Urinary iodine excretion and maternal thyroid function

During pregnancy and postpartum

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ABSTRACT

Objectives: To evaluate urinary iodine excretion during the course of pregnancy and postpartum in relation to maternal and neonatal thyroid function parameters in Saudi women living in Jeddah, Kingdom of Saudi Arabia.

Methods: A prospective longitudinal study was conducted on Saudi normal pregnant women during the course of pregnancy (N=80), at term and 6-10 weeks postpartum (N=65), during the period January 1997 through to December 2000. Maternal urinary iodine excretion was determined together with serum levels of total thyroxine, total tri-iodothyronine, free thyroxine, free tri-iodothyronine, thyrotropin, reverse tri-iodothyronine, thyroxine-binding globulin and thyroglobulin. A group of non-pregnant woman (N=200) were included for comparative purposes. Data were also analyzed for significant trends using ANOVA. Neonatal serum levels of total thyroxine, total tri-iodothyronine, free thyroxine, thyrotropin, thyroxine-binding globulin, and thyroglobulin were also measured.

Results: Changes in urinary iodine excretion and in serum thyroid function parameters during the course of pregnancy, at term and postpartum have been demonstrated. Subclinical iodine deficiency was evident in 28.8% of pregnant women at term and 11.5% of women at 6-10 weeks postpartum. Serum total thyroxine and total tri-iodothyronine levels increased in the first trimester ($P<0.001$) and remained elevated at term ($P<0.001$). Serum free thyroxine levels showed a significant decrease by the 2nd trimester ($P<0.001$) and continued to decrease in the 3rd trimester ($P<0.001$). Serum free tri-iodothyronine showed continuous decrease throughout gestation. Thyrotropin levels were decreased during the first and 2nd trimesters ($P<0.001$) but then increased to be comparable to non-pregnant values. Serum reverse tri-iodothyronine increased during the first and 2nd trimesters ($P<0.001$). There was a significant increase in serum thyroxine-binding globulin and thyroglobulin levels during the course of pregnancy. A significant negative correlation between thyrotropin and human chorionic gonadotropin levels was observed throughout pregnancy ($r=-0.31$, $P<0.001$). The observed correlation was stronger ($r=-0.37$; $P<0.001$) in the first trimester as compared to that in the second ($r=-0.164$; $P<0.001$) or the third ($r=-0.125$; $P<0.269$) trimester. There was a negative correlation between maternal free thyroxine and neonatal thyrotropin ($r=-0.70$; $P<0.001$). Positive correlation was found between neonatal total thyroxine and birth weight ($r=0.61$; $P<0.001$) and maternal urinary iodine concentration ($P<0.001$).

Conclusion: The changes in urinary iodine excretion during the course of pregnancy were documented. The decrease in free thyroxine and free tri-iodothyronine and the increase in reverse tri-iodothyronine concentrations during pregnancy resemble the changes in thyroid hormones seen in non-thyroidal illness. Moreover, the changes in thyrotropin in relation to that of human chorionic gonadotropin support the view that the thyroid gland is not primarily thyrotropin driven in early pregnancy. The results suggest that a more complex control may finally regulate maternal thyroid activity; the pituitary and the chorionic systems both function in an independent way in response to possible different feedback stimuli. This could be a physiological adaptation enabling energy conservation during the high metabolic demands of pregnancy. Finally, the results of the present study point to the need of an increased iodine supply in Saudi pregnant women living in Jeddah, Kingdom of Saudi Arabia to decrease the potential consequences of low iodine intake on maternal thyroid economy.

Keywords: Iodine, thyroid function, pregnancy, postpartum.

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Adequate nutritional iodine supply is important particularly during pregnancy, where the iodine requirement is increased due to enhanced renal clearance and the transfer of iodine from the mother to the fetus, as well as, a greater need of iodine for thyroid hormone synthesis.[1,2] In iodine-sufficient areas, physiological losses are not associated with significant changes in the maternal thyroid economy. Conversely, in moderately or marginally low iodine intake areas, pregnancy leads to a relative iodine deficiency state, and constitutes a stimulus for the maternal thyroid function as indicated by relative hypothyroxinemia, increased thyrotropin (TSH) levels during the 2nd part of pregnancy, increased serum thyroglobulin (Tg) and increased maternal thyroid volume.[3] However, in severely-iodine-deficient areas, the changes are more pronounced as evidenced by marked hypothyroxinemia, and increased TSH levels which are accompanied by intense maternal and neonatal thyroid stimulation.[4,5] The regulation of maternal thyroid function during pregnancy is complex and varies with each stage of pregnancy.[2,6] In addition, several studies have indicated a profound influence of maternal thyroid status early in pregnancy on fetal brain and nervous system development,[7-9] emphasizing the need for a greater understanding of thyroid physiology and the critical importance of the control mechanisms regulating maternal thyroid function. Biochemical data on free thyroxine (FT_4) and TSH levels in pregnancy have often been contradictory. Maternal FT_4 concentrations have been variously reported as unchanged, increased, or decreased during pregnancy.[10-14] Serum TSH concentrations have been reported to be higher in late than in early pregnancy;[15-16] however the concentrations of the hormone in the first trimester have been shown to be higher,[16-17] lower[12] or unchanged,[18] relative to normal control levels. Greater uncertainty of the changes in free-tri-iodothyronine (FT_3) in late pregnancy is also commonly reported.[16,19] In addition, changes in other thyroid hormone function tests [including total thyroxine (TT_4), total tri-iodothyronine (TT_3), reverse-T_3 (r-T_3), thyroxine-binding globuline (TBG)] are variable.[2,6,10-14] Moreover, few longitudinal studies have considered the maternal and fetal interaction during the course of pregnancy, in relation to maternal iodine status. Finally, there is very little information on the changes of maternal thyroid function tests in relation to fetal outcome and maternal iodine status in Saudi pregnant women. Thus, the main objective of the present study is to examine urinary iodine (UI) excretion together with the functional activity of maternal thyroid throughout pregnancy, at term and postpartum in healthy Saudi women living in the Jeddah area in relation to fetal outcome.

