hormones, especially in 2nd and 3rd trimesters which has direct positive correlation with increase in the levels of antioxidant enzyme SOD, MDA, insulin, c-peptide and IR; however the increase in lipid profile has indirect correlation with thyroid hormones through hyperinsulinaemia.

CONCLUSION

Hormonal variations during normal pregnancy results in increased metabolic rate of the body which cause impaired lipedimic and glycemic status and increased production of ROS that ends up in oxidative stress condition which may affect the growth of fetus. Therefore antioxidant supplementation and regular check up of lipedimic and glycemic index of normal pregnant females should be recommended.

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