

STUDYING SOME IMMUNOLOGICAL PARAMETERS IN PREGNANT WOMEN WITH THYROID GLAND PROBLEMS

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ABSTRACT

This study was carried out to determine some hormonal and immunological parameters in women who attended Maternity and Children teaching hospital in Ramadi city , from 1st of August 2014 to 1st of April 2015. Ages of patients ranged from 16 - 49 years . The study included 358 blood samples from pregnant and non-pregnant women . sixty five samples were found to be abnormal thyroid function . The remaining 293 samples from healthy pregnant and not pregnant women were used as a controls . Women under study were divided into three groups: Pregnant Women with thyroid disorder(Group I) , Pregnant Women without thyroid disorder (Group II ,control 1) , and non- Pregnant Women without thyroid disorder (Group III , control 2). The study showed that there was no significant differences in T3 ,T4and FTI levels in patient compared with control 1 (p-value >0.05) while it showed significant difference between patient and control 2 with p-value (<0.05). the means of T3,T4 and FTI in patient were 4.08 ng/ml, 91.09 µg/dl, 69.55 respectively. While there was no significant difference in level of TSH in cases and control 1 and control 2 with p-value >0.05. The mean level of TSH of patient was 18.65 µIU/ml . A comparison in Immunological parameters between the three groups with each other showed that there was significant increase in levels of Anti thyroxin peroxidase abs and Anti thyroglobulin abs in cases compared with control 2 with p-value <0.05, but didn't show significant difference between cases and control 1 with p-value >0.05. The mean levels of patients for Anti TPO abs was 302.10 IU/ml and for Anti TG abs was 143.21 IU/ml.

Introduction:

Pregnancy is a physiological state accompanied by a high-energy demand and an increased oxygen requirement which leads to complex alteration in metabolic and hormonal changes in the physiology of maternal-fetal system and the request for thyroid hormones is increased during gestation ^(1,2) . Due to specific conditions related to the pregnancy period, There are various alteration accompanied with this phase of life. Because autoimmune thyroid disease is common in women during the childbearing period, it is important to understand both the expected changes in thyroid function in normal pregnancy and how pregnancy may affect pre-existing Graves' disease, hypothyroidism and thyroiditis. Etc. ⁽³⁾.

Thyroid disorders may affect both the pregnant woman and the developing fetus; where thyroid hormones having essential role in embryogenesis and fetal development. As fetus is completely dependent on the mother for thyroid hormone ⁽⁴⁾ .

Uncorrected thyroid dysfunction in gestation has adverse effects on fetal and maternal well-being (before and after delivery). The deleterious effects of thyroid dysfunction can also extend beyond pregnancy and delivery to affect neurointellectual development in the early life of the child ⁽²⁾ , and also lead to maternal, fetal, and neonatal morbidity, and mortality. Maternal complications involves miscarriage, pregnancy induced hypertension, placental abruption, preterm labor, heart failure, and thyroid storm. Fetal and neonatal complications include low birth weight, stillbirth, hyperthyroidism, goiter, and hypothyroidism. ^(5,6)

Autoimmune thyroid disease is common in gestation. and characterized by the presence of

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antithyroid antibodies, specifically anti thyroglobulin (TG-abs), and anti thyroid peroxidase (TPO -abs). The presence of these antibodies is connected with a significant increment in miscarriages.⁽⁷⁾

The present study was designed to achieve the following aims :

- 1-To measure thyroid hormones (tT3 and tT4)in pregnant women to find out the prevalence of thyroid disorders with measurement of TSH and some indexes such as Free Thyroxine Index .
- 2- To assess some immunological parameters in those pregnant women with thyroid diseases with focusing on anti-thyroglobulin antibodies and thyroid peroxidase antibodies levels to investigate the role of these auto antibodies as cofactors in causing abortions and pregnancy problems .

Materials and Methods :

1-Patients and Controls :

The study included Pregnant women in reproductive age who were suffering of thyroid problems , who attended Maternity and Children teaching hospital in Al-Anbar governorate during the period extended from 1st of August 2014 to the 1st of April 2015 . Patients were selected randomly for pregnancy stage and their ages were from 16 - 49 to years. Blood samples from apparently healthy non pregnant women and pregnant women with normal thyroid function were used as controls . Ten milliliters (10ml) of venous blood were collected from each of patients and controls to be centrifuged to get pure serum samples .