Methods. A total of 80 Saudi pregnant women living in the Jeddah area participated in the present study. Age and anthropometric data of the women studied are presented in **Table 1**. The study was carried out during the period of January 1997 through to December 2000, in the Jeddah area. All women had resided in the Jeddah area for more than 5 years and were recruited from women attending antenatal clinics at King Abdulaziz University Hospital (KAUH) and New Jeddah Clinic Hospital (NJCH), Jeddah, KSA. Women with hepatic, renal or with evident endocrine disorders, history of immunosuppresive therapy, history of thyroid dysfunction or on any form of drug treatment were excluded from the present study. In addition, all pregnant women included were: 1. Screened negative for both anti-thyroperoxidase (Anti-TPO) and anti-thyroglobulin (Anti-Tg); and 2. Not smoking cigarette or shessha. Originally, a total of 189 women were examined, but only 80 women fulfilled the criteria for selection. In addition, only 65 women were studied out of the 80 at postpartum, as 15 dropped out of the postpartum part of the study. The group was studied at presentation to the antenatal clinics (range 8-14 weeks gestation, mean ± standard deviation (SD): 11.6 ± 1.62 weeks); at the 2nd trimester (17-27 weeks; mean ± SD: 21.5 ± 3.61); the 3rd trimester (29-35 weeks, mean ± SD: 32.62 ± 1.81); and at terms (36-42 weeks, mean ± SD: 39.7 ± 1.29); and 6-10 weeks after delivery (mean ± SD: 8.54 ± 2.1). At the first appointment, all pregnant women had a general physical and obstetric examination and measurements of their height and weight were made. In addition, they underwent an ultrasound scan in order to confirm the pregnancy and assess fetal age and maturity. At each visit and at delivery, blood samples were collected for the measurement of TT_4, FT_4, TT_3, FT_3, r-T_3, TSH, TBG, Tg and human chorionic gonadotropin (hCG). Urinary iodine excretion was also determined in urine samples collected at each visit. At birth all newborns were examined for the following observations: 1. Apgar score, birth-weight, head-circumference and fetal length; 2. Birth weight centile; which were determined using locally developed birth weight standards;[20] 3. Pediatric estimation of pregnancy age and its correlation; with gestational age estimated by ultrasound and from menstrual data; 4. The presence of any congenital malformations; and 5. Complications at birth such as cyanosis, bradycardia, convolutions and apnea. A total of 200 non-pregnant Saudi women who were randomly selected also participated in the present study, as a reference population for comparative purposes. They were healthy, not lactating and had not been lactating during the previous 2 years, were not pregnant neither had been during the previous 2 years, were not using oral contraceptives, and had regular menstrual cycles. The mean (± SD) age was

27.3 ± 4.6 years and mean body mass index (BMI) 23.22 ± 1.66 kg/m^2. Maternal and cord blood samples together with maternal urine samples were collected as indicated above. Also samples were collected from non-pregnant women. Collected blood samples were immediately transferred to the laboratory and sera were separated by centrifugation. Collected sera and urine samples were divided into multiple aliquots and stored at –130°C until analysis. All assays and determinations were performed in batches, to eliminate variability within assays.

Determination of urinary iodine excretion. Urinary iodine was measured by dry ashing the samples and then estimating the catalytic effect of the I^- on the reduction of ceric ions to cerous ions in the presence of arsenous ions which were oxidized to arsenic. The change in color of the former was measured from the standard curve. Measurements were carried out in duplicates after the method of Wilson & Van Zyl.[21] Recovery of added ^{125}I and ^{127}I was above 95% and not corrected for. The lower detection limit for the assay was 0.15 umol/L. The mean co-efficient of variance for 0.18 umol/L was 16.2%, for 0.78 umol/L was 7.1% and for 1.3 umol/L was 5.5%. Results were expressed both as microgam of iodine per gram creatinine (ug/g) or directly as umol of iodine per liter of urine (umol/L). Creatinine was measured by the standard technique described by Jaffe.[22]

Determination of total thyroxine, total tri-iodothyronine, free thyroxine, free tri-iodothyronine and thyrotropin. Total thyroxine, TT_3, FT_4, FT_3, TSH and hCG levels were determined in sera using the electrochemi luminescence immunoassay (ECLIA) technique using Elecsys 2010 system Autoanalyzer (Boehringer Mannheim Laboratory Diagnostics, Boehringer Mannheim GmbH, D-68298 Mannheim, Germany). All the kits and reagents were obtained from same supplier. Intra and inter-assay precisions for the various assays were indicated by the coefficients of variance (% CV), as follows: Total thyroxine (2.8% and 3.9 %); TT_3 (4.1% and 4.6%); FT_4 (1.8% and 3.2 %); FT_3 (2.1% and 2.7%); and TSH (1.9% and 2.5 %).

Determination of reverse tri-iodothyronine, thyroxine-binding globulin and thyroglobulin. Reverse-T_3 levels were determined in sera using the radioimmunoassay technique (Diagnostic Products Corp., Los Angeles CA, United States of America, (USA)). Thyroxine-binding globulin levels were determined by radioimmunoassay technique (Corning Medical and Scientific, Medfield, MA, USA) and Tg levels were determined by radioimmunoassay technique (Diagnostic System Laboratory, Inc, Webster, TX, USA). Intra- and inter-assay precisions for the various assays were indicated by (% CV) as follows: r-T_3 (5.2% and 3.6%), Tg (4.6% and 4.1%) and TBG (3.6% and 2.7%).

Table 1 - Maternal age, body mass-index, gravida, week of delivery, fetal birth weight, fetal length and fetal head circumference of pregnant women.

Clinical and Anthropometric data	Values
Age (years)	26.46 ± 5.72
Body mass-index (kg/m^2)	28.21 ± 3.65
Gravida	4.20 ± 2.85
Week of delivery	39.65 ± 1.29
Fetal birth weight (g)	3390 ± 444
Fetal length (cm)	51.90 ± 3.80
Fetal head circumference (cm)	34.75 ± 2.10

Values are presented as means ± standard deviation for 80 pregnant women at delivery

Determination of anti-thyroperoxidase and anti-thyroglobulin. Anti-thyroperoxidase and Anti-Tg levels in sera were determined using coated well ELISA technique by kits supplied by Diagnostic System Laboratory Inc, Webster, TX, USA.

Statistical analysis. Results are presented as means (±S.D.) Data were analyzed using statistical package for social sciences (SPSS) - Statistical Package (version 10 for Windows Smart Viewer) supplied by SPSS Inc. 2000, Mapinfo Corp. Tokyo, NY, USA. Results that were not normally distributed were log-transformed before analysis. Analysis of variance was used to examine differences among the groups for different variables, and the Bonferroni criterion was used when significance tests were made. Correlations were carried out using regression analysis.

Results. A total of 80 Saudi women were studied longitudinally during the course of pregnancy, with successful delivery of their neonates. Relevant clinical and anthropometric data on the mothers and neonates are presented in **Table 1.** None of the babies delivered showed any form of neonatal complications or morbidity.

