



Maternal thyroid hormones and human chorionic gonadotrophin concentrations of Sudanese pregnant woman

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Abstract

A prospective study was conducted during the period 2001 to 2003 at Omdurman maternity, Khartoum teaching and Khartoum North teaching hospitals. One hundred and twenty Sudanese pregnant women at different stages of pregnancy were chosen for this study. Forty non-pregnant women of the same age without endocrine disease were chosen as control. Thyroid hormones (free T₄ and T₃) were measured. In addition hCG was also measured in both groups. The Study group was divided according to the gestational age (weeks) into three subgroups: - first trimester 5 - 12 weeks, second trimester 13 - 27 weeks and third trimester 28 - 40 weeks. Determination of serum hormones concentration was carried out using a highly sensitive specific RIA technique.

The results of this study showed increased levels of thyroid total hormones (T₄ and T₃) during three trimesters of pregnancy TSH remained within normal range. The results of this study also showed that there was an increase in concentration of free thyroid hormones (T₄ and T₃). The hCG increased during first trimester, then decreased sharply in the second and third trimesters of pregnancy

Introduction

Thyroid status is frequently assessed during pregnancy both to evaluate suspected thyroid abnormalities and monitor the status of pre-existing thyroid diseases⁽¹⁾. The Production, circulation and disposal of thyroid hormones are all altered during pregnancy. Changes in serum thyroxine binding globulin (TBG) concentration occur in the first trimester, but accelerated thyroid hormone metabolism by the placenta is seen later in pregnancy T₄ is transported bounded principally to thyroxine binding globulin (TBG) and secondarily to thyroxine binding prealbumin (TBP A) and

albumin⁽²⁾.

Because of the strong affinity of TBP for T₄ over 99.9% of plasma T₄ is in this form of TBP-T₄ and means plasma (total T₄) effectively in the plasma ,TBP-T₄⁽³⁾. In normal pregnancy, plasma total T₄ increased by 30 - 50% than in non-pregnant women⁽²⁾. Total T₄ may be increased due to estrogen therapy including women taking estrogen containing oral contraceptives⁽⁴⁾. Triiodothyrimine T₃ is a hormone synthesized and stored in thyroid gland; more than 99% of T₃ in the blood is bound reversibly to plasma proteins. The concentration of T₃ is much lower than

that of T₄, but its metabolic potency is much greater ⁽⁵⁾. T₃ levels increased during pregnancy, oral contraceptive or estrogen treatment paralleled thyroxine binding globulin (TBG) increases.

TSH is responsible for the control of synthesis and secretion of thyroid hormones T₃ and T₄. TSH is regulated by thyrotrophin releasing hormone (TRH). Pregnancy is associated with variation in TSH concentrations, but remains within the normal range. Before the development of highly sensitive TSH assay, investigators noted blunted TSH response to TRH in the first trimester of pregnancy, in some women, suggesting thyroid hormone excess and consequent pituitary suppression ⁽⁶⁾.

Free thyroid hormones (FT4 and FT3) levels

The serum concentration of free thyroxine and triiodothyronine (FT4 and FT3), which are presumably the biologically active form of thyroid hormone ⁽⁷⁾. Free thyroxine (FT4) and free triiodothyronine (FT3) concentrations in pregnancy remain within the normal range. Thus measurement of free thyroxine concentration correlates better with clinical status than total thyroxine level ⁽⁷⁾. Free thyroxine concentration typically to T₄ uncovers. Patients' actual clinical status (hypo and hyperthyroid conditions), in particular, the free T₄ is not affected by alteration in plasma (TBG); such as occur in pregnancy and women taking estrogen containing oral contraceptives. About 20% of women in the third trimester of pregnancy tend to

have low plasma (free T₄), but without any pathological significance necessarily attaching to these findings, reference values appropriate to the stage of pregnancy should be used ⁽³⁾. There was a close correlation between the reduction in mean-serum TSH concentration and the maximal elevation of hCG in the first trimester. The mean serum free T₄ concentration was also significantly higher in the first trimester compared with the second or third trimester ⁽⁸⁾. The heterodimeric glycoprotein, TSH and human chorionic gonadotrophin hCG are composed of a common alpha subunits and shared considerable similarity in their unique beta subunits, furthermore TSH and hCG /LH receptors are also quite similar. Excess hCG in the first trimester is thought to result in a hormone syndrome in which high concentration of hCG stimulate TSH receptor ⁽⁹⁾.

