# THYROID FUNCTION AND URINARY IODINE EXCRETION IN PREGNANT WOMEN AT JAKKARAT, NAKORNRAJASIMA, THAILAND

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**Thesis**

**Entitles**

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THYROID FUNCTION AND URINARY IODINE EXCRETION IN PREGNANCY WOMEN AT JAKKARAT, NAKORNRAJASIMA, THAILAND.

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ABSTRACT

In Thailand, the prevalence of IDD of 37 % (n = 4500) in pregnant women was reported by the Ministry of Public Health in the year 2000. Nakornrajasima Province is located in the Northeastern of the country which is an area of iodine deficiency problems. The purpose of this study was to determine thyroid function in 350 of the normal pregnancies at the clinics of Jakkarat Hospital. Demographic data were collected for each subject. Urinary and venous blood samples were taken in various trimesters. Serum TSH and FT4,FT3 levels were measured by using in house IRMA and DPC solid-phase RIA method, respectively. Urine iodine was analyzed by modified ammonium persulfate.

The median and interquatile range of TSH concentration was increased from the first trimester of 0.911(0.46-1.31) mIU/L to the second trimester of 1.20(0.88-1.84) mIU/L and the third trimester of 2.02(1.34-4.45) mIU/L. In contrast, both FT4 and FT3 concentration were decreased from the first to the second and the third trimester of pregnancy. The FT4 values were 1.32(1.20-1.60), 1.25(1.09-1.42) and 1.15(1.04-1.34) ng/dl, while FT3 were 3.42(2.80-4.10), 3.17(2.30-4.0) and 2.27(1.05-3.08) pg/ml respectively. Urine iodine concentration throughout pregnancy were 297.11(119.80-358.00), 396.62(128.16-481.96) and 375.44(181.58-616.86) μg/L respectively and higher than reference range.

In conclusion, the present study demonstrates that the women who received iodated salt (1:20000) during pregnancy showed high normal mean ± SD values of TSH but low normal FT3 and FT4 while UI was higher than normal in the third trimester. The highest degree of correlation between the FT3 and TSH was found ($r = -0.593$, $P<0.01$, $y = 3.136-0.316x$). This suggested that UI determination alone gives inadequate information. Serum FT3 and TSH seems to be an important determinant of mechanism of thyroid gland response to ensure the extra iodine needed by the growing fetus.

KEY WORDS: PREGNANCY / THYROID FUNCTION / IODINE DEFFICIENCY

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การทำงานของต่อมธัยรอยด์และระดับไอโอดีนในปัสสาวะหญิงตั้งครรภ์ที่โรงพยาบาลจักราช อำเภอจักราช จังหวัดนครราชสีมา (THYROID FUNCTION AND URINARY IODINE EXCRETION IN PREGNANCY WOMEN AT JAKKARAT, NAKORNRAJASIMA, THAILAND)

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บทคัดย่อ

จังหวัดนครราชสีมา ตั้งอยู่ในภาคตะวันออกเฉียงเหนือของประเทศไทย ซึ่งเป็นพื้นที่ที่กระทรวงสาธารณสุขได้ทำการสำรวจหญิงตั้งครรภ์ 4500 ราย ในปี พ.ศ. 2545 และได้รายงานอัตราความชุกของโรคขาดสารไอโอดีน ถึงร้อยละ 47 การศึกษาในครั้งนี้ มีวัตถุประสงค์เพื่อศึกษาการทำหน้าที่ของต่อมธัยรอยด์ในหญิงตั้งครรภ์ปกติจำนวน 350 ราย ที่มาฝากครรภ์ที่โรงพยาบาลจักราช โดยการบันทึกประวัติ เก็บปัสสาวะและเจาะเลือดทุกราย โดยแบ่งการเก็บตัวอย่างเป็นสามไตรมาสจนสิ้นสุดการตั้งครรภ์ ได้ทำการวิเคราะห์หาระดับซีรั่ม TSH โดยวิธีอินเฮาส์ ไอาร์เอ็มเอ และระดับของ FT4, FT3 โดยวิธีดีพีซี โซลิด-เฟส อาร์ไอเอ และปริมาณของสารไอโอดีนในปัสสาวะใช้วิธีโมดิฟายด์ แอมโมเนียม เปอร์ซัลเฟต

ผลการศึกษาพบว่าในระหว่างการตั้งครรภ์ทุกไตรมาสระดับของ TSH, FT4 และ FT3 มีค่าอยู่ในพิสัยปกติ ในขณะที่ UI มีค่าสูงกว่าค่าพิสัยปกติ โดยในไตรมาสแรกค่ามัธยฐานและพิสัยควอไทล์ของ TSH เท่ากับ 0.91(0.46- 1.31) mIU/L และเพิ่มสูงขึ้นในไตรมาสที่สอง; 1.20(0.88-1.84) และไตรมาสที่สาม; 2.02(1.34-4.45) mIU/L ตามลำดับ ในทางตรงกันข้าม ทั้ง FT4 และ FT3 มีระดับลดลงจากไตรมาสแรกไปสู่ไตรมาสที่สองและสาม โดย FT4 มีค่าเท่ากับ 1.32(1.20-1.60), 1.25(1.09-1.42) และ 1.15(1.04-1.34) ng/dl ในขณะที่ FT3 มีค่าเท่ากับ 3.42(2.80-4.10), 3.17(2.30-4.0) และ 2.27(1.05-3.08) pg/ml ตามลำดับ สำหรับระดับไอโอดีนในปัสสาวะ พบว่ามีค่าสูงกว่าค่าพิสัยปกติทุกไตรมาส โดยมีค่าเท่ากับ 297.11(119.80), 396.62(128.16-481.96) และ 375.44(181.58-616.86) μg/l ตามลำดับ