Urinary iodine excretion. Urinary iodine excretion during the course of pregnancy and postpartum are presented in **Figure 1** (see also **Table 2).** Urinary iodine excretion levels showed a wide individual scatter. In pregnant women, UI excretion was significantly elevated, compared with non-pregnant state values, as early as the first trimester (1.22 + 0.41 umol/L versus 01.03 + 0.15 umol/L) ($P < 0.001$), but decreased during the 2nd and 3rd trimesters as compared to the first trimester values. By term, UI excretion decreased by 21.4% as compared to non-pregnant values. The postpartum

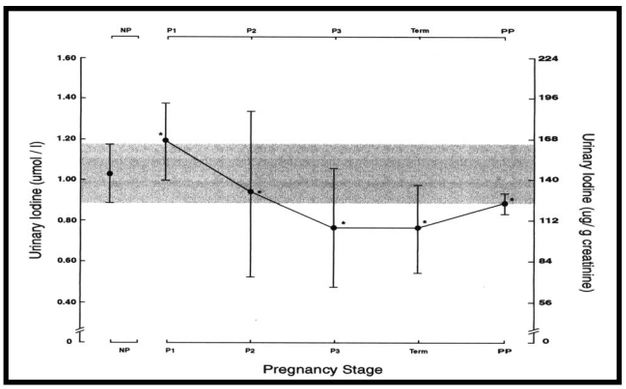

Figure 1 - Changes in urinary iodine excretion in non-pregnant (NP), and pregnant women (P_1=first trimester; P_2=2nd trimester; P_3=3rd trimetser) and at 6-10 weeks postpartum (PP). Points represent mean ± standard deviation (SD) for pregnant women (N=80 and for PP, N=65). Denotes statistical significance as described in the text. The shaded area represents the reference range obtained from non-pregnant women (N=200).

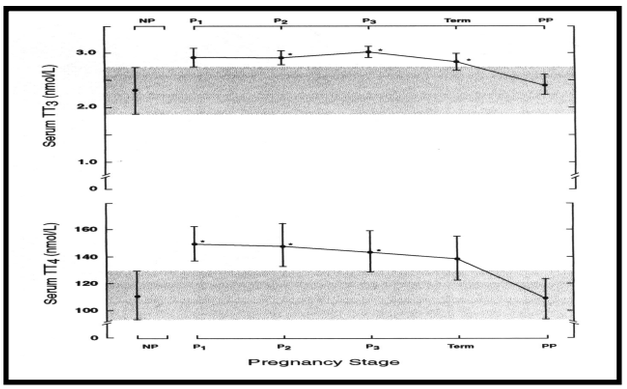

Figure 2 - Changes in serum total thyroxine (TT_4) and total tri-iodothyronine (TT_3) levels in non-pregnant (NP) and pregnant women (P_1=first trimester; P_2=2nd trimester; P_3=3rd trimester); and 6-10 weeks postpartum (PP). Points represent means ± standard deviation (SD) for pregnant women; (N=80 and for PP, N=65). Denotes statistical significance as described in the text. The shaded area represents the reference range obtained in the non-pregnant women. (N=200).

values were essentially similar to those seen in non-pregnant women. Using World Health Organisation (WHO)/United Nations International children's Emergency Fund (UNICEF)/International Council for the Control of Iodine Deficiency Disorders (ICCIDD) criteria of <0.79 umol/L as the cut-off for iodine deficiency, 28.8% and 11.5% of the pregnant women by the 3rd trimester had UI excretion below the cut-off value at term and 6-10 weeks postpartum. Correlations of UI excretion with maternal blood thyroid function tests showed significant negative correlations between maternal TSH (r=-0.228, P< 0.05), TBG (r=-0.238, P<0.05), Tg (r=-0.229, P< 0.05), and hCG (r=--0.139, P<0.05) and UI excretion, **(Table 3).** Serum TT_4, TT_3, FT_4, and FT_3: The changes in serum TT_4, TT_3, FT_4 and FT_3 levels as a function of gestation time are presented in **Figures 2 & 3.** At initial presentation, TT_4 levels ranged between 122.0 and 177.0 nmol/L, with a mean value of 149.9 nmol/L. The serum TT_4 levels significantly

Table 2 - Maternal concentrations of serum TT_4, TT_3, FT_4, FT_3, TSH, r-T_3, TBG and Tg together with urinary iodine (UI) excretion studied during first, 2nd and 3rd trimesters, at term and 6-10 weeks post-partum.

Parameter	NP (N=200)	P_1 (N=80)	P_2 (N=80)	P_3 (N=80)	Term (N=80)	PP (N=65)	Probability (F)
Total thyroxine (TT_4) (nmol/L)	111.51 ± 28.13	149.9 ± 13.10*	148.1 ± 16.91	144.5 ± 15.62*	139.6 ± 16.22	110.2 ± 15.30	0.001
Total tri-iodothyronine (TT_3) (nmol/L)	2.33 ± 0.55	2.92 ± 0.33*	2.92 ± 0.34*	3.02 ± 0.26*	2.85 ± 0.31*	2.41 ± 0.36*	0.0001
Free T_4 (FT_4) (pmol/L)	15.17 ± 1.80	14.77 ± 1.47*	12.99 ± 1.32*	11.22 ± 1.13*	12.40 ± 1.40*	14.61 ± 1.58*	0.001
Free T_3 (FT_3) (pmol/L)	5.21 ± 0.57	4.71 ± 0.47*	4.08 ± 0.46*	4.37 ± 0.42*	4.12 ± 0.53*	5.02 ± 0.77	0.010
Thyrotropin (TSH) (mIU/ml)	1.94 ± 0.4	1.26 ± 0.29*	1.46 ± 0.25*	1.66 ± 0.24*	2.10 ± 0.32*	2.01 ± 0.51	0.001
Reverse-T_3 (r-T_3) (nmol/L)	0.31 ± 0.09	0.38 ± 0.06*	0.37 ± 0.07*	0.32 ± 0.07*	0.35 ± 0.10*	0.32 ± 0.08	0.010
Thyroxine-binding globulin (TBG)(mg/L)	19.83 ± 1.78	38.81 ± 2.76*	46.10 ± 5.01*	44.28 ± 5.07*	52.10 ± 6.66*	28.16 ± 6.52*	0.000
Thyroglobulin (Tg) (ug/L)	16.03 ± 1.24	28.81 ± 2.79*	33.46 ± 3.57*	49.13 ± 3.79*	55.60 ± 4.44*	23.44 ± 3.89*	0.000
Urinary Iodine (umol/L) (ug/g creatinine)	1.03 ± 0.15* 138.5 ± 19.3 *	1.22 ± 0.41* 172.3 ± 35.6*	0.94 ± 0.38* 132.7 ± 19.6*	0.86 ± 0.32* 121.6 ± 24.1*	0.81 ± 0.20* 125.5 ± 30.9*	0.89 ± 0.15* 123.7 ± 26.4*	0.000 0.000

* Statistically significant from corresponding controls
Values are presented as means ± standard deviation SD, NP - non pregnant, P_1, P_2, P_3 - first, 2nd and 3rd trimesters of pregnancy, PP - 6-10 weeks post-partum.

