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Measurement of Serum Free Thyroxine by RIA in Normal Populations and During Pregnancy

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การวัดระดับฟรีธัยร็อกซินฮอร์โมนโดยวิธี RIA ในคนปกติและในหญิงมีครรภ์ปกติ

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มีรายงานการศึกษาถึงระดับฟรีธัยร็อกซินฮอร์โมนในหญิงมีครรภ์ปกติ พบว่ามีค่าแตกต่างจากค่าของคนปกติที่ไม่ได้ตั้งครรภ์ ทำให้เกิดปัญหาในการวินิจฉัยและติดตามผลการรักษาโรคของต่อมธัยรอยด์ในหญิงมีครรภ์เป็นอย่างมาก จึงได้ศึกษาระดับฟรีธัยร็อกซินโดยวิธี RIA ชนิด Amersham และ Diagnostic products ในหญิงมีครรภ์ปกติจำนวน 177 ราย ตามไตรมาสต่าง ๆ เทียบกับคนปกติ พบว่าค่าของฟรีธัยร็อกซินชนิด Amersham มีค่าต่ำกว่าค่าของคนปกติอย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) ในไตรมาสที่สองและสาม ส่วนค่าที่หาโดยชนิด Diagnostic Products มีค่าต่ำกว่าของคนปกติในไตรมาสที่สองและสามเช่นกัน แต่ไม่มีความแตกต่างกันทางสถิติ

เนื่องจากค่าของฟรีธัยร็อกซินในหญิงมีครรภ์มีค่าแตกต่างจากค่าของคนปกติ เพื่อความถูกต้องจึงควรใช้ค่าปกติของหญิงมีครรภ์ ซึ่งแบ่งตามไตรมาสต่าง ๆ มาใช้ในการวินิจฉัยและติดตามผลการรักษาโรคของต่อมธัยรอยด์ในหญิงมีครรภ์

Serum Free thyroxine (FT_4) concentrations have been variously reported as either constant or falling in pregnancy. In this study, 177 serum samples from apparently normal pregnancies were used to derive trimester related euthyroid ranges throughout pregnancy for free T_4 measured by Diagnostic Products and Amersham kits. Serum free T_4 concentrations as determined by Amersham kits were shown to be significantly reduced in the second and third trimester ($P > 0.05$). The free T_4 concentrations measured by Diagnostic Products kits fell with increasing gestational age especially in the third trimester, but the range of values were not significantly reduced from normal non-pregnant women.

The observation that serum FT_4 concentrations may fall as gestation progressed, as demonstrated by both the Amersham and Diagnostic Products radioimmunoassay technique, suggests that diagnosis and management thyroid disorders in pregnancy, trimester related reference ranges for FT_4 must be used in pregnancy if the test is to correctly interpreted.

INTRODUCTION

The most commonly used test for evaluating thyroid function is the serum thyroxine (T_4) concentration. Since circulating T_4 is almost totally bound (99.97%), primarily by the thyroxine-binding globulin (TBG) but also by albumin and prealbumin, measurement of serum total T_4 concentration will be influenced by both the quantity of T_4 secreted by the thyroid gland and the concentration of TBG. It is generally accepted that the free or unbound T_4 concentration (FT_4) is more readily available to the tissues for metabolic action, so it is considered to be the physiologically active form. Since it is not altered by the concentration of the thyroxine-binding proteins, especially TBG. It is more useful for evaluating thyroid function than that of total thyroid hormone, particularly during pregnancy which thyroxine binding protein is high. Many methods have been described for measuring of free thyroid hormone. Previous methods are equilibrium dialysis which are time-consuming and technically more difficult to perform. Another methods are indirect measurement of the serum FT_4 by calculating the product of the T_4 and T_3 resin uptake and is reported as a free T_4 index (FTI). These indirect methods do have clinical utility but do not give a quantitative FT_4 concentration. Recently a direct measurement of serum FT_4 can be detected by radioimmunoassays (RIA) which is rapid, reproducible, and simple method. Several commercial RIA kits are now available and had equal diagnostic efficiency in patients with suspected thyroid disease from subclinical thyroid disease to overt disease[4]. All of the FT_4 kits resolved the borderlilne cases with subclinical thyroid disease more clearly than did T_4 and T_3 measurement[4]. Since all these advantages, FT_4 measurements by RIA are commonly used routinely[2]. However there are several reports of discrepancies among these kits in pregnancy.[3,5,6] Some kits showed results that decreased significantly as gestation progressed but some kits showed same results similar to normal populations.[11,13] It will be misleading if we use the FT_4 values obtained from normal populations to diagnosed and manage thyroid disorders in pregnancy. In practice, trimester-related reference ranges for FT_4 must be used in pregnancy if the test is to be correctly interpreted.