Human chorionic gonadotrophin β-hCG levels

Human chorionic gonadotrophin (hCG) belongs to the glycoprotein hormone with a molecular weight of about 3KD family together with luteinizing hormone (LH), follicular stimulating hormone FSH and thyroid stimulating hormone TSH ⁽¹⁰⁾. hCG is synthesized by trophoblastic cells of the placenta during pregnancy and stimulates the growth of corpus luteum . Thyrotrophin secreted by the maternal pituitary gland, the placenta produces large amounts of human chorionic gonadotrophin hormone, which has some thyrotrophin like

bioactivity⁽¹¹⁾. The production of human chorionic gonadotrophin occurs during the first week after fertilization and causes a transient increase in serum free T₄ concentrations, which in turn decreases serum thyroid stimulating hormone (TSH) concentrations during the first trimester⁽¹²⁾.

Materials and methods

Pregnant women:

One hundred and twenty Sudanese pregnant women were randomly selected from Omdurman Maternity, Khartoum Teaching and Khartoum North Teaching Hospitals. Their ages ranged between 19-45 years, and they were classified according to gestational age per week as follows:

1/ forty subjects in first trimester, gestational age (5-12 weeks)

2/ forty subjects in second trimester, gestational age (13-27 weeks)

3/ forty subjects in third trimester, gestational age (28-39 weeks)

Gestational age was estimated from the date of last menstrual period (L.M.P). Each subject was asked about occurrence of thyroid diseases and examined for the presence of thyroid enlargement and other signs of thyroid abnormalities. Cases of thyroid abnormality were excluded before sample collection.

Non-pregnant women (control)

Forty non-pregnant healthy women from Omdurman Islamic University, with approximately similar ages (18 - 45 years) were selected for this

investigation.

Blood samples:

Five ml of venous blood were collected in a dry tube, allowed to clot and immediately centrifuged at 2000 r.p.m for 5 minutes and separated sera were stored at -20°C until analyzed.

Specific reagents:

All radioimmunoassay specific reagents for the measurement of thyroid hormones were obtained from China Institute for Atomic Energy (CIAE), Department of Isotopes (*Beijing China*). The reagents include tracer, standard and antibodies and separating agents for the different hormones.

Measurement of hormones by Radioimmunoassay (RIA) techniques

Principle of RIA: Antigen (Ag) and tracer (Ag*) compete with a limited amount of antibody (Ab) to form Ag-Ab or Ab-Ag* complexes. This reaction counts radioactivity using gamma counter.

Total serum thyroxin (T₄)

All assay tubes were set in duplicates using the following protocol: Add and mix properly after each addition. The assay was carried out using tubes containing the sample, standard, quality control (QC), nonspecific binding (with no antibody) and total count which contained only tracer, were counted in a gamma counter for 60 seconds.

Assay procedure:

1/ Tubes were labeled and arranged in assay rack.

2/ 50µl sample and standard were pipetted into

labeled tubes.

3/ 500µl tracer T4 were dispensed into labeled tubes.

4/ 500µl antibody suspension and NSB reagent were dispensed into labeled tubes

5/ Tubes were vortexed, mixed thoroughly and incubated at 37°C for 45 minutes

6/ Test tubes rack was placed on a magnetic separator and allowed for 10 minutes, then the supernatant was decanted and allowed to drain for minutes.

7/ All tubes were counted in a gamma counter.

Assay procedure for measurement of total and free T3, free T4 was the same as that of total T4.

TSH and hCG were measured using highly sensitive immunoradiometric assay (IRMA)

Specific reagents:

1/ labeled antibody (tracer)

2/ standards

3/ separating agents

4/ IRMA washing buffer

Results

The results of this study covers one hundred and twenty Sudanese normal pregnant women and forty non-pregnant healthy - at Omdurman Islamic university - as control. Their ages ranged between (18 - 45) years at Khartoum and Khartoum north teaching hospital and Omdurman maternity hospital whose serum hormonal levels were

analyzed during all trimesters of pregnancy: Total (T₄, T₃, and TSH), for normal pregnant and non-pregnant women were assayed.