Table 3 - Pearson's correlation between various maternal thyroid function tests during the entire course of gestation in women studied.

Parameter	TT_4 (nmol/L)	TT_3 (nmol/L)	FT_4 (pmol/L)	FT_3 (pmol/L)	r-T_3 (nmol/L)	TSH (mIU/ml)	TBG (mg/L)	Tg (ug/L)	hCG (mIU/ml)
TT_4 (nmol/L)	-	-	-	-	-	-	-	-	-
TT_3 (nmol/L)	0.01	-	-	-	-	-	-	-	-
FT_4 (pmol/L)	0.183†	-0.106	-	-	-	-	-	-	-
FT_3 (pmol/L)	0.131*	-0.102	0.331†	-	-	-	-	-	-
r-T_3 (nmol/L)	0.115	-0.199†	0.320†	0.034	-	-	-	-	-
TSH (mIU/ml)	-0.081	0.073	-0.330†	-0.145*	0.196†	-	-	-	-
TBG (mg/L)	-0.042	0.005	-0.321†	-0.323†	-0.245†	0.141*	-	-	-
Tg (ug/L)	0.053	0.108	-0.511†	-0.217†	-0.220†	0.351†	0.290†	-	-
hCG (mIU/ml)	0.025	-0.022	0.104	0.035	0.054	-0.305†	-0.021	-0.092	-
Urinary iodine (umol/L)	0.032	0.041	0.227†	0.078	0.108	-0.228†	-0.238†	-0.229†	0.139*

*- significance at 0.05 level (2 - tailed),
†- significance at the 0.001 level (2 - tailed),
TT_4 - total thyroxine, TT_3 - total tri-iodothyronine, FT_4 - free T4, FT_3 - free T_3, r-T_3 - reverse -T_3, TSH - thyrotropin, TBG - thyroxine-binding globulin, Tg - thyroglobulin, hCG - human chorionic gonadotropin

increased in the first trimester of pregnancy (by 34.4%, P<0.001) and remained thereafter elevated at term by 25.2%, P<0.001) as compared to non-pregnant state values. At initial presentation, serum TT_3 showed a marked increase as compared to non-pregnant (by 25.3%, P<0.001) or postpartum (by 21.2%, P<0.001) values. Serum TT_3 levels were also increased in the 2nd (by 25.3%, P <.001) and 3rd (by 29.6%, P<0.001) trimesters and at term (by 22.3%, P<0.001), as compared to non-pregnant state values **(Figure 2 & Table 2).** Serum FT_4 levels showed a significant decrease in the 2nd (by 14.4%, P<0.001) and continued to decrease in the 3rd (by 26.1%, P<0.001) trimester, as compared to non-pregnant state. At term, serum levels of FT_4 were decreased by 18.3 % (P<0.001) as compared to non-pregnant values. However, there was no significant difference between first trimester and non-pregnant values. Serum FT_3 levels showed a continuous decrease throughout gestation that became significant in the

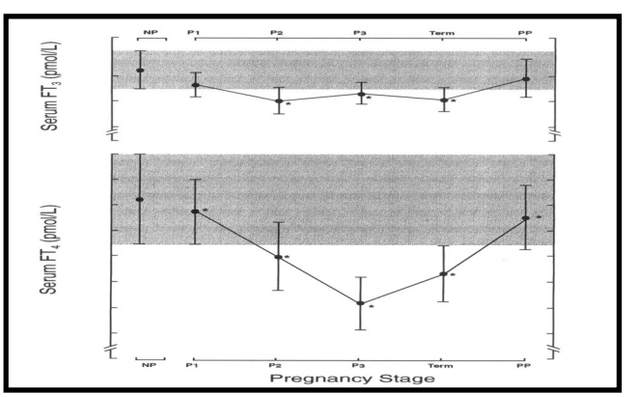

Figure 3 - Changes in serum free T_4 (FT_4) and free T_3 (FT_3) levels in non-pregnant (NP) and pregnant women (P_1=first trimester, P_2=2nd trimester, P_3=3rd trimester), at term and 6-10 weeks postprtum (PP). Points represent means ± standard deviation (SD) for pregnant women, (N=80 and for PP, N=65). Denotes statistical significance as describe in the text. The shaded area represents the range obtained in the non-pregnant women (N=200).

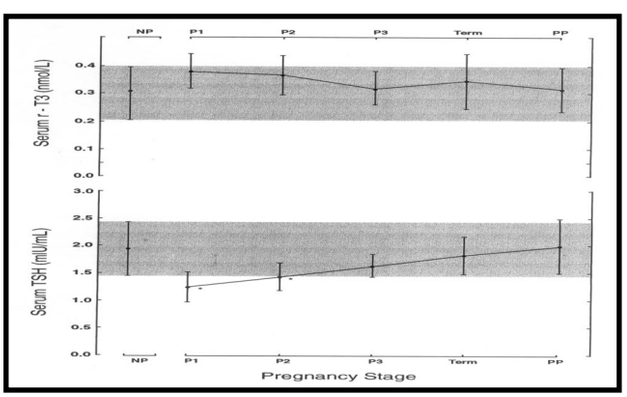

Figure 4 - Changes in serum thyrotropin (TSH) and reverse T_3 (r-T_3) levels in non-pregnant (NP) and pregnant women (P_1=first trimester, P_2=2nd trimester, P_3=3rd trimester), at term ad 6-10 weeks postpartum (PP). Points represent means ± standard deviation for pregnant women, N=80 and PP, N=65). Denotes statistical significance as described in the text. The shaded area represents the reference range obtained in the non-pregnant women (N=200).

Table 4 - Thyroid function tests in neonates born to women studied.

Parameter	N	Mean ± SD
Total thyroxine (TT_4) (nmol/L)	65	141 ± 6
Total tri-iodothyronine (TT_3) (nmol/L)	65	0.97 ± 0.05
Free T_4 (FT_4) (pmol/L)	65	13.88 ± 1.67
Thyrotropin (TSH) (mIU/ml)	65	10.21 ± 3.65
Thyroxine-binding globulin (TBG) (ml)	65	22.75 ± 4.44
Thyroglobulin (Tg) (ug/L)	65	110 ± 12

N- number
SD - standard deviation

2nd (by 21.7%, P< 0.001) and the 3rd (by 16.1%, P<0.001) trimesters. Also, FT_3 values were significantly decreased at term (by 20.9%, P<0.001) as compared to non-pregnant values **(Figure 3 & Table 2)**.