การศึกษาในครั้งนี้แสดงให้เห็นว่าหญิงตั้งครรภ์ในถิ่นที่มีปัญหาโรคขาดไอโอดีนและได้รับเกลือไอโอเดต (1:20000) เป็นประจำทุกวัน ในไตรมาสที่สาม ค่าเฉลี่ย ± ค่าเบี่ยงเบนมาตรฐาน ของTSH อยู่ในระดับปกติไปทางสูง แต่ FT4 และ FT3 จะมีระดับ ปกติไปทางต่ำ ในขณะที่ค่า UI จะมีค่าสูงกว่าพิสัยปกติ นอกจากนี้ยังพบว่าสัมประสิทธิ์สหสัมพันธ์ระหว่าง FT3 และ TSH มีค่าสูงสุด($r = -0.593$, $P<0.01$, $Y = 3.136-0.316X$) จึงอาจกล่าวได้ว่าการตรวจระดับไอโอดีนในปัสสาวะอย่างเดียวจึงยังไม่เพียงพอ และเพื่อให้แน่ใจว่าทารกในครรภ์จะได้รับปริมาณสารไอโอดีนในปริมาณที่มากพอ ต่อการเจริญเติบโต มารดาจึงควรได้รับการตรวจหาระดับ TSH และ FT3 ร่วมด้วย

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| Ab | Antibody |
| Ag | Antigen |
| Ag* | Labeled antigen |
| Ag-Ab | Antigen-antibody complex |
| $As^{5+}$ | Arsenic |
| $As^{3+}$ | Arsenite |
| B | Bound from or antigen-antibody complex of standard or unknow |
| $B_0$ | Bound form or antigen-antibody complex of zero standard |
| BSA | Bovine serum albumin |
| r | Correlation coefficient |
| cpm | Count per minute |
| cm | centimeter |
| °C | Celsius decree |
| DPC | Diagnostic Product Coorporation |
| Fig | Figure |
| FT3 | Free Triiodothyronine |
| FT4 | Free tetraiodothyronine |
| g | Gram |
| hr | Hour |
| IDD | iodine deficiency disorder |
| IRMA | Immunoradiometric assay |
| $I^-$ | iodide ion |
| μl | Microlitre |
| min | Minute |
| mIU | milli international unit |
| ml | millilitre |
| M | Molar or mole per litre |

# LIST OF ABBREVIATIONS (continued)

| | |
|---|---|
| ng/dl | nanogram per decilitre |
| pg/dl | pigogram per deciliter |
| NSB | Non-specific binding |
| QC | Quality control |
| RIA | Radioimmunoassay |
| sec | second |
| sd | standard deviation |
| TSH | Thyroid stimulating hormone or thyrotropin |
| T3 | Triiodothyronine |
| T4 | Tetraiodothyronine or thyroxin |
| TBA | Thyroxin binding albumin |
| TBG | Thyroxine binding globulin |
| TBPA | Thyroxine binding pre-albumin |
| UI | Urinary iodine |

# CHAPTER 1
# INTRODUCTION

Iodine deficiency is considered largely a problem of developing countries. Iodine deficiency in endemic areas can cause maternal hypothyroxinemia which during pregnancy can result in impaired psychoneurological development of infants. It is the leading cause of preventable mental retardation. The World Health Organization's standard for iodine-deficiency disorders in population surveys recommends that a median urinary iodine concentration above 100 μg/L is evidence against significant iodine deficiency in that population. Iodine is a chemical trace element. Human need it to make thyroid hormones, tetra-iodothyronine or thyroxine ($T_4$) and triiodothyronine ($T_3$).Thyroid gland produced these hormones. After manufacture in the thyroid gland, thyroid hormones travel in the blood and control many chemical processes in different parts of body. Thyroid hormones are essential for normal growth, physical and mental development, and for maintenance the metabolism of body heat and energy. Dietary source of iodine include products from the sea (fish, squid, crab, lobster etc.), milk, eggs, and iodized products (e.g., salt). When people do not have enough iodine, they cannot make thyroid hormone. This deficiency of iodine has several important health consequences that together are called "iodine deficiency disorders" or IDD. These consequences are series of functional and develop mental abnormalities occur, including thyroid function abnormalities and, when iodine deficiency is severe, endemic goiter and cretinism, endemic mental backwardness, decrease fertility rate, increase perinatal death, socioeconomic retardation and childhood mortality. Iodine deficiency is one of important problem of Thailand because of its widespread prevalence and its destructive effects on human health. Proper supplement with iodine completely prevents these consequences.

In the 1990 WHO, an estimated 1.572 billion people (28.9% of the world's population) were at risk of IDD, 655 million (12%) were affected by goiter, up to 11.2

million (2%) by cretinism, the extreme from of mental retardation due to iodine deficiency, and another 43 million people had some degree of mental impairment. By 1999, 81% of 130 countries where IDD was a public health problem had a national intersectional coordinating body, 78% had an action plan for IDD control, 75% had salt iodization legislation in place and another 9% had it in draft from, 65% had laboratory facilities for program monitoring, 68% of households had access to iodized salt, 73% of countries were monitoring salt quality, and 61% were monitoring iodine nutrition in population(1, 2, 3, 4, 5, 6)

IDD has long been public health problem especially in the North and the North – Eastern of Thailand. In 2002, the prevalence of IDD of 47 % (n = 4500) in the pregnant women was reported by Nutrition Division, Ministry of Public Health. The severity of IDD was only 8.6 %( n = 385) while moderate and mild IDD were 15.8 %( n = 710) and 25.6 %( n = 1011) respectively. Median urine iodine (MUI) was 10.7 μg/dl. In the North East pregnant women, MUI of 9.32 μg/dl (n = 1198) was found to be the lowest level (7).

Pregnant woman with a normal thyroid gland is faced with a triple challenges. First, important modifications occur in thyroid function due to the marked increase in the circulating level of the major T4 transport protein, thyroxine-binding globulin (TBG) in response to high estrogen levels. Secondly, several thyroid stimulatory factors of placental origin (mainly hCG) are produced. Third, pregnancy is accompanied by a decrease in the availability of iodine for the maternal thyroid gland, due to increased renal clearance, and loss to the fetoplacental complex, resulting in a relative iodine deficiency state. In areas with borderline dietary iodine intake, the adaptation of thyroid function to pregnancy is impaired. In these areas the incidence of goiter during pregnancy is increased, and the expected serum increase in total T4 and T3 does not occur. The brain development of the fetus is dependent on maternal thyroxin. Maternal hypothyroxinemia during pregnancy as a consequence of iodine deficiency can be responsible for impaired psychoneurological development observed in children from endemic goiter areas. One of the major goals of a universal iodination program has been the provision of adequate iodine during pregnancy. The study of

thyroid function of pregnant women can serve as a test of the adequacy of iodine supply of this important group. During the last decade, as a result of the national iodination program, nearly all of the population was provided with iodized salt. World Health Organization (WHO) recommended the ideal dietary allowance of iodine is 200 μg of iodine per day for pregnant women (8, 9, 10, 11, 12).