Total serum thyroid hormones (T₄ and T₃) concentrations:

Mean serum concentrations of total thyroid hormones (T₄ and T₃) in pregnant and control (non-pregnant women are presented in table (1) and Figs (1 and 2). Serum T₄ and T₃ concentrations in pregnant women increased rapidly and significantly higher (P< 0.05) in all trimesters compared to the mean concentration in non-pregnant women. The T₄ concentration continued to increase thereafter, although at slower rate (Fig. 1). The average concentration of T₄ during the second trimester (202 ± 11.7 ng/ml) was significantly higher than that in the first trimester (188.6 ± 9.6 ng/ml) and third trimester (196.4 ± 10.2 ng/ml) (± SEM), but statistically not significantly different. An apparent correlation was observed between increase in gestational age and the increase in T₃ concentration in pregnant women (Fig. 2), the average serum T₃ concentration (3.1 ± 0.13 ng/ml) during the third trimester was significantly higher (P< 0.05) than the mean concentrations (2.6 ± 0.21 ng/ml) and (2.3 ± 0.11 ng/ml) in the first and second trimesters respectively

Serum thyroid stimulating hormone (TSH) concentration

Table (2) and Fig (3) presented the mean concentrations of TSH in pregnant women

throughout all trimesters ($1.4 \pm 0.18 \mu\text{U/L}$), ($1.3 \pm 0.12 \mu\text{U/L}$) and ($1.5 \pm 0.09 \mu\text{U/L}$) respectively, which were similar to the mean concentration of TSH in non-pregnant women ($1.7 \pm 0.17 \mu\text{U/L}$) ($P < 0.05$). TSH concentration remained within the normal range throughout all gestational age. However, 12.5% of pregnant women in first trimester were observed to have suppressed serum TSH concentration ($0.1 - 0.3 \mu\text{U/L}$) below the normal concentration ($0.4 - 4.0 \mu\text{U/L}$).

Free thyroid (FT_4 and FT_3), β -hCG for normal pregnant and non-pregnant women were assayed. The mean \pm standard error mean (SEM) of thyroid hormones free (FT_4 and FT_3), hCG were calculated from all different trimester and non-pregnant women.

Serum free thyroid hormones (FT_4 and FT_3) concentrations

The mean concentration of FT_4 in pregnant women ($10.0 \pm 1.22 \text{ pmol/L}$) in the first trimester was significantly higher ($P < 0.05$) than the mean concentration of FT_4 ($7.9 \pm 0.63 \text{ pmol/L}$) in non-pregnant women. However the concentration of the same hormone ($5.9 \pm 0.34 \text{ pmol/L}$) in the second and ($4.2 \pm 0.19 \text{ pmol/L}$) in the third trimesters were significantly lower ($P < 0.05$) than mean

concentration ($7.9 \pm 0.63 \text{ pmol/L}$) in non-pregnant women (table 1 and Fig 1). The increase of FT_3 concentration ($3.7 \pm 0.23 \text{ pmol/L}$) in pregnant women in the first trimester was not significantly higher than the mean concentration of FT_3 ($3.5 \pm 0.13 \text{ pmol/L}$) in control (non-pregnant women). However, the concentrations of the same hormone ($2.8 \pm 0.18 \text{ pmol/L}$) and ($1.9 \pm 0.11 \text{ pmol/L}$) in the second and third trimesters respectively were significantly lower ($P < 0.05$) than control concentrations, as in table (1) and Fig (2). Apparent inverse relationship was observed between FT_4 and FT_3 concentrations throughout all trimesters.

Serum human chorionic gonadotrophin (β -hCG) concentration

Mean concentrations of serum hCG in pregnant women showed in table (2). Average hCG concentration for the three trimesters were first trimester ($61859.4 \pm 14265.9 \text{ IU/L}$), second trimester ($28902 \pm 3678 \text{ IU/L}$) and third trimester ($26246 \pm 3439.5 \text{ IU/L}$), the SEM was relatively large. Highly significant increase ($P < 0.01$) of mean hCG concentrations that limited in the first trimester and decreased significantly and sharply in the second and third trimesters.