Serum thyrotropin, reverse tri-iodothyronine, thyroxine-binding globulin and thyroglobulin. The changes in serum levels of TSH, r-T_3, TBG and Tg, as a function of gestation time are presented in **Figures 4 & 5.** Serum TSH levels were decreased significantly during the first (by 35.1%, P<0.001) and 2nd (by 24.7%, P<0.001) trimesters, as compared to non-pregnant state. At term there was no significant changes in TSH values as compared to non-pregnant state. Serum r-T_3 showed a significant increase during the first (by 22.6%, P<001) and the 2nd (by 19.4%, P<0.001) trimesters, as compared to non-pregnant state **(see Table 2).** Serum TBG levels continuously increased throughout pregnancy and were significantly higher than non-pregnant state values: increased by 96.3%, 132.5% and 173.7% in the first, 2nd and 3rd trimesters. At term, TBG values were 1.6-fold higher than the non-pregnant values. The TT_4/TBG ratio decreased with gestation age (being 3.85, 3.21 and 2.67 at the first, 2nd and 3rd trimesters). Serum levels of Tg increased significantly throughout pregnancy: by 78.4%, 109.01% and 206.01% at first, 2nd and 3rd trimesters **(see Table 2)** as compared to non-pregnant state. At term, serum Tg levels continued to be elevated as compared to non-pregnant state (by 2.47-fold, P< 0.001). Person's correlation between various maternal thyroid function tests during the entire course of gestation is presented in **Table 3.**

Serum human chorionic gonadotropin. Serum hCG levels, peaked significantly in the first trimester [88090 + 6984 mIU/ml, mean + SD] and then decreased [46745 + 9253 and 14213 + 2819 mIU/ml] in 2nd and 3rd trimesters. When measured 6-10 weeks post-partum, hCG levels were undetectable. A significant negative correlation between TSH and hCG levels was observed throughout pregnancy (r =-0.31; P<0.000) **(Figure 6).** The observed correlation was stronger (r=-0.37; P<0-000) in the first trimester as compared to that in the 2nd (r =0.164; P<0.146) or the 3rd (r=-0.125; P=0.269) trimesters. No significant correlation was found between hCG and TT_4, TT_3, FT_4, FT_3 levels throughout pregnancy. When hCG was used as a dependent variable in relation to other thyroid function tests, only TSH showed a significant effect (r_2 = 0.09; P<0.000).

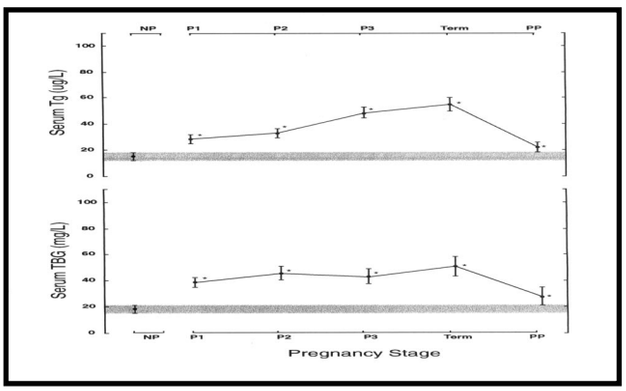

Figure 5 - Changes in serum thyroxine-binding globulin (TBG) and thyroglobulin (Tg) levels in non-pregnant (NP) and pregnant women (P_1=first trimester, P_2=2nd trimester, P_3=3rd trimester), at term and 6-10 weeks postpartum (PP). Points represent means ± standard deviation (SD) for pregnant women (N=80 and for PP, N=65). Denotes statistical significance as described in the text. The shaded area represents the reference range obtained in the non-pregnant women (N=200).

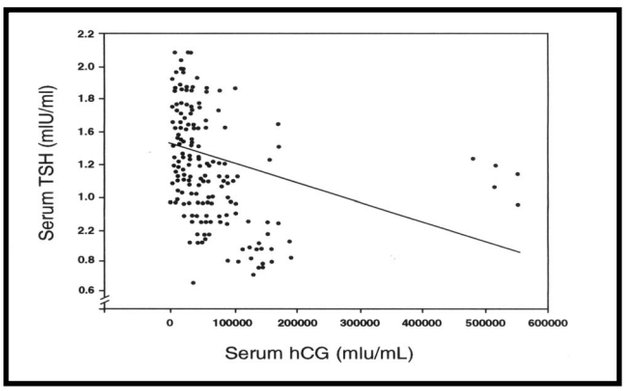

Figure 6 - The relationship between serum thyrotropin (TSH) and human chorionic gonadotropin (hCG) values obtained throughout the course of gestation (r=-0.31, P<0.000).

Table 5 - Person's correlation between various maternal thyroid function tests during the entire course of gestation and fetal thyroid function tests in women studied.

Parameter	Fetal total thyroxine (TT_4) (nmol/L)	Fetal total tri-iodothyronine (TT_3) (nmol/L)	Fetal free thyroxine (FT_4) (pmol/L)	Fetal thyrotropin (TSH) (mIU/ml)	Fetal thyroxine-binding globulin (TBG) (mg/L)	Fetal thyroglobulin (Tg) (ug/L)
Maternal TT_4 (nmol/L)	-0.454†	-	-	-	-	-
Maternal TT_3 (nmol/L)	0.267*	-	-	-	-	-
Maternal FT_4 (pmol/L)	-0.650†	-0.174*	-	-	-	-
Maternal FT_3 (nmol/L)	-0.035	-0.087	-0.075	-0.081	-	-
Maternal r-T_3 (nmol/L)	-0.092	-0.025	-0.066	-0.022	-	-
Maternal TSH (mIU/ml)	-0.701†	-0.051	0.007	-	0.117	0.179*
Maternal TBG (mg/L)	0.770†	0.153*	0.210	0.251*	-	-
Maternal Tg (ug/L)	0.651†	-0.006	-0.024	-0.016	0.000	-
Maternal hCG (mIU/ml)	0.029	0.008	-0.106	-0.136	-0.192	-0.245*
Maternal urinary iodine (umol/L)	-0.750†	0.010	0.141*	0.080	-0.167*	0.058

* significance at 0.05 level (2-tailed)
† - significance at the 0.01 level (2-tailed)
r-T_3 - reverse T_3, hCG - human chorionic gonadotropin

Thyroid function tests in neonates. The results for the neonates based on cord blood samples are presented in **Table 4.** The values obtained for serum TT_4, TT_3, FT_4, TSH, TBG and Tg were within the range of normality for neonatal thyroid function. There was a negative correlation between maternal FT_4 and the neonatal TSH (r=-0.70; P<0.001). Positive correlations were found between neonatal TT_4 and birth weight (r=0.61; P<0.001). Also, there were positive correlations between neonatal TT_4, and maternal UI excretion (r=0.75; P<0.001), FT_4 (r= 0.65; P < 0.001), TBG (r=0.77; P<0.001 and Tg (r= 0.65; P < 0.001) **(see Table 5).**