# CHAPTER 2
# OBJECTIVES

The objectives of this study are as followed:

1. To determine serum FT3, FT4 and TSH levels among normal pregnant women presenting for prenatal at the clinics of Jakkarat hospital, Jakkarat district Nakornrajasima province by using Diagnostic product corporation (DPC) solid-phase RIA and in-house immunoradiometric assay (IRMA), respectively.

2. To measure urine iodine levels in these pregnant women by modified ammonium persulfate method.

3. To analyze serum TSH, FT4, FT3 and UI by using SPSS version 11.5 program.

# CHAPTER 3
# LITERATURE REVIEW

## Anatomy of thyroid gland

The thyroid gland is the largest endocrine gland in human located in the front of the neck attached to the lower part of the larynx and to the upper part of the trachea. It has two sides or lobes. These lobes are connected together by a thin band of connective tissue called the isthmus. Weighing of thyroid gland is approximately 10-20 grams in an adult. Each lobe is about 4 cm long and 1 to 2 cm wide. The gland lies in front of cervical 5, 6, 7 and thoracic one vertebrae. Isthmus, 1.2 cm x 1.2 cm embraces the $2^{nd}$ and $3^{rd}$ tracheal rings. Each lobe 5 cm x 2.5 cm extends from middle of thyroid cartilage to $5^{th}$ tracheal ring. The name "thyroid" comes from the Greek word which means "shield". The thyroid gland has a rich blood supply and the right lobe more vascular and often is larger. The artery arises from the common or external carotid artery, the inferior thyroid artery from the thyrocervical trunk of the subclavian artery, and the small thyroid artery from the brachiocephalic artery at the aortic arch. Venous drainage is via multiple surface veins coalescence into superior, lateral, and inferior thyroid veins. The blood flow to thyroid gland is about 5 ml/g/min.

The thyroid tissue is made up of two types of cells: follicular cells and parafollicular cells. Most of the thyroid tissue consists of the follicular cells, which secrete iodine containing hormones called thyroxine (T4) and triiodothyronine (T3).

The parafollicular cells secrete the hormone calcitonin. The thyroid needs iodine to produce the hormones (13, 14, 15, 16).

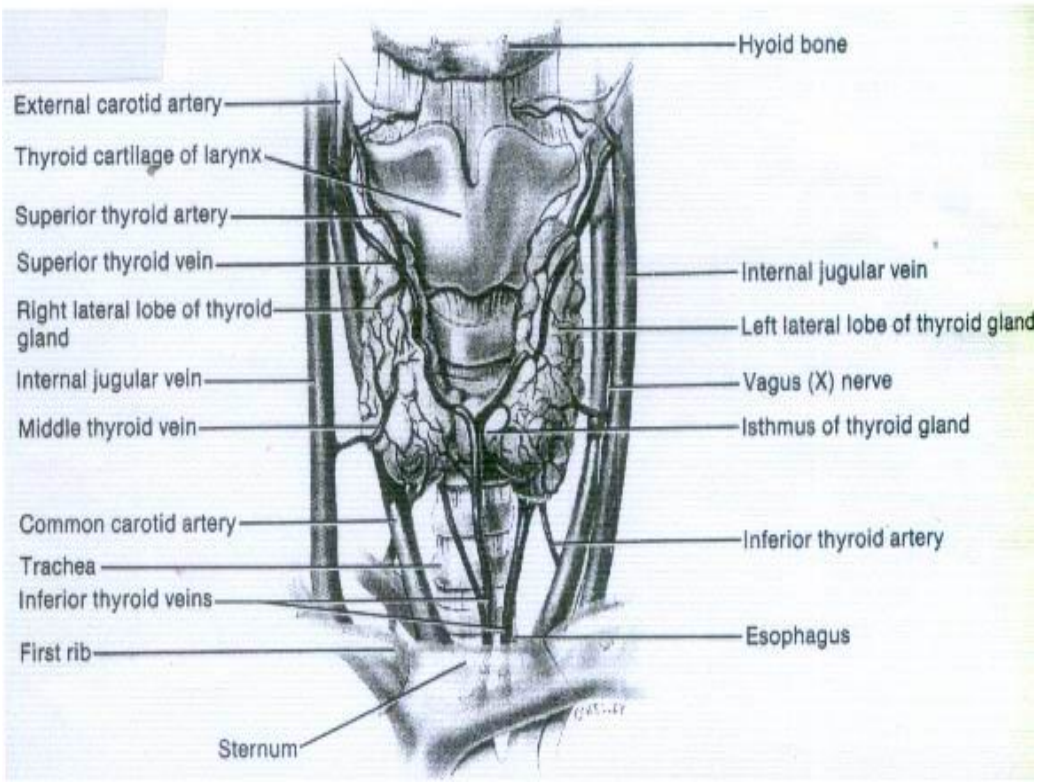



**Fig. 1** Thyroid gland (14)

**Functions of the thyroid gland**

The thyroid controls the body's metabolism when it is working correctly. The speed of the metabolism affects the working of organs such as the heart and brain. The metabolism acts on your digestive system to control how efficiently your burn calories and to ensure your muscles and nerves are in good condition; it can influence how you think and feel. The thyroid gland regulates your metabolism by making thyroid hormone, which is a chemical that carries a message from the thyroid gland to the rest of the body through the blood stream. The thyroid gland makes thyroid hormone from iodine absorbed from the food you eat-the larger the amount of thyroid hormone produced, the faster the cells work; when less thyroid hormone is produced, the cells work slower. Levels of hormones secreted by the thyroid are controlled by the pituitary gland's thyroid-stimulating hormone, which in turn is controlled by the hypothalamus.