Table (1): Serum total thyroid hormones concentrations:

No. of Samples	Trimesters	Weeks of gestation	Concentrations of T ₄ (ng/ml)	Concentrations of T ₃ (ng/ml)
40	First trimester	5 -12	188.6 ± 9.6	2.6 ± 0.21
40	Second trimester	13 - 27	202 ± 11.7	2.3 ± 0.11
40	Third trimester	28 - 39	196.4 ± 10.2	3.1 ± 0.13
40	Control (non-pregnant)	-	116±2.8	1.8 ± 0.075

Fig (1): Serum free T₄ mean concentration during trimesters of pregnancy compared with control (non-pregnant) women.

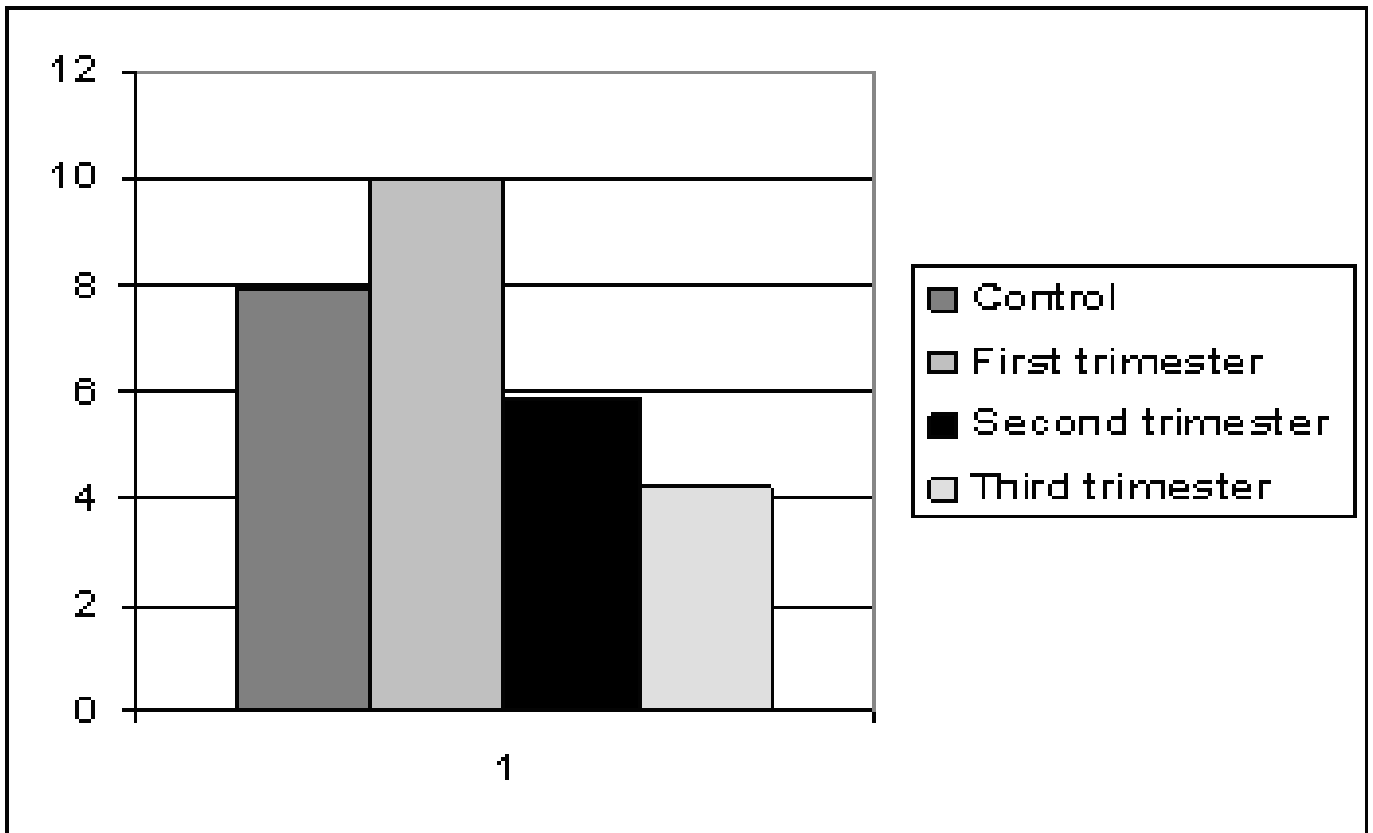


Table (2): Serum free thyroid hormones (FT₄ and FT₃) concentrations

No. of Samples	Trimesters	Weeks of gestation	Concentrations of free T ₄ (pmol/L)	Concentrations of free T ₃ (pmol/L)
40	First trimester	5 -12	10 ± 1.22	3.7 ± 0.23
40	Second trimester	13 - 27	5.9 ± 0.34	2.8 ± 0.18
40	Third trimester	28 - 39	4.2 ± 0.19	1.9 ± 0.11
40	Control (non-pregnant)	-	7.9 ± 0.63	3.5 ± 0.13

Values are mean ± SEM

Fig (2): Serum total T₃ mean concentration during trimesters of pregnancy compared with control (non-pregnant) women.