The purposes of this study are to determine the FT_4 values obtained from normal populations and during pregnancy in various trimesters and look for any differences between two values. Furthermore, we want to look for any differences among these values obtained from pregnant women in various trimester to assess the clinical utility of these tests. If the data from studies shows statistically significant different results, then the clinical applications can be made for using trimester-related reference ranges for FT_4 to diagnose and manage thy-

roid diseases in pregnancy instead of using normal population ranges because it will be misleading.

Materials and Methods

Procedures

We studied FT_4 RIA kits from the following manufacturers which are routine laboratory use in Srinagarind Hospital : Amerlex Magnetic (Amerlex-M, Amersham U.K.); Coat-A-count (Diagnostic Products U.K.). All assays were performed by one operator.

Subjects

Normal populations : Clotted blood 10 ml was drawn from 60 normal female controls living in North-East part of Thailand, ages ranged from 20-40 years.

Pregnant women : Samples were collected from 177 pregnant women attending an antenatal clinic at various trimesters of pregnancy.

We used Student's unpaired t-tests for statistical analysis.

Results

Concentrations of FT_4 measured by two kit methods are shown in Table 1. Diagnostic products FT_4 concentrations (1.30 ± .32 ng/dl) were similar to Amersham FT_4 concentrations (1.23 ± .28 ng/dl) in the normal subjects. FT_4 concentrations measured by the Diagnostic Products method in the pregnant subjects were lower than FT_4 in the normal subjects in the second and especially third trimester (1.26 ± .22, 1.17 ± .18 ng/dl) but were not statistically significant. FT_4 concentrations measured by the Amersham method in the pregnant subjects were significantly lower ($p < 0.05$) than FT_4 in the normal subjects in the second and third trimester (1.07 ± .08, 1.08 ± .18 ng/dl).

DISCUSSION

In this study, the serum free T_4 in pregnant subjects was within the non-pregnant normal range in the first trimester, although the mean was slightly higher and the distribution was narrower. As pregnancy progressed, the free T_4 concentrations measured by the Amersham kits were shown to be Signcficanty reduced in the second and third trimester. This change is in good agreement with the results of other workers who have used the same free T_4 assay method.[3,8,9]

Table 1 Mean (± 2SD) free thyroid hormone levels in normal and pregnant subjects

		Pregnant subjects (trimester)		
Source	**Normal subjects**	**first**	**second**	**third**
Numbers	**58**	**59**	**59**	**59**
Diagnostic product (ng/dl)	1.30 ± .32	1.38 ± .26	1.26 ± .22	1.17 ± .18
Amersham (ng/dl)	**1.23 ± .28**	**1.30 ± .24**	**1.07 ± .08***	**1.08 ± .18***

Difference from normal subjects : *$p < 0.05$

The observed decline in free T_4 concentration as pregnancy progresses might be explained by supposing that the method of measuring free T_4 is susceptible to increases in the TBG concentrations that occur during pregnancy. However this is not the case. Because there are several studies of Amerlex free T_4 concentrations in women taking oral contraceptives (where TBG levels are expected to be increased) where Amerlex free T_4 values were within the same range as the distribution of normal euthyroid cases.[9] Furthermore, there are studies of Amerlex free T_4 concentrations in non-pregnant euthyroid subjects with elevated TBG which gave Amerlex free T_4 values in good agreement with a control group of normal TBG euthyroid subjects.[7,8,10] The results from these studies show clearly that the Amerlex free T_4 assay is not susceptible to methodological interference by raised TBG levels. Some studies suggested that elevated non-esterified fatty acid (NEFA) concentrations encountered in pregnancy, together with the reduction of serum albumin concentrations in pregnancy, may contribute to the low Amerlex FT_4[1], but this has been refused by another studies which have demonstrated that no correlation between serum NEFA, serum albumin and Amerlex FT_4 concentrations in the pregnancy group.[4]. There have not been any good reasons to explain the low FT_4 levels detected by these methods. Sridama et al. reported a study of ultrasensitive TSH in the pregnancy group with low Amerlex FT_4 where no TSH elevation was found. So the low FT_4 concentrations is possibly artifactually low.

From a practical viewpoint, the FT_4 levels measured by Amersham kits in non-pregnant normal population should not be used in diagnosis and treatment thyroid diseases in pregnancy, since this method gave misleading results. Although there are no statistical significant differences between the FT_4 levels measured by Diagnostic products kits in non-pregnant normal population and pregnant group, interpretation of FT_4 levels in pregnancy by using FT_4 valves obtained from non-pregnant normal subjects might be misleading especially in the third trimester. In practice, trimesterrelated reference ranges for FT_4 should be used in pregnancy if the test is to be correctly interpreted.

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