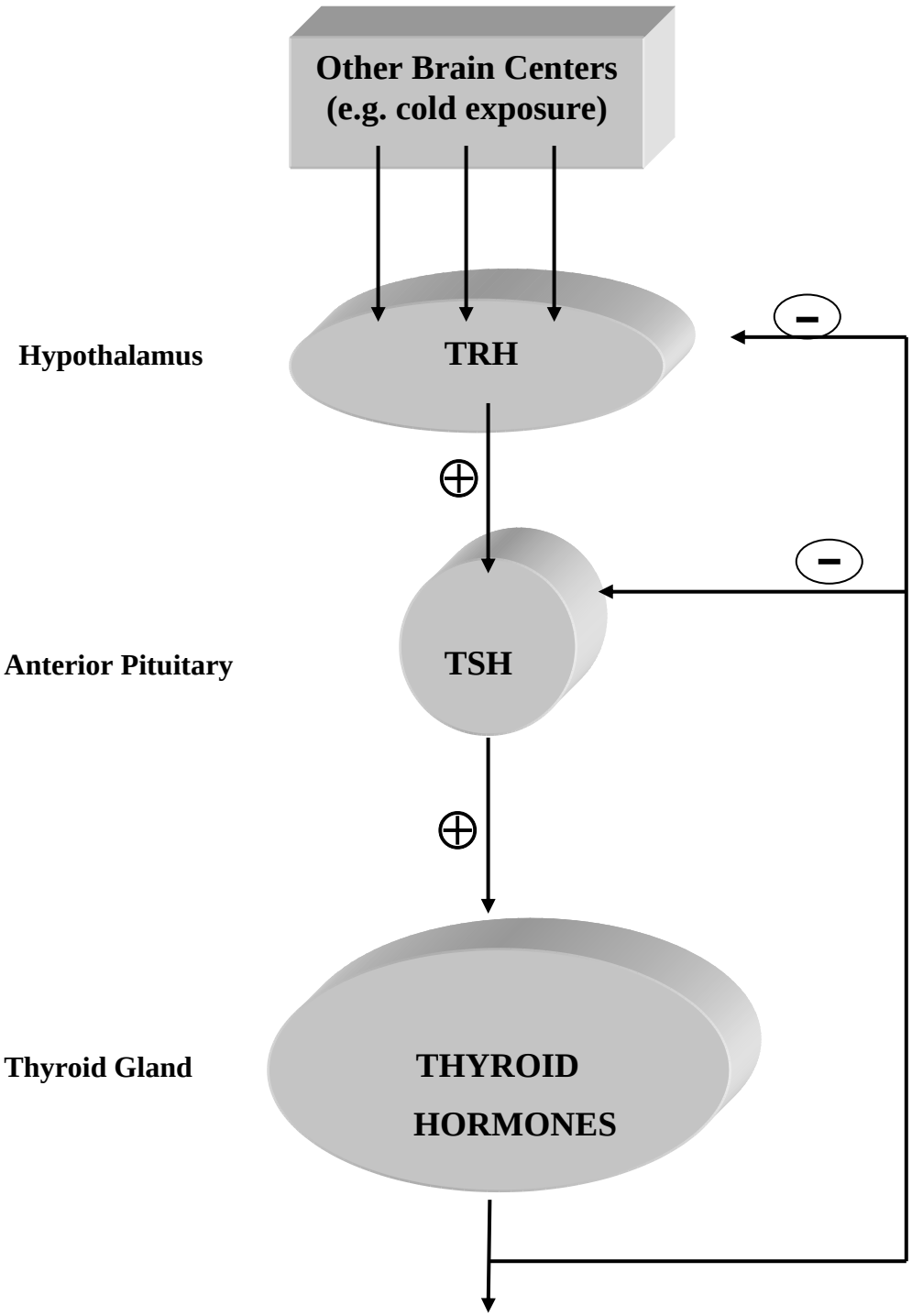



**Fig.2** The control of Thyroid hormones (16)

**Synthesis and Secretion of Thyroid Hormones**

Thyroid hormones are synthesized by mechanisms fundamentally different from what is seen in other endocrine systems. Thyroid follicles serve as both factory and warehouse for production of thyroid hormone.