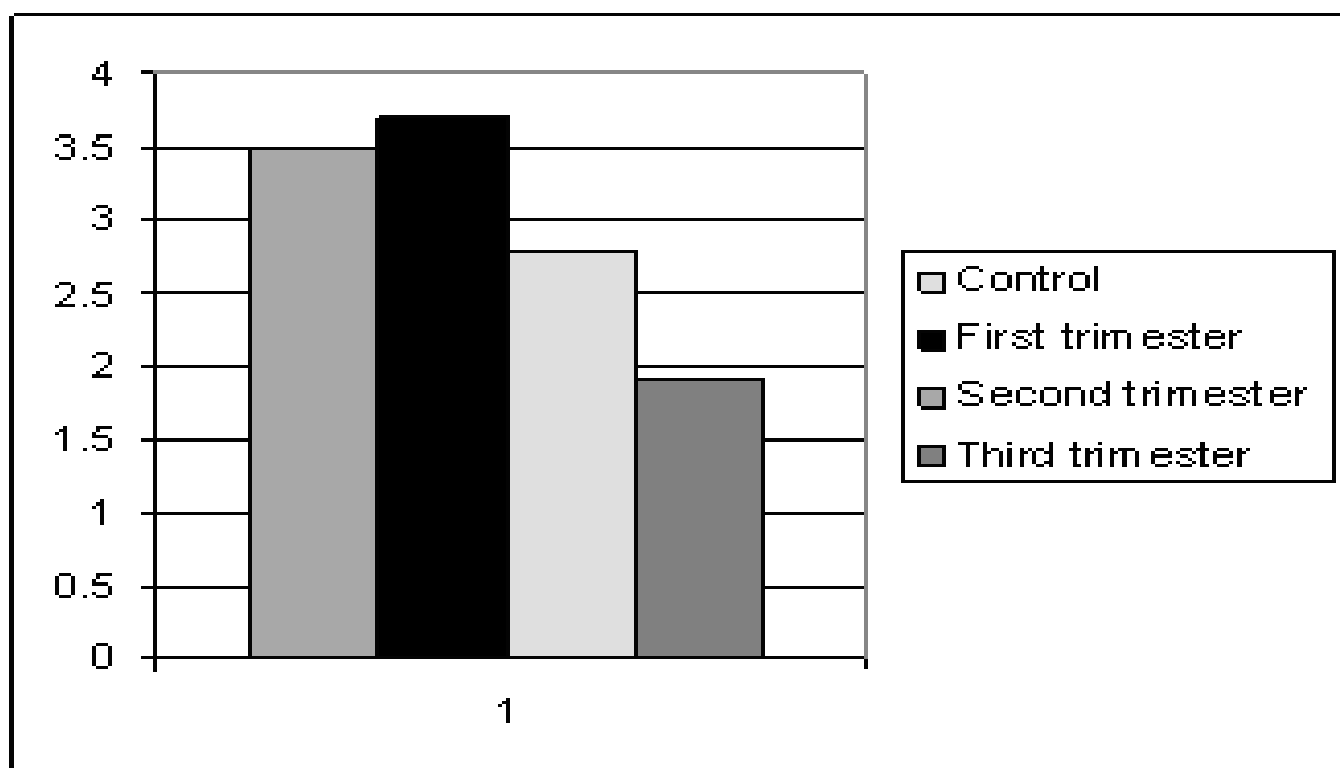


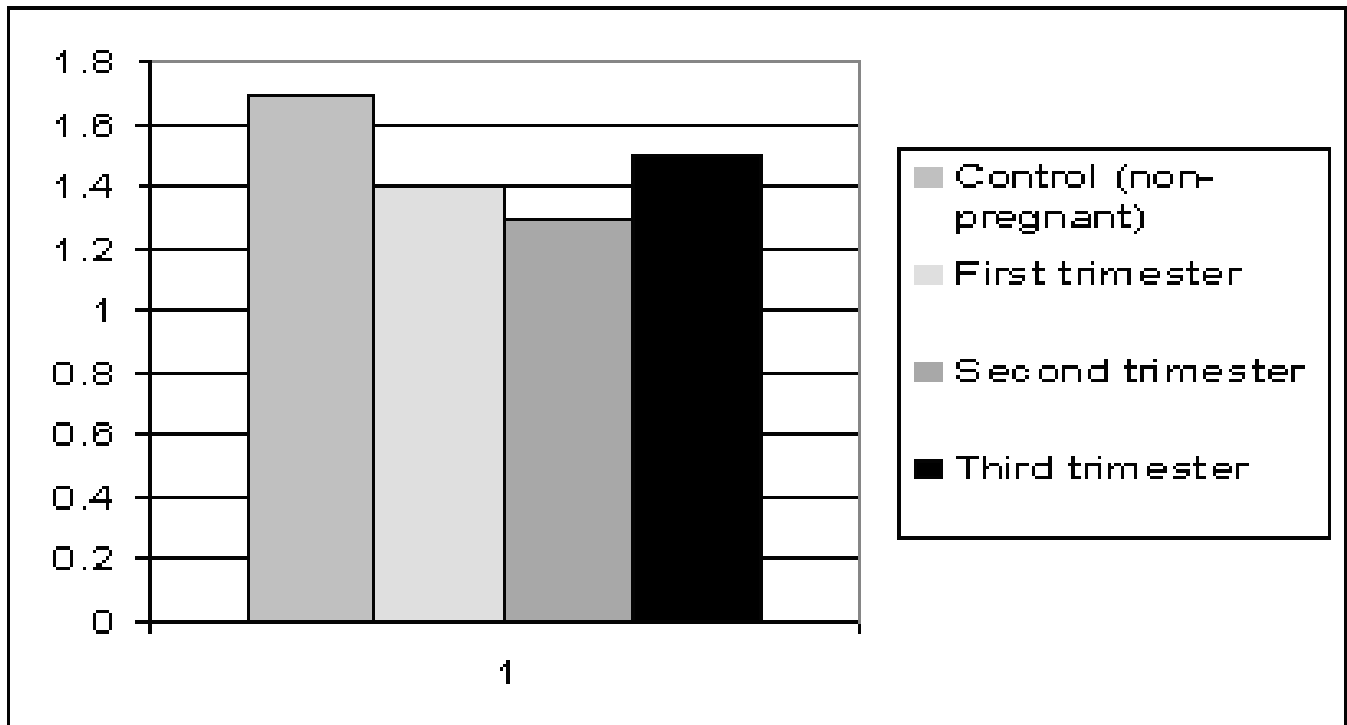
Table (3): Serum human gonadotrophin (β -hCG) concentrations

No. of Samples	Trimesters	Weeks of gestation	Concentrations of β -hCG (IU/L)
40	First trimester	5 -12	61859.4 \pm 14265.9
40	Second trimester	13 - 27	28402 \pm 3678
40	Third trimester	28 - 39	26246 \pm 3439.5

Table (4): Serum thyroid hormone concentration (TSH)

No. of Samples	Trimesters	Weeks of gestation	Concentrations Of TSH (Mu/L)
40	First trimester	5 -12	1.4 \pm 0.18
40	Second trimester	13 - 27	1.3 \pm 0.12
40	Third trimester	28 - 39	1.5 \pm 0.09
40	Control (non-pregnant)	-	1.7 \pm 0.17

Fig (3): Serum TSH mean concentration during trimesters of pregnancy compared with control (non-pregnant) women.