Discussion. In the present study, the changes in UI excretion and in thyroid hormones and related parameters during the course of normal pregnancy, at term and 6-10 weeks postpartum have been demonstrated. The results showed that pregnancy with marginal iodine deficiency was associated with significant alterations in thyroid function. Pregnant women exhibited an increased UI excretion occurring as early as the first trimester and then showed a decline reaching the lowest at term with 28.8% of studied women exhibiting values of UI excretion < 0.79 umol/L. These results are consistent with some reports,[24-26] but contrasted with other reports showing either increased[27-29] or unchanged[30-32] UI excretion as compared with non-pregnant values. These differences could be explained by the existence of UI-threshold, which is termed "iodostat" and was suggested that the level at which the iodostat is set depends upon customary dietary iodine.[33] In pregnancy, the iodostat may not change to conserve iodine despite the enhanced UI excretion. The latter may result in depletion of thyroidal iodine stores, which does not seem to have deleterious effects for the mother or the neonate as long as euthyroidism is maintained throughout gestation.[29] However, if iodine intake during gestation was decreased or the pre-gestational iodine stores were inadequate, the repercussions could be more deletarious.[2] In the present study, we observed an increase in the serum levels of TT_4 and TT_3 with some significant changes in that of r-T_3 during the course of pregnancy. These changes are related to the greatly increased levels of TBG, such findings are consistent with previous reports.[3,6,11,15,16,33,34] Studies on the changes of FT_4 and

FT_3 levels during pregnancy are conflicting, with some showing an increase,[36] a decrease[34] and, in others, no change.[29] These discordant results can be partly related to the methodologies employed, and the studied populations.[37] However, in the present cohort the mean serum levels of FT_4 decreased during the course of pregnancy (by 26.1%, P<0.001 by the 3rd trimester), which is consistent with previous studies.[6,15,16,18,38] Therefore, at this stage, we confirm a higher TT_4 output by the thyroid in response to markedly increased serum TBG levels. The most common cause of acquired TBG excess is related to either exogenous estrogen (such as use of oral contraceptives or postmenopausal replacement therapy) or the endogenous increase of estrogen observed during pregnancy.[39] The mechanism for the estrogen-mediated increase in TBG, as observed in the present study, was originally thought to be the result of enhanced hepatic TBG synthesis, as well as, to diminished peripheral TBG degradation rate.[37] However, recent studies have shown that the extent of sialylation of TBG directly influences its rate of clearance from circulation. Thus, estrogen seems to stimulate TBG sialylation leading to prolongation of its half-life in circulation, and consequently, elevated serum levels. In addition, there is at present no data suggesting an increase in thyroid hormone production during pregnancy in order to satisfy the maternal and fetal demands. Although renal iodine clearance is enhanced during pregnancy, absolute thyroidal iodine uptake remains unchanged.[27] Moreover, relative iodine deficiency cannot fully explain the decrease in FT_4; since, the latter is observed in both iodine-deficient and iodine-repleted areas.[5] The decrease in FT_4 is also associated with similar changes in FT_3, which also argues against an iodine-related phenomenon, as in iodine deficiency, TT_3 values are normal or even increased. Lastly, the net TT_4 turnover and presumably also thyroid hormone requirements are unaltered in human pregnancy being 90 and 97 ug per day in non-pregnant and pregnant women.[40] In a prospective study by Berghout et al,[35] as is the finding of the present study, a gradual decline in FT_4 and FT_3 levels during pregnancy was observed, which was associated with elevated levels of $r\text{-}T_3$. The concentration ratios of FT_3/FT_4 were increased. Similar changes were observed in patients with non-thyroidal illness,[41] but not in normal pregnant women living in the southwest of France.[31] It is also possible that the changes in thyroid hormones can be related to energy balance during pregnancy. It can be calculated that the extra energy needs of pregnancy can be 1020 kJ/day, which comprises the energy required for the synthesis of new tissues together with the related increments in basal metabolism. However, in pregnancy, the increase in energy intake was found to be very small, approximating 80 kJ/day, giving rise to an estimated energy gap of 940 kJ/day. This is only partially compensated for by a decrease in physical activity, thus, saving 355 kJ/day. A shortfall of 585 kJ/day has still to be met, and it is in this respect that down-regulation of thyroid hormone action as indicated by the decreases in the levels of FT_4 and FT_3 (as in non-thyroidal illness) may contribute to the saving of energy.[37] Studies on the changes of TSH concentrations during the course of pregnancy are conflicting with studies reporting unchanged or slightly increased TSH levels[18,42] These contradictory results are partly explained by the methodologies employed, and probably by the iodine status of the populations studied.[2,37] In the present study, the decrease in FT_4 was accompanied by an increase in serum TSH levels. However, serum TSH levels exhibited a decrease (by 35%, P<0.001) only during the first trimester, but returned to non-pregnant values during the 2nd and 3rd trimesters and at term. These changes are consistent with previous studies[35,43,] but contrast with the work of Rasmussen et al,[44] who found no changes in TSH levels during pregnancy as compared with 12 months postpartum values. Moreover, serum TSH levels, as measured by sensitive assays in the present study is reciprocally diminished at the time of the hCG peak. These changes reflect thyroidal stimulation by hCG, which peaks in the first trimester and decreases during the 2nd and 3rd trimesters, such changes are consistent with previous studies,[3,6,11,36,44] and consistent with the observed negative correlation between TSH and hCG levels in the first trimester which was more pronounced in women in the earliest weeks of gestation (such as 8-10 weeks gestation). This is not surprising, since both TSH and hCG, are heterodimeric glycoproteins composed of a common α-subunit, and they share considerable similarity in their β-subunits with similar receptors.[2] Furthermore, there exists thyroid-stimulating activity (TSA) in sera of normal pregnant women, and the TSA shows a significant correlation with hCG levels.[45] This effect was also seen in vitro: Human chorionic gonadotropin extracts from normal pregnant sera by mean of anti-hCG monoclonal antibodies, induced c-AMP accumulation in Fisher Rat Thyroid cell Line-5 (FRTL-5) cells, and the effect correlated with hCG immunoreactivity.[46] Thus, maternal thyroid glands may secrete thyroid hormones during early pregnancy in response to the thyrotropic activity of hCG that overrides the normal operation of the hypothalamic-pituitary-thyroid feedback system.[6] Our results confirm that, at delivery, subclinical iodine insufficiency exists amongst 28.8% pregnant women living in the Jeddah area. This subclinical state of iodine deficiency affects maternal and fetal thyroid function. This is evidenced by a negative correlation between maternal TSH and UI excretion ($r = -0.228$, P<0.05); and greater increases in TSH and Tg levels in neonates whose mothers had decreased UI excretion compared to those neonates