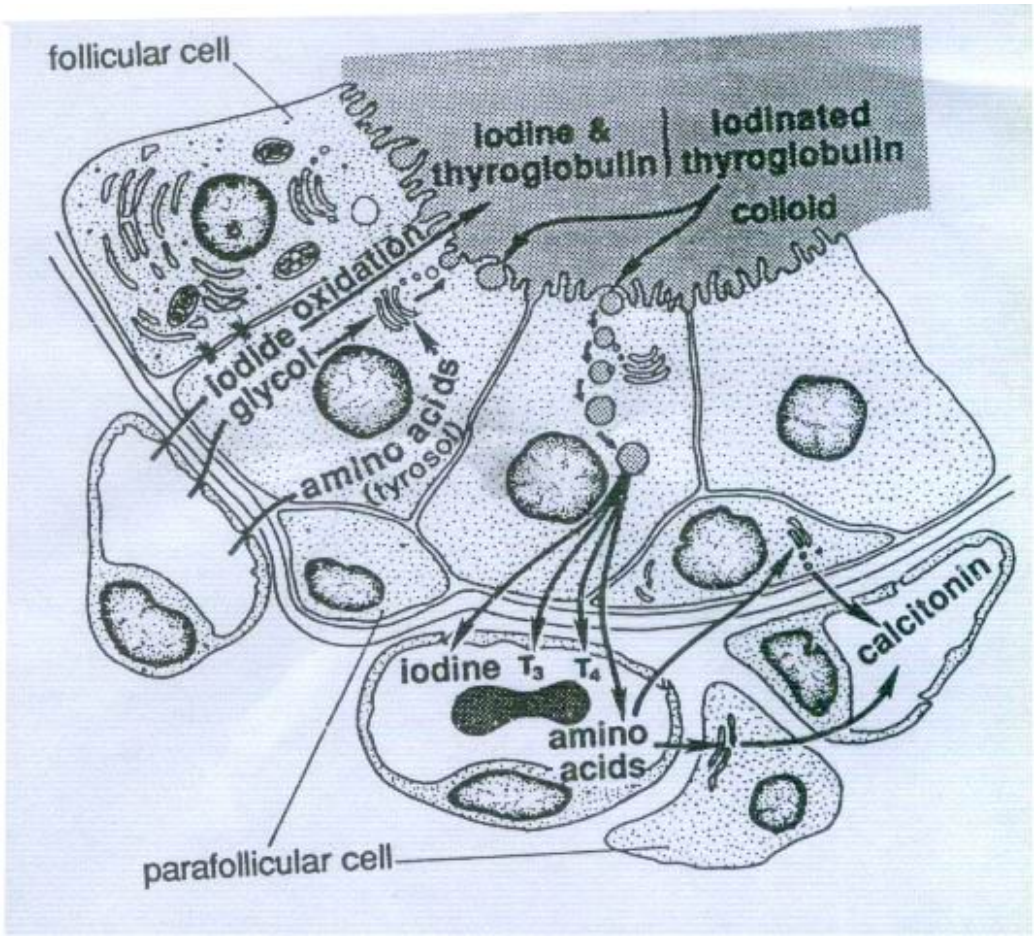



**Fig .3** The biosynthesis of Thyroid hormones (17)

The thyroid gland produces thyroid hormone in a mechanism by which it combines the element iodine with the amino acid tyrosine. In this process, one or two iodine molecules attach to a tyrosine molecule to form iodinated tyrosine (iodotyrosines). Compounds containing one iodine or two iodine molecules then combine with one another in a process known as coupling to form the primary thyroid hormones, T4 (tetraiodothyronine or thyroxine with 4 iodine molecules) and T3 (triiodothyronine with 3 iodine molecules).

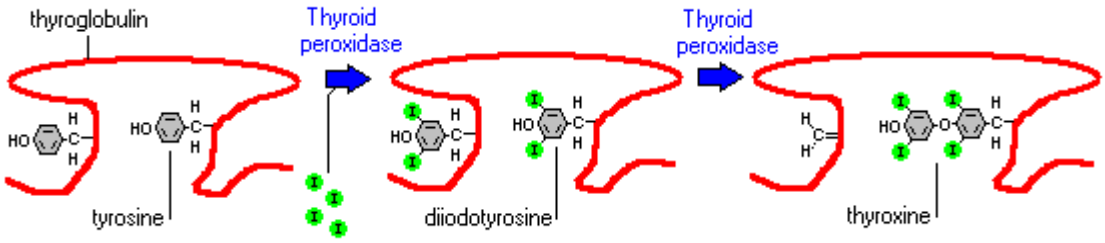



**Fig. 4** The synthesis of thyroxine (19)

The basic compounds with only one iodine molecule (T1) or two iodine molecules (T2) are also secreted into the blood circulation although they have never traditionally been considered as significant as T3 or T4. However, several recent studies indicate that T2 may be more important than originally thought. In fact, T2 is considered necessary for production of the deiodinase enzyme that helps convert T4 into T3 in the body. T1's physiological role is still being evaluated.

Normally, the thyroid gland secretes an abundance of T4 along with smaller amounts of T3 and even smaller amounts of tyrosine, T1 and T2. When T4 reacts with the cells that comprise the body's organs, including the pituitary, liver, brain and skeletal muscle, it loses an iodine atom to become T3. The majority of T3 found in the blood circulation is formed by this peripheral (away from the thyroid gland) conversion of T4 to T3. T3 is approximately 10 times more potent than T4, although both T4 and T3 serve vital functions. And consequently most of the T3 found in your blood is produced from peripheral conversion of T4 into T3 (17, 18, 19,20).

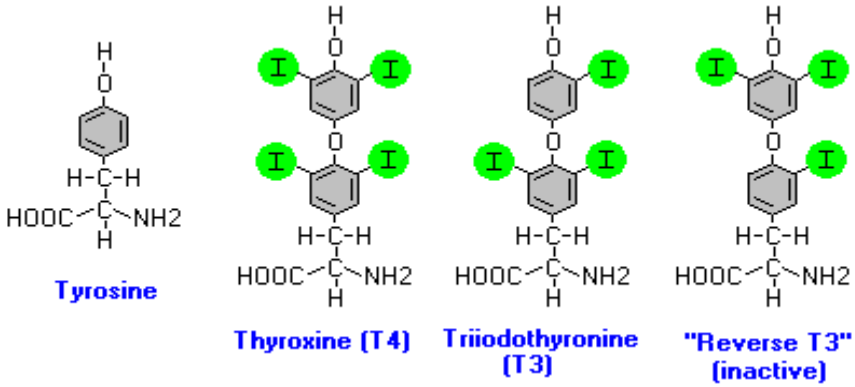



**Fig.5** The structure of thyroid hormones (19)

**Thyroid Function during Pregnancy**

Normal pregnancy entails are critical substantial changes in thyroid function. Major alterations in the thyroid system during pregnancy include: mild thyroid gland enlargement, including a two- to threefold increase in thyroxine-binding globulin (TGB) concentration under the influence of elevated of estrogen concentration is seen within the first two weeks of pregnancy and persists until delivery increased levels of TBG lead to lowered free T4 concentrations, a 30-100% increase in total triiodothyronine(T3) and thyroxine concentrations, increase serum thyroglobulin, increase renal iodide clearance, increased of FT4, reduction in TSH during the first trimester and TSH returns to normal after the first trimester. Furthermore, chorionic gonadotropin (hCG) has mild thyroid stimulating activity. Pregnancy produces an overall increase in thyroid activity, which allows the healthy individual to remain in a net euthyroid state (21, 22, 23).

**Palpation of thyroid gland size**

The examination of pregnant women, the examiner sits facing the subject, places two thumbs on other side of the subject's trachea several centimeter below the notch of thyroid cartilage and rolls thumbs gently over the thyroid, which lies next to the windpipe. The first decision should be whether or not the subject has a goiter. If each lobe of the thyroid is smaller than the part of the subject's thumb beyond the last joint (the terminal pharynx), the thyroid is classified as Grade 0, no goiter. If each lobe is not visible but palpable larger than the terminal pharynx of the subject's thumb, the goiter is classified as Grade 1. If it can be seen with the subject looking straight ahead, it is called Grade 2 (24).