Discussion:

The present study showed that the increase in thyroid total hormones (T₄ and T₃) concentrations that occur during pregnancy did not proceed at steady rate. Serum T₄ and T₃ concentrations rose rapidly and were significantly higher than non-pregnant women during all trimesters of pregnancy. T₄ concentration continued to increase, thereafter although at slower rate. In contrast T₃ increased in early pregnancy and slightly decreased in the second trimester and then continued to increase until the end of pregnancy. The increase of T₄ and T₃ may be due to the progressive increase of thyroxine binding globulin (TBG) in the first trimester of pregnancy. As a result of reduced clearance by the liver, due to increase of estrogen secretion throughout pregnancy⁽¹³⁾. These results were similar to these reported by^(1, 6 & 14). The increase of T, after first trimester of pregnancy was associated with increased requirement of T₄ and T₃ during pregnancy, these mostly common in women with hypothyroidism who were treated with T₄ therapy if they become pregnant. In those women the dose of T₄ has been increased by 25 - 50 % to maintain normal serum thyroid stimulating hormone (TSH) level during pregnancy^(15 & 16). The specific reason for this increase need for T₄ is unknown. The increase of T₄ to about 30mg which is largely achieved during first trimester, since the increase of T, requirement continues until delivery.

In this study, TSH remained within the normal range (0.4 - 4.0 mU/L) during all trimesters of

pregnancy. Although pregnant women had serum TSH level was suppressed below the normal in the first trimester (12.5%), but within the normal range of TSH. The increase in T₄ level in pregnancy is probably due to non-pituitary stimulation of thyroid hormones, on the other hand the negative feedback control system of hypothalamic pituitary thyroid axis functions normally during pregnancy. The result of the present work was similar to that previously reported by⁽¹⁷⁾. The results showed suppressed TSH in first trimester, which might be due to severity of morning sickness in women with hyperemesis gravidarum in which hCG level was higher than normal. These results were in accord with those reported by⁽⁶⁾. This was associated with a decrease in serum TSH, which suggested a physiological activation of thyroid gland⁽⁶⁾.

The maternal free thyroid hormone concentration has been variously reported to be unchanged⁽¹⁹⁾ or decreased⁽²⁰⁾ or increased^(21 and 22).

Serum free thyroid hormones (FT₄ and FT₃) changes

Figs (I and 2) showed that there was increase of free T₄ and free T₃ only in the first trimester and then decreased gradually in the second and third trimesters, although the increase of free T₄ was limited in the first trimester is statistically significant, while the increase of free T₃ is not significantly different compared to non-pregnant women. While level of FT₄ and FT₃ in second and third trimesters remain within the normal range. The reason of the decrease of free thyroid hormones is

not clear, but the interrelationship of TSH, estrogen and TBG may be important. These findings agreed with (14 and 18). Thyroid hormone test in the first trimester has been slightly different from that in the second or third trimesters, therefore, the stage of pregnancy should also be considered when evaluating thyroid function in pregnancy, these results similar to reports by (6, 8 and 21).

Serum human chorionic gonadotrophin (hCG):

In the present study it has been shown that serum hCG concentration increased only in the first trimester of pregnancy (Table 2) and then decreased progressively in the second and third trimesters of gestation. The difference between the first and second trimesters values were significant as was the difference between the first and third trimesters. It was well known that excess hCG has a thyroid stimulating effect when TSH was within the normal level. The significant correlation between serum hCG and FT₄ concentrations suggested that thyroid gland is physiologically activated by hCG in early pregnancy. These results confirmed those reported by (6, 18 and 23) who considered that hCG has intrinsic thyrotrophic activity. There is considerable evidence that hCG has intrinsic thyrotrophic activity (24). These results reflected the effect of hCG on thyroid hormones in the first trimester.

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