2-Diagnostic Kits and Chemical Reagents:

The following diagnostic kits and chemical reagents were used :

Enzyme Linked Immuno Sorbent Assay (ELISA) Biomerieux France , was used to measure Total Triiodothyronine (tT3),Total T4 , and thyroid stimulating hormone (TSH). IMMULITE 2000 system , Siemens Healthcare Diagnostics Inc. United Kingdom was used for measurement of Free Thyroxine Index(FTI) , Thyroid Uptake (TU) , Anti-Thyroid Peroxidase Antibody(Anti-TPO Antibody) ,and Antithyroglobulin , and Anti-Thyroglobulin Antibody (Anti-Tg Antibody).All reagents and kits were prepared and applied according to the companies instructions .

Results and Discussion :

Pregnancy , a normal physiological condition is interplay of numerous metabolic and hormonal

parameters to meet the demands of growing fetus. Pregnancy causes stress to the maternal thyroid gland. maternal thyroid hormone excess or deficiency can influence the outcome for mother and fetus at all stages of pregnancy. Maternal hypothyroidism is the most common disorder Pregnancy causes significant changes in metabolism, fluid balance, organ function and blood circulation which are driven by estrogen⁽⁸⁾ .

Population Study:

The total number of women included in the study was 358 (147 pregnant and 146 non pregnant).Out of the total number of pregnant women 65 (18.1%) were found to have some abnormalities in thyroid function while the remaining number was found to be normal .Two groups were used as controls , the first group included those pregnant women with normal thyroid function which was considered control 1 , and those who are not pregnant were considered control 2 .

According to ages , study population was grouped into 7 groups as described in table (1).The fourth group (31 – 35 years) and the fifth group (36 – 40 years) represented the highest number of patient and the highest number of abnormal findings .

Distribution of Pregnant Women According to Gestational Age:

The percentage of pregnant women with problems in thyroid gland function in 1st trimester was 32.3% (10 patients), and percentage of pregnant women with problems in thyroid gland function in 2nd trimester was 27.4% (34 patients) and percentage of pregnant women with problems in thyroid gland function in 3rd trimester was 36.8% (21patients) , While The percentage of pregnant women without problems in thyroid gland function in 1st trimester was 67.7% (21 patients), and percentage of pregnant women without problems in thyroid gland function in 2nd trimester was 72.6% (90 patients) and percentage of pregnant women without problems in thyroid gland function in 3rd trimester was 63.2% (36 patient). (Table 2)

The present study showed the prevalence of thyroid disorder in pregnant especially in 2nd trimester more than other stages the explanation for that the embryogenesis and fetal development during pregnancy attributed with increased demand thyroid hormones and iodine with the progression of pregnancy for that Pregnancy is a stress test to the maternal thyroid gland, to increase in thyroxin binding

globulin, and thyroid stimulation by HCG⁽⁹⁾. As Fetus depends in the first 12 weeks on the mother for thyroxine⁽¹⁰⁾. After this period the fetus thyroid become gradually active at the end of first trimester of gestation therefore, The latter physiological condition, indicate the additional request of iodine during pregnancy in the diet to provide the iodine required by fetus thyroid and protect the maternal system⁽¹¹⁾.

Distribution of Cases Under Study According to Type of Disorder in Thyroid Function:

The number of pregnant women with Hypothyroidism was forty patient (61.5%), and number of pregnant women with hyperthyroidism was 7 patient (26.2%), while the number of pregnant women with Euthyroid was 8 patient (12.3%). As shown in Table (3)

Hypothyroidism in present study was observed in 61.5%, and hyperthyroidism observed in 26.2% the present results are higher than those reported for pregnant Palestine women in Radi,⁽¹²⁾ studying which was observed in observed hypothyroidism found in 2.2% and hyperthyroidism in 1.0%. However, the difference between present study and that may be due to the fact that present study samples were collected randomly from the all trimester of pregnancy. While the samples of other study collected during only the first-trimester of pregnancy.