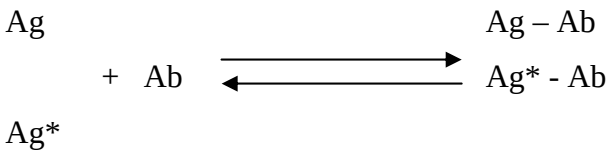
**Table 1.** Classification of goiter from WHO/UNICEF/ICCIDD Consultation on Indicators for Assessing Iodine Deficiency Disorders and control Programs: Geneva 1992 (25).

| | |
|---|---|
| Grade 0 | No palpable or visible goiter. |
| Grade 1 | A mass in a neck that is consistent with an enlarge thyroid. That is palpable but not visible when the neck is in the normal position. It moves upward in the neck as the subject swallows. Nodular alteration(s) can occur even when the thyroid is not enlarged. |
| Grade 2 | A swelling in the neck that is visible when the neck is in a normal position and is consistent with an enlarged thyroid. When the neck is palpated. |

**Principle of Radioimmunoassay**

Radioimmunoassay (RIA) is the method by which a radionuclide label (tracer) is used to the concentration of biologic molecules (hormones, steroids, vitamins, drug, etc.). This technique was first developed in 1960 by Berson and Yalow, and leading to improvements in the diagnosis of endocrinopathies and in our understanding of hormonal secretion. RIA is based upon the competition between labeled and unlabelled antigens for specific antibody sites, forming antigen antibody complexes. It involves use of a minus scale amount of labeled antigen in solution with a high affinity antibody to that antigen. Unknown sample, potentially containing unlabelled antigen may be added to this system and will compete with labeled antigen for binding site on the antibody (25, 26, 27, 28, 29, 30, 31).

This reaction by the expression:




Where: Ag is unknown antigen in patient sample (Or in solution of standards).

Ag* is radiolabeled antigen.

Ab is antibody.

Ag-Ab is unlabeled antigen – antibody complex.

Ag*-Ab is labeled antigen – antibody complex.

At equilibrium, after incubation for an optimal time, the complex form (bound) is separated from the free form. For the determination of antigen in an unknown sample, the degree of competitive inhibition observed is compared with that obtained in known standard solution under the same assay conditions (32, 33, 34).

**Separation Method**

At the end of the incubation period, the reaction mixture contains both free and bound antigen. A separation step of the antigen-antibody complex (bound fraction: B) and free antigen (free fraction: F) is required. Many types of separation techniques have been studied. At present a large number of separation techniques are being uses which are different in principle, and the choice is influenced by such features as easy, speed, completeness of separation, minimal influence by non specific factors (which can vary from one specimen to the other) and capacity to handle large numbers of test serum (35).

**Ideal separation techniques**: Any separation procedure need to meet several criteria to contribute the accurate results. Major requirements for successful separation are as follow:

1. Separation of bound and free components must be essentially complete. The approach selected should not affect the equilibrium between bound and free antigen during the separation process.

2. The technique should be easily and quickly performed. All equipment and reagents need to be inexpensive and simple to use.

3. The technique must not be affected by serum or plasma.

**Separation techniques**

1. Electrophoresis
2. Dialysis and Filtration
3. Gel filtration (permeation) chromatography
4. Centrifugation
5. Solid-phase adsorption
6. Chemical precipitation
7. Double-antibody (Immunoprecipitation) and related technique
8. Solid phase
   8.1 Magnetic particle
   8.2 Double-antibody solid phase
   8.3 Antibody-coated disc and tubes

Antibody-coated disc and tubes: Antibody is previously adsorbed on to a solid surface or inside of tubes. Solid phase that have been used include polypropylene or polystyrene tube walls, glass beads both large and small, cross linked dextran (sephadex) particles, agarose, paraamino cellulose, polyacrylamide gel, or polymeric antibody and magnetic particles. In 1967 Catt and Tregear introduced solid phase RIA based on antibodies adsorbed on to plastic surfaces or the inside of plastic tubes. The coated plastic takes a variety of forms including plastic disc, tubes, balls, rods and microtitre well. The assay is performed simply by adding labeled and unlabeled antigen to the antibody-coated surface, incubating, decanting the supernatant or free fraction leaving the bound fraction on the plastic surface. The advantages are simple, rapid, irreversible, non-centrifugation and minimal NSB. However, the disadvantages are the non-uniformity of the coating of the tube walls which may lead to lot-to-lot variation in the result, large amounts of antibody used, the complex preparation of tubes and the slower reaction rates. (36, 37, 38, 39, 40).

**Serum TSH Assays**

First generation TSH assays have detection limits of about 5 to 10 mU/L. Since the normal range for TSH is about 0.5 to 5.0 mU/L, these assays often miss mild hypothyroidism (where the TSH is usually just above 5) and are totally inadequate for assessment of hyperthyroidism (where the TSH is usually below 0.5). As a result, most laboratories have stopped using the first generation TSH assay.

Second generation TSH assays have a lower detection limit of about 0.1 mU/L. These assays distinguish normal from hypothyroid patients with a high degree of accuracy. Since the detection limit is just below the normal range for TSH of about 0.5 to 5.0 mU/L, these assays can also be used as screening tests to distinguish hyperthyroidism from normal thyroid function. Second generation assays are currently in wide use.

Third generation TSH assays have become available with detection limits of about 0.01 mU/L. Because of the considerably lower detection limit, these assays can reliably distinguish between normal and hyperthyroid patients. Because the distinction between normal and hyperthyroid patients is usually not a problem, these assays have limited value and are not widely utilized.

A few exceptions need to be kept in mind when interpreting TSH values. In situations where patient's thyroid function is changing rapidly, the TSH may lag behind. Patients who are recovering from hyperthyroidism may continue to have suppressed TSH for a variable amount of time after recovery. There are rare patients who have TSH secreting pituitary tumors and will have a minimally elevated TSH in the face of hyperthyroidism.

**Principle of the FT4**

The FT4 procedure is a solid –phase radioimmunoassay, wherein $^{125}$I-labeled T4 analog competes for a fixed time with FT4 in the sample for sites on T4-specific antibody. Because the antibody is immobilized to the wall of a polypropylene tube, simply decanting the supernatant suffices to terminate the competition and to isolate the antibody-bound fraction of the radiolabeled free T4. Counting the tube in a gamma counter then yields a number, which converse by way of a calibration curve to a measure of the free T4 present in the sample (41).

**Principle of the FT3**

The FT3 procedure is a solid –phase radioimmunoassay, wherein $^{125}$I-labeled T3 analog competes for a fixed time with FT3 in the sample for sites on T3-specific antibody. Because the antibody is immobilized to the wall of a polypropylene tube, simply decanting the supernatant suffices to terminate the competition and to isolate the antibody-bound fraction of the radiolabeled free T3. Counting the tube in a gamma counter then yields a number, which converse by way of a calibration curve to a measure of the free T3 present in the sample (42).