whose mothers had a normal UI excretion. These observations are consistent with that reviewed recently by Glinoer et al.[34] In concurrence with such studies, none of neonates exhibited hypothyroxinemia despite maternal hypothyroxinemia, suggesting that the fetus was somehow protected at the expense of the mother as a result of avid transfer of iodine across the placenta.[37] In the present study, neonatal birth weights correlated positively with cord blood FT_4, but negatively with maternal UI excretion values. This negative correlation is interesting as it has been well documented that severe iodine deficiency leads to low birth weight; however, the implications of such correlations need further study. Several studies showed that maternal and fetal function during pregnancy deteriorated with an increasing degree of iodine deficiency. Severe iodine deficiency throughout pregnancy can result in neonatal goiter and hypothyroidism with the long-term consequence of mental retardation and neurological sequalea.[5,42,47] Also, in areas of moderate iodine deficiency the consequences on maternal and fetal thyroid function have been described,[48,49] demonstrating a high frequency of maternal and fetal goiter and an increase in neonatal hyperthyrotropinemia.[50]

In conclusion, this prospective study shows that pregnancy with marginal iodine deficiency was associated with significant changes in thyroid function. The main results as compared with non-pregnant and postpartum values or both were: 1. Marginal iodine deficiency was evident in 28.8% pregnant women at term and 11.5% in women 6-10 weeks postpartum according to WHO/UNICEF/ ICCIDD criteria for iodine deficiency; 2. Relative hypothyroxinemia, indicated by a less than adequate rise in TT_4 concentrations (inappropriate for the increases in TBG levels) and the gradual lowering of FT_4 levels during the course of pregnancy; 3. Some preferential TT_3 secretion, as indicated by the slightly increased molar concentration ratio of TT_3/TT_4; 4. The gradual rise, within the limits of normality, of serum TSH levels beyond the first trimester; and 5. A significant increase in serum Tg levels especially at term. The results of the present study point to the need for an increased iodine supply in Saudi pregnant women living in the Jeddah area to decrease the potential consequences of low iodine intake on maternal thyroid economy.

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References

1. Lazarus JH, Kokandi A. Thyroid disease in relation to pregnancy: A decade of change. *Clin Endocrinol* 2000; 53: 265-278.
2. Glinoer D. The regulation of thyroid function in pregnancy: Pathways of endocrine adaptation from physiology to pathology. *Endocr Rev* 1997; 18: 404-433.
3. Glinoer D, De Nayer P, Bourdoux P, Lemone M, Robyn C, Van Steirteghem A et al. Regulation of maternal function during pregnancy. *Clin Endocrinol Metab* 1990; 72: 276-287.
4. Silva JE, Silva S. Interrelationships among serum thyroxine, tri-iodothyronine, reverse tri-iodothyronine, and thyroid-stimulating hormone in iodide-deficient pregnant women and their offspring. Effects of iodide supplementation. *Clin Endocrinol Metab* 1981; 52: 671-677.
5. Thilly Ch, Delange F, Lagasse R, Bourdoux P, Ramioul L, Berquist H et al. Fetal hypothyrodism and maternal thyroid status in severe endemic goiter. *Clin Endocrinol Metab* 1978; 47: 354-360.
6. Ballabio M, Poshyachinda M, Ekin RP. Pregnancy-induced changes in thyroid function: Human chorionic gonadotropin as putative regulator of maternal thyroid. *Clin Endocrinol Metab* 1991; 73: 824-831.
7. Man EB, Jones WS, Holden RH, Mellits ED. Thyroid function in human pregnancy. VIII. Retardation of progen aged 7 years; relationship to maternal age and maternal thyroid function. *Am J Obstet Gynecol* 1771; 111: 905-916.
8. Woods RJ, Sinha AK, Ekins RP. Uptake and metabolism of thyroxine by the rat foetus in early pregnancy. *Clin Sci* 1984; 67: 356-363.
9. De Escobar GM, Obregon MJ, Escobar del Rey F. Fetal and mternal thyroid hormones. *Horm Res* 1987; 26: 12-27.
10. Osathanondth R, Tulchinsky D, Chopra IJ. Total and free throxine and tri-iodothyronine in normal and complicated pregnancy. *Clin Endocrinol Metab* 1976; 42: 98-104.
11. Yamamoto T, Amino N, Tanizawa O, Ichihara K, Azukizawa M, Moyoi K. Longitudinal study of serum thyroid hormones, chorionic gonadotropin and thyrotropin during and after normal pregnancy. *Clin Endocrinol (Oxf)* 1979; 10: 459-468.
12. Harada A, Hershman JM, Reed AW. Comparison of thyroid stimulators and thyroid hormone concentrations in the sera of pregnant women. *Clin Endocrinol Metab* 1979; 48: 793-797.
13. Braverman LE, Abreau CM, Brook P. Measurement of serum free thyroxine by RIA in various clinical states. *J Nucl Med* 1980; 21: 233-239.
14. Skjoldebrand L, Brundin J, Carlstom A, Petterssson T. Thyroid associated components in serum during normal pregnancy. *Acta Endocrinol (Copenh)* 1982; 100: 504-511.
15. Pacchiarotti A, Martino E, Barlalena L. Serum thyrotropin by ultrasensitive immunoradiometric assay and serum free thyroid hormones in pregnancy. *Clin Chem* 1986; 35: 275-278.
16. Price A, Griffiths H, Morris BW. A longitudinal study of thyroid function in pregnancy. *Clin Chem* 1989; 35: 275-278.
17. Kannan V, Sinha MD, Devi PK, Rastogi GK. Plasma thyrotropin and its response to thyrotropin releasing hormone in normal pregnancy. *Obstet Gynecol* 1973; 42: 547-549.
18. Weeke J, Dybkjaer L, Granlie K. A longitudinal study of serum TSH, and total and free iodothyronines during normal pregnancy. *Acta Endocrinol (Copenh)* 1982; 101: 531-537.
19. Kurtz A, Dwyer K. Elkins R. Serum free throxine in pregnancy. *Br Med J* 1979; 2: 550-551.