Also Lazarus,⁽¹³⁾ reported thyroid disorders during pregnancy and the prevalence of hypothyroidism (2.5%) is higher than hyperthyroidism (0.2%) which support the findings of the present study.

Hormonal assay Results:

The present study showed no significant difference in T3 and T4 levels in pregnant women with problems in thyroid gland (cases) compared with pregnant women without problems in thyroid gland (control 1) with p-value was (>0.05) while it showed significant difference between cases and non-pregnant pregnant women without problems in thyroid gland (control 2) with p-value was (<0.05). and the mean for T3 and T4 of cases higher than control 1 and control 2 the means for three groups of T3 respectively were 4.08 ng/ml, 2.2 ng/ml, 1.84 ng/ml. while the mean for three groups of T4 respectively were 91.09 μ g/dl, 92.46 μ g/dl, 74.61 μ g/dl.

When compare the levels of T3 according to age among three groups show There was no significant

difference in level of T3 in cases and control 1 and control 2. with p-value (<0.05). shown in tables 4.

When comparing the levels of T3 and T4 according to gestational age between cases and control 1 showed There was significant difference in levels of T3 and T4 with p-value (<0.05). while showed increase of T3 and T4 in 2ed and 3rd trimesters compared with 1st trimester and the mean for cases in T3 according to gestational age respectively were 1.51 ng/ml, 2.19 ng/ml, 2.86 ng/ml. while for control 1 were 1.58 ng/ml, 1.79 ng/ml, 1.79 ng/ml.

While the mean for cases in T4 according to gestational age respectively were 69.51 μ g/dl, 96.27 μ g/dl, 118.12 μ g/dl. and for control 1 were 84.76 μ g/dl, 89.17 μ g/dl, 101.97 μ g/dl. (table 4).

The present study is in consistent with Khandakar et al.⁽⁹⁾, who showed that the levels of thyroid hormones significant increase with the progression of pregnancy especially in 2nd and 3rd trimesters with ($p<0.01$), as compared to non-pregnant females. This increase may be due to the role of these hormones in embryogenesis and fetal development during this period.

The etiology of this increase in total circulating thyroid hormones involved production of type III deiodinase from the placenta, this enzyme, which converts T4 to reverse T3, and T3 to diiodotyrosine (T2), has extremely high activity during fetal life.^(13,15) another Cause for this increase is due to increased concentrations of plasma TBG⁽¹⁰⁾.

Because of the fetus needs thyroxin for brain development, growth, and lung maturation, the thyroxin is transfer across the placenta more over Placental de-iodinases can convert T4 to T3. Thus if maternal levels of thyroxin are not well maintained in pregnancy, fetus is at risk⁽¹⁴⁾.

Moreover the increased activity of the thyroid gland during pregnancy may be to Thyroglobulin production which increase during gestation. The increase in thyroglobulin can be seen as early as the first trimester, but it is more manifest in the latter part of pregnancy⁽¹⁶⁾. The structural homology between hCG and TSH lead to the hCG may act as a thyrotropic action causing the large amount of thyroid hormone in this period. HCG possesses an intrinsic thyroid-stimulating activity and perhaps even a direct thyroid growth promoting activity⁽¹⁷⁾

Also the present study consistent with study of Kaur et al.,⁽¹⁸⁾ which showed T3 and T4 hormones also

significant increase in their levels by the progression of pregnancy which reported that T3 levels increased to 1.3 ng/ml at $p \leq 0.05$ in 2nd trimester to 1.4 ng/ml at $p \leq 0.001$ in 3rd trimester with respect to 1st trimester. On the other hand T4 levels in blood showed an insignificant increase from 10.4 $\mu\text{g/dl}$ in 1st trimester to 15.2 $\mu\text{g/dl}$ in 2nd trimester but slightly significant increase to 16.02 $\mu\text{g/dl}$ at $p \leq 0.5$ in 3rd trimester. In 2nd and 3rd trimester the levels of T3 and T4 were significantly high at $p \leq 0.05$ compared with non-pregnant.