**Urinary iodine excretion**

Determining the state of iodine nutrition has been the measurement of the excretion of iodine in the urine. Approximately 90% of ingested iodine are excreted in the urine. While it is not generally possible to obtain twenty-four hour samples of urine. Keying iodine excretion to creatinine excretion with nutritional status so that ratio is no longer used. The advantage of assessment by this method are that the method entirely objectives, it is non-invasive, and it provides information on the one factor that can be addressed directly. The problem with iodine excretion is that it requires a laboratory geared to providing accurate determinations, it incurs some expense, and skilled technician specially trained in this technique. The details of the laboratory are the change in color of chemical called ceric ammonium sulfate by another chemical, arsenous acid, is accelerated by iodine, which acts as a catalyst. The rate of color change depends upon the amount of iodine present; it can be measured in an instrument called a colorimeter, and the amount of iodine in the urine can be calculated by comparison with standard solutions of known iodine content.

First step urine sample is digested in ammonium sulfate at high temperature (110°C) to remove other substance which are interfere chemical reaction. Following that step, iodine is measured by catalytic action on the reduction of ceric ion ($As^{4+}$) to the cerous ion ($Ce^{3+}$) and the oxidation of arsenite ($As^{3+}$) to arsenic ($As^{5+}$). The oxidation-reduction is called “Dandell-Kolthoff”.

$$2Ce^{4+}(\text{ceric}) + 2I^{-} \longrightarrow 2Ce^{3+}(\text{cerous}) + I_2$$

$$I_2 + As^{3+}(\text{arsenite}) \longrightarrow As^{5+}(\text{arsenic}) + 2I^{-}$$

The ceric ion ($As^{4+}$) is yellow but the cerous ion ($Ce^{3+}$) is colorless. Thus, the reaction can be followed by disappearance of yellow color as the ceric is reduced. The speed of this color disappearance is directly proportional to the amount of iodide catalyzing it, while other reactants are stable. Thus optical density of the color changing will be measured at wave length of 420 nm by spectrophotometer (43, 44).

**Research review**

In 2001, Glinoer D. presented women during pregnancy the hormonal changes and metabolic result in profound alterations in the biochemical parameters of thyroid function. The main events occurring pregnancy are a marked increase in serum thyroxine-binding globulin levels; in iodine – sufficient areas a marginal decrease in free hormone concentrations that is significantly amplified when there is iodine restriction or overt iodine deficiency; a frequent trend toward a slight rise in basal thyrotropin (TSH) values between the first trimester and term: a transient stimulation of the maternal thyroid gland by elevated levels of human chorionic gonadotropin (hCG) resulting in a rise in free hormone and decrement in serum TSH concentrations during the first trimester; and finally, modifications of the peripheral metabolism of the maternal thyroid hormones (45).

In 2000, Eltom A. et al. reported concentration of the median TSH in the third trimester of pregnancy was 1.0 ( 0.8 – 1.9 ) mU/l and this had increased to 2.2 (1.7-2.7) m U/l three months after delivery. In the third trimester of pregnancy, the median T3 concentration was 2.6 nmol/l and this had decrease to2.2 nmol/l in three months after delivery. The median FT4 concentration increase from 9.7 pmol/l three months after delivery to 10.4 pmol/l nine months after delivery. Both these level were significantly higher than the median value in the third trimester of pregnancy ($P< 0.001$, $P<0.001$, respectively) (46).

In 1999, Pop VJ. et al. presented normal pregnant women in the first trimester there is an increase of FT3 and FT4 with a moderate suppression of TSH, both related to increase in hCG levels. Total t3 and T4 levels are increase throughout pregnancy, due to an increase in serum thyroxine binding globulin. The increase in FT4 and FT3 during early pregnancy seems to be highly relevant for the fetus which produces no thyroxine during this stage of gestation (47).

In 1998, Elnagar B. et al. reported the study suggests that determination of urinary iodine alone gives in adequate information about the capacity of an individual to utilize an available iodine supply and it also shows the existence of different patterns of thyroid response during pregnancy. The history of iodine availability prior to and during pregnancy seems to be an important determinant of the mechanism of thyroid gland response to ensure the extra iodine needed by the growing fetus. The twenty- four hour urinary output at the different times during gestation were higher among the Swedish women, with mean value (95% confidence interval) of 1.40 (1.19-1.61), 1.33 (1.14-1.51), 1.45 (1.06-1.84) and 1.14(0.88-1.39) μmol/d. No significant changes in daily urinary iodine loss were observed in the two groups with progression of pregnancy. In the Swedish women the mean free T4 concentration fell from 11.81 pmol/l at the beginning of pregnancy to 8.82 pmol/l and the mean TSH rose from 1.11-1.95 mU/l between the beginning and end of pregnancy. Such changes were not detected among the Sudanese women, who had significantly lower mean TSH values than the Swedish women in weeks 36-39 of pregnancy ($P<0.02$), and significantly higher FT4 values than the Swedish women both in weeks 20- 24 and in weeks 36-39 ($P< 0.005$ and $P <0.001$) respectively (48).

In 2000, Kung AWC. et al. reported there was a progressive significant reduction in the serum free T4, free T3, free thyroxine index levels as pregnancy advanced. The percentage of women with FT4, FT3 and FTI results below the normal range for non pregnant adults increased significantly from 14.3%, 15.6% and 0.3% at first trimester to 5302%, 61.1% and 4.8% near term, respectively (P all< 0.01). The molar FT3/FT4 ratio increased significantly, suggestion preferential T3 secretion as pregnancy progressed. In the first trimester, 13.9% of subjects suppressed TSH of <0.03 mIU/l, probably due to stimulation by chorionic gonadotropin. A TSH level increased progressively and was almost doubled near term ($P <0.05$). TSH remained with in the normal range except for one subject who had raised TSH level. This patient was negative for anti thyroglobulin and antiperoxidase antibody. In the first trimester found median urine iodine concentrations were higher than non pregnant controls (0.84 vs. 0.79 mmol/l, $P<0.05$). The concentration increased further as pregnancy

advanced. The percentage of women having urine iodine concentration <0.4 mmol/l decreased from 11.3% at first trimester to 4.7% near term (49).