20. Al-Frayh A, Abdul Jabar F, Haque K. Survey of auxological variance and growth standards in Saudi newborns at various gestational ages and the pre-school gestational ages and the pre-school children in the Kingdom of Saudi Arabia. King Abdulaziz City for Science and Technology, Publication #33. Riyadh (KSA): King Saud University Press, 1987. p. 38-46.
21. Wilson B, Vanzyl A. The estimation of iodine in thyroidalamino acids by alkaline ashing. *S Afr J Med Sc*i 1967; 32: 70-82.
22. Jaffe M. Uberden Niederschlagden Pikrinsaure in normalen Harn erzeugt und uber die Reaktion des Kreatinins. *Z Physiol Chem* 1986; 10: 391-400.
23. World Health Organization (WHO). UNICEF, ICCIDD Report. Indicators for assessing iodine deficiency disorders and their control through salt iodization. WHO/NUT; Document No. WHO/NUT/94.6: Geneva; 1994.
24. Bauch KMW, Ulrich FE, Grosse E, Kempe R, Scinemann F, Sterzel G et al. Thyroid status during pregnancy and postpartum in regions of iodine deficiency and endemic goitre. *Endocr Exp* 1986; 20: 67-77.
25. Pedersen KM, Laurberg P, Iversen E, Knudsen PR, Gregeren HE, Rasmussen OS et al. Amelioration of some pregnancy-associated varations in thyroid function by iodine supplementation. *Clin Endocrinol Metab* 1993; 77: 1078-1083.
26. Glinoer D, De Nayer P, Delange' F, Lemone M, Topper V, Spehl M et al. A randomized trial for the treatment of mild iodine deficiency during pregnancy: Maternal and neonatal effects. *Clin Endocrinol Metab* 1995; 80: 258-269.
27. Aboul-Khair SA, Crooks J. Turnbull AC, Hytten FE. The physiological changes in thyroid function during pregnancy. *Clin Sci* 1964; 27: 195-207.
28. Smyth PPA, Smith DF, Radeliff M, O'Herlihy C. Maternal iodine status and thyroid volume during pregnancy: Correlation with neonatal intake. *Clin Endocrinol Metab* 1997; 82: 840-843.
29. Smyth PPA. Variation in iodine handling during normal pregnancy. *Thyroid* 1999; 9: 637-642.
30. Liesenkotter KP, Gopel W, Bogner U, Stach B, Gruters A. Earliest prevention of endemic goiter by iodine supplementationn during pregnancy. *Eur J Endocrinol* 1996; 134: 443-448.
31. Caron PHM, Bazzi S, Dufor A, Faure G, Ghandour I, Lauzu P et al. Urinary iodine excretion during normal pregnancy in healthy women living in the Southwest of France: Correlation with maternal thyroid parameters. *Thyroid* 1997; 7: 749-754.
32. Elnagar BEA, Wide L, Gebre-Medhin M, Karlsson FA. Iodine status, thyroid function and pregnancy: Study of Swedish and Sudanese women. *Eur J Clin Nutr* 1998; 52: 351-355.
33. Dworkin HJ, Jacquez JA, Beierwalzes WH. Relationship of iodine ingestion to iodine excretion in pregnancy. *Clin Endocrinol (Oxf)* 1966; 26: 1329-1336.
34. Franklyn JA, Sheppard MC, Ramsden DB. Serum free thyroxine and free tri-iodothyronine concentrations in pregnancy. *Brit Med J* 1983, 287: 394-396.
35. Berghout A, Ender E, Ross A, Hogerzeil H, Smits NJ, Weirsinga WM. Thyroid function and thyroid size in normal pregnant women living in an iodine replete area. *Clin Endocrinol (Oxf)* 1994; 41: 375-379.
36. Guillaume J, Schussler GC, Goldman J. Components of the total serum thyroid hormone concentrations during pregnancy: High free thyroxine and blunted thyrotropin (TSH) response to TSH-releasing hormone in the first trimester. *Clin Endocrinol Metab* 1985; 60: 678-684.
37. Glinor D, De Nayer P. Thyroid and its disease in pregnancy. In: Monaco F, Satta MA, Shapiro B, Troncone L (editors). Thyroid Diseases. Clinical Fundamental Therapy. New York (NY): CRC Press; 1993. p. 517-527.
38. Panesar NS, Li CY, Rogers MS. Reference intervals for thyroid hormones in pregnant Chinese women. *Ann Clin Biochem* 2001; 38: 329-332.
39. Refetoff S. Inherited thyroxine-binding glolubin abnormalities in man. *Endocr Rev* 1989; 10: 847-853.
40. Dowing JT, Appleton WGJT. Thyroxine turnover during human pregnancy. *Clin Endocrinol Metab* 1967; 27: 1749-1750.
41. Docter R, Krenning EP, de Jong M, Hennemann G. The sick euthyroid syndrome: changes in thyroid hormone serum parameters and hormone metabolism. *Clin Endocrinol (Oxf)* 1993; 39: 499-518.
42. Burrow GN. Thyroid status in normal pregnancy. An editorial. *Clin Endocrinol Metab* 1990; 71: 274-275.
43. Chan BY, Swaminathna R. Serum thyrotropin concentration measured by sensitive assays in normal pregnancy. *Br J Obstet Gynecol* 1988; 95: 1332-1336.
44. Rasmussen NG, Hornnes PJ, Hegedus L Ultrasonically determined thyroid size in pregnancy and postpartum: The goitrogenic effect of pregnancy. *Am J Obstet Gynecol* 1989; 160: 1216-1220.
45. Yoshikawa N, Nishikawa M, Horimot M. Thyroid-stimulating activity in sera of normal pregnant women. *Clin Endocrinol Metab* 1989; 69: 891-895.
46. Yoshimura M, Hersman JM. Thyrotropic action of human chorionic gonadrotropin. *Thyroid* 1995; 5: 542-434.
47. Bleichrodt N, Escobar del Rey F, Morreale de Escobar G, Garcia I, Rubio C. Iodine deficiency implications for mental and psychomotor development in children. In: De Long GR, Robbins J, Condliffe PG, editors. Iodine and the brain. New York (USA): Plenum Press; 1989. p. 269-287.
48. Phaoah POD, Connolly KJ, Ekins RP, Harding AG. Maternal thyroid hormone levels in pregnancy and the subsequent cognitive and motor performance of the children. *Clin Endocrinol (Oxf)* 1984; 21: 265-270.
49. Liu JL, Tan Yb, Zhuang ZJ, Shi ZF, Chen BZ, Zhang JX. Influence of iodine deficiency of human fetal thyroid gland and brain. In: DeLong GR, Robbins J, Condliffe PG, editors. Iodine and the brain. New York (USA): Plenum Press; 1989. p. 249-57.
50. Glinoer D, Delange F, Labourer I. Maternal and neonatal thyroid function at birth in an area of marginally low iodine intake. *Clin Endocrinol Metab* 1992; 275: 800-805.