The present study showed no significant difference in level of TSH in cases and control 1 and control 2 with p -value (>0.05). The mean of TSH level of cases women was higher than that of control 1 and non-pregnant women and the means for three groups respectively were 18.65 $\mu\text{IU/ml}$, 2.38 $\mu\text{IU/ml}$, 3.27 $\mu\text{IU/ml}$.

When compare the levels of TSH among patient and control 1 and control 2 according to age showed There was significant difference with p -value (<0.05), as shown in table 4.

When compare the levels of TSH according to gestational age between cases and control 1 showed There was significant difference with p -value was (<0.05) while showed increase of TSH level in 2ed and 3rd trimester compared with 1st trimester. and the means for cases in TSH according to gestational age respectively were 12.45 $\mu\text{IU/ml}$, 12.54 $\mu\text{IU/ml}$, 14.76 $\mu\text{IU/ml}$ while for control 1 were 1.72 $\mu\text{IU/ml}$, 2.69 $\mu\text{IU/ml}$, 2.76 $\mu\text{IU/ml}$.

The present study agree with study of Khandakar et al.⁽⁹⁾ and Pasupathi,⁽¹⁹⁾ which did not show a significant difference between pregnant and non-pregnant women. That may be due to the patient involved both cases hypothyroidism and hyperthyroidism and also prevalence hypothyroidism more than hyperthyroidism in present study.

The changes in TSH levels may be explained by the fact that the HCG increase in pregnancy and its structure quite similar to TSH, in addition it has thyrotropic activity during the first trimester of gestation that lead to TSH does decrease slightly in the first trimester⁽¹⁷⁾. These findings support our results.

Also Radi,⁽¹²⁾ showed there was no significant association between increasing in gestational age and TSH with ($p=0.09$). Moreover Khandakar et al.⁽⁹⁾, observed There was significant increase in TSH levels with ($p<0.01$) at 1st, 2nd and 3rd trimesters of

pregnancy when compared to that of normal non-pregnant females.

Results of the control group showed significant relationship between age and TSH That agree with other studies such as Bocos-Terraz et al.⁽²⁰⁾ who reported that TSH showed significant differences ($P < .005$) according to the age of the mother.

levels of Free Thyroxin Index (FTI) :

The present study showed significantly decrease in FTI levels in cases compared with control 2 with p -value was (<0.05). but didn't show significant difference between cases and control 1 with p -value was (>0.05) and the means for three groups respectively were 69.55, 74.55, 120.89.

When comparing the levels of FTI according to age among three groups show There was no significant difference in level of FTI in cases and control 1 and control 2. with p -value (>0.05), as shown in table 5.

When compare the levels of FTI according to gestational age between patient and control 1, There was significant difference in level of FTI. with p value was (<0.05). while showed increase of FTI in 2ed and 3rd trimester compared with 1st trimester and the mean for cases in TSH according to gestational age respectively were 54.34, 74.76, 78.83. while for control 1 were 31.8, 54.54, 118.78. as shown in tables 5.

The present study consistent with Ball et al.⁽²¹⁾ and Glinoe et al.⁽¹⁷⁾ studies they showed lower free hormone concentrations at term than non-pregnant women. Also other studies have confirmed that serum free T4 and T3 are ~25% lower in women at delivery than non-pregnant women.

Das et al.,⁽²²⁾ found a very significant rise in FTI at 3rd trimester ($p<0.01$) compared with 1st trimester, that consistent with present study.

That may be due to with the advancing pregnancy and particularly around third trimester, the presence of some unknown metabolites arising from the pregnancy disturbe the binding of T4 with the rising TBG molecules, which can displace and bind T4 molecules (competitively or otherwise) from the rising TBG molecules. The displaced and bound-T4 moiety, thus becomes physiologically ineffective. This would, therefore, cause a less proportionate rise in the level of serum thyroxine binding capacity (TBK). This displacement phenomenon, therefore, tends to constitute a rise in serum free thyroxine index level in the pregnant women.

By contrast Elizabeth et al.,⁽²³⁾ showed there was no significant association between increasing in gestational age and FT4 ($p=0.575$). that does not consistent with present study .

Results of the control group showed no significant relationship between age and FT4.that doesn't agreement with Radi,⁽¹²⁾ This may be due to fact that the mean age of the control 1 (31 years) and control 2 (31years) which is less than the mean age of cases (32 years). While other study detected that the mean age of the control (29.32years) which is more than the mean age of cases (24.89 years).

Immunological data(levels of Anti TPO abs and Anti TG abs):

The present study showed significant increase in levels of Anti TPO abs and Anti TG abs in cases compared with control 2 with p-value was (> 0.05).but didn't show significant difference between cases and control 2 .

The mean levels of Anti TPO abs for three groups were 302.10 IU/ml , 32.1 IU/ml , 11.11 IU/ml respectively , and the mean levels of Anti TG abs for three groups were 143.21 IU/ml ,34.99 IU/ml ,13.19 IU/ml respectively .

When comparing the levels of Anti TG abs according to age among three groups show There was no significant difference in level of Anti TG abs in cases and control 1 and control 2 with p value (>0.05). as shown in tables 6 .

When comparing the levels of Anti TPO abs and Anti TG abs according to gestational age between patient and control 1 showed significant difference between their with p -value was (<0.05). while showed increase of Anti TPO and Anti TG abs in 2ed and 3rdtrimester compared with 1st trimester and the means of Anti TPO abs for cases according to gestational age respectively were 166.9 IU/ml, 319.87 IU/ml, 434.09 IU/ml. while for control 1 were 32 IU/ml, 32.7 IU/ml ,33.4 IU/ml .While the means of Anti TG abs for cases according to gestational age respectively were 178.60 IU/ml, 291.11 IU/ml ,324.69 IU/ml . while for control 1 were 34.25 IU/ml ,35.56 IU/ml ,35.8 IU/ml (tables 6).

This study showed a significant difference between pregnant and non-pregnant women. In relation to Anti-TPO and Anti TG means . During pregnancy the growing fetus can release enough antigens to stimulate the maternal immune system for that thyroid auto-antibodies are elevated during this

peroid , afterward produce enough antibodies stimulate the maternal thyroid and inducing the thyroid abnormalities⁽²⁴⁾ ,that accordance with present study

It has been shown that the TPO-Ab level was associated with the TSH level, women who have TPO-Ab and TG-Ab have significant increase TSH level .^(25,26)

The present study showed that in the first trimester, the levels of antithyroid antibodies can decrease, but after that TPO-Ab and TG-Ab levels increase until delivery that may be explained by immunosuppression during first trimester of pregnancy. These findings is different than Kun et al.⁽²⁷⁾ who reported that the levels of antithyroid antibodies can increase in the first trimester, but after that TPO-Ab level may decrease the enzyme very important for metabolism of cells through increasing chemical reactions there for any defect happens in cells that influence to this enzymes at last influence in processes produced by these enzyme⁽²⁸⁾ .

Thyroid peroxidase is used in the synthesis of thyroid hormones that lead to increasing its antigenicity ,and anti TPO used in investigation some of cases disease through increasing its in serum when defect happen in thyroid gland, and also pregnancy influence into the function and size of thyroid gland there for lead to increasing this antibodies which corresponding with Anti TG by increasing thyroglobulin in pregnancy which lead to increasing the antibodies against it .

Table 1: Distribution of Study Population According to Age Groups

Age groups In years	Total no.	Abnormal thyroid No (%)	Normal thyroid No (%)
15 – 20	35	5(14.3)	30(85.7)
21 – 25	45	6(13.3)	39(82.7)
26 – 30	54	12(22.2)	42(77.8)
31 – 35	75	21(28)	54(72)
36 – 40	68	16(23.5)	52(76.5)
41 – 45	40	4(10)	36(90)
46 – 50	41	1(2.4)	40(81.9)
Total	358	65(18.1)	293(81.9)

Table 2 : Distribution of Pregnant Women According to Gestational Age

Gestational age	Total no.	Abnormal thyroid No (%)	Normal thyroid No (%)
1 st trimester	31	10 (32.3)	21 (67.7)
2 nd trimester	124	34 (27.4)	90 (72.6)
3 rd trimester	57	21 (36.8)	36 (63.2)
Total	212	65 (30.7)	147 (69.3)

Table 3 : Distribution of Cases Under Study According to the Type of Thyroid Disorder

Type of disorder	Number	%
hypothyroidism	17	26.2

hyperthyroidism	40	61.5
Euthyroid	8	12.3
Total	65	100

Table 4 : mean level of tT3 ,tT4 , and TSH in patients and controls

Type	No.	Mean tT3 (ng/ml)	Mean tT4 (µg/dl)	Mean TSH (µIU/ml)
Group 1(Patients)	65	4.08	91.09	18.65
Group 2 (control 1)	147	2.20	92.46	2.38
Group 3 (control2)	146	1.84	74.61	3.27
P value		1&2=7.7 1&3=0.01	1&2=0.8 1&3=0.04	1&2=3.6 1&3=1.0

Table 5 : Mean levels of Free Thyroxin Index (FTI) in patient and controls

Type	No	Mean FTI
Group 1(Patients)	65	69.55
Group 2 (control 1)	147	74.55
Group 3 (control2)	146	120.89
P value		1&2=0.7, 1&3=0.007

Table 6 Mean levels of thyroid peroxidase Ab , and antityroglobulin antibodies in patients and Controls :

Type	No	Mean anti-TPO(IU/ml)	Mean anti-TG(IU/ml)
Group 1(Patients)	65	302.10	143.21
Group 2 (control 1)	147	32.1	34.99
Group 3 (control2)	146	11.11	13.19
P value		1&2=0.08 1&3=0.004	1&2=0.07 1&3=0.04

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دراسة بعض المتغيرات المناعية عند النساء الحوامل المصابات باضطرابات الغدة الدرقية

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الخلاصة:

تضمنت الدراسة تحديد بعض التغيرات الهرمونية والمناعية عند الحوامل المراجعات الى مستشفى الرمادي التعليمي للنسائية والاطفال للفترة من ٢٠١٤ /٨/١ الى ٢٠١٥/٤/١ . شملت الدراسة ٣٥٨ نموذج دم من نساء حوامل وغير حوامل. وجد ان ٦٥ امرأة حامل من بين العدد الكلي يعانين من بعض الاضطرابات بالغدة الدرقية بينما وجد ان العدد المتبقي لا يعانين من اي اضطراب. تراوحت اعمار النساء بين ١٦ - ٤٩ سنة. شملت الدراسة ايضا العدد المتبقي من النساء اللاتي لا يعانين من مشاكل بالغدة الدرقية والبالغ عددهن ٢٩٣. قسم العدد الكلي للنساء قيد الدراسة الى ثلاث مجاميع : المجموعة الاولى شملت الحوامل اللاتي ثبت معاناتهن من اضطراب الغدة الدرقية ، والمجموعة الثانية شملت النساء الحوامل اللاتي ثبت عدم معاناتهن من اضطراب الغدة كسيطرة اولى ، اما المجموعة الثالثة فقد شملت النساء غير الحوامل ولا يعانين من مشاكل بالغدة الدرقية كسيطرة ثانية . بينت الدراسة عدم وجود فروق معنوية في مستويات T3 و T4 و FTI عند مقارنة المجموعة الاولى والثانية بينما تبين وجود فروق معنوية واضحة عند مقارنه المجموعة الاولى بالثالثة. توجد ان معدلات مستويات T3 و T4 و FTI للمجموعة الاولى 4.08 ng/ml و 91.09 µg/dl و 69.55 ng/ml على التوالي ، بينما تبين عدم وجود فروق معنوية في مستويات TSH بين المجموعة الاولى والثانية اذ بلغ معدل هذا الهرمون لدى المجموعة الاولى 18.65 µIU/ml عند مقارنة المتغيرات المناعية بين المجاميع الثلاثة تبين وجود فروق معنوية بمستويات الاجسام المضادة Anti thyroxin peroxidase و Anti thyroglobulin بين المجموعة الاولى والمجموعة الثالثة بينما لا يوجد فرق معنوي بين المجموعة الاولى والثانية ، كما تبين ان مستويات هذين النوعين من الاجسام المضادة 302.10 IU/ml و 143.21 IU/ml على التوالي.