In 1999, Elton A. et al. reported the urinary iodine concentration (UIC) in pregnant goitrous women (mean ± SD) was 0.53 ± 0.39 μmol/dL in weeks 10-13, 0.34 ± 0.16 μmol/dL in weeks 30-24, and 0.32 ± 0.20 μmol/dL in weeks 32-39 and was significantly lower than that during weeks 10-13 (P<0.05). In weeks 10-13 the mean (UIC) did not differ significantly from that in the control group, but in weeks 20-24 and weeks 32-39 it was significantly lower than the value in the control group (P< 0.003, p<0 .001). The proportions of these women with UIC BELOW 0.16 μmol/dL with the progression of pregnancy. None of the subjects showed a UIC value above 0.79 μmol/dL. The mean TSH( ± SD) levels in the goitrous group in weeks 10-13, weeks 20-24 and weeks 32-39 were 1.42 ± 1.60 μmol/dL, 1.28 ± 0.91 mU/L and 1.33 ± 0.71 mU/L. This level does not show significant changes with the progression of pregnancy, they were different from the mean for the non-pregnant control. TSH values in all the pregnant women were all within the reference range. The mean T3 levels during weeks 10-13, 20-24 and 32-39 of pregnancy in the goitrous pregnant women were 2.5 ± 0.8 nmol/L, 2.8 ± 0.8 nmol/L, 2.6 ± 0.4 nmol/L. There is no statistically significant difference between the pregnant and non-pregnant groups in this respect. Regarding, there were no significant differences seen between the two study groups during weeks 10-13 of pregnancy. However, in weeks 20-24 and weeks 32-39, the goitrous pregnant group had significantly lower mean FT4 levels than the control group (P<0.03 and P<0.05). The proportions of the pregnant women with F below the references range were 31, 50 and 40% in weeks 10-30, 20-24 and 32-39 of pregnancy. There was no correlation between UIC and F in weeks 10-13, 20-24 and 32-39 of pregnancy (P=0.9, r=-0.1), (p=0.2, r=0.4), (p=0.8, r=0.1). There was no correlation between UIC and TSH in weeks 10-13, 20-24 and 32-39 of pregnancy (P =0.2, r = 0.2), (p =0.2, r =0.4), (P= 0.1, r =0.3) (50).

# CHAPTER 4
# MATERIALS AND METHODS

## MATERIALS

### Instruments

| No | Name | Supplier |
|---|---|---|
| 1. | Automatic gamma counter | Wallac, 1470-010 Wizard |
| 2. | Refrigerated centrifuge machine | Beckman J6-MI, USA |
| 3. | Spectrophotometer | Milton Roy 1001 plus |
| 4. | Heating Block | Fisher Scientific |
| 5. | Fume Hood for perchloric acid | Labconco Corporation, USA |
| 6. | Milli - Q plus water system | ZD 52 11584, USA |
| 7. | Deminalizer | Ablauf Corporation |
| 8. | pH meter | Orion Research microprocessor Ionanalyzer /901, USA |
| 9. | Vortex mixer | Vertex-2 Genie, Scientific Industries, USA |
| 10. | Analytical balance | Metler H 31 AR & P1210 |
| 11. | Hot plate with stirrer | Monotherm, Germany |
| 12. | Laboratory thermometer | |
| 13. | Repeated automatic pipettes; | Eppendorf, Germany |
| 14. | 14. Polystyrene tubes: size 12 x 75 mm | Plastic Inc. Pennsylvania |
| 15. | Glass tubes : 13 x 100 mm | |
| 16. | Test tube rack | |
| 17. | Magnetic separator | Serono (Switzerland) |
| 18. | Rotator | LEEC Limited, UK |
| 19. | Glass bottle, volumetric flash, beaker | Pyrex |

**Chemicals**

| No | Name | Supplier |
|---|---|---|
| 1. | TSH-kit | National Institute of Health (NIH) Ministry of Public Health Bangkok, Thailand. |
| 2. | FT4-kit | Diagnostic Product Coporation (DPC), USA |
| 3. | FT3- kit | Diagnostic Product Coporation (DPC), USA |
| 4. | Control sample | Siriraj's Nuclear Chemistry Laboratory |
| 5. | Distilled water | Thai Otsuka pharmaceutical Co., Ltd. |
| 6. | Arsenic Trioxide ($As_2O_3$) | Merck 119, Germany |
| 7. | Sulfuric Acid ($H_2SO_4$) | Merck 731, Germany |
| 8. | Ceric ammonium sulfate (Ce $(NH_4)_4(SO_4)_4.2HO_2$) | Merck 2273, Germany |
| 9. | Potassium iodate ($KIO_3$) | Merck 505, Germany |
| 10. | Nitric acid ($NHO_3$) 65% | Merck 456, Germany |
| 11. | Deionized water | |
| 12. | Sodium chloride (NaCl) | Merck 731, Germany |
| 13. | Ammonium persulfate $(NH_4)_2S_2O_8$ | Merck 64217, Germany |

**Reagents**

**TSH**

1. Assay buffer: 0.05 M Phosphate buffer with 0.1% Sodium azide, 0.05% Tween-20 and 1% BSA
2. Labeled monoclonal anti-TSH or $^{125}$I-mAb
3. Anti-TSH – Solid phase
4. Washing buffer: 0.05 M Phosphate buffer pH 7.4 with 0.1% Sodium azide, 0.05% Tween-20
5. Standard serum of 0, 0.2, 1.5, 3.0, 10, 30, 60 mIU/L for TSH
6. Control serum of low (1.0 mIU/L), medium (20.0 mIU/L) and high (33.0 mIU/L)

**FT4 radioimmunoassay by DPC solid-phase RIA**

1. FT4 Ab-coated tubes

Polypropylene tubes coated with antibodies to thyroxine and packaged in zip-lock plastic bags. Store refrigerated and protected from moisture, carefully resealing the bags after opening. Stable at 2-8° C until the expiration date marked on the bag. Color: pale yellow.

2. $^{125}$I FT4

Lyophilized tracer, consisting of an iodinated thyroxine analog. Reconstitute each vial by adding measured 110 ml distilled water. Let stand for 10 minutes. Then mix by gentle inversion. Stable at 2-8°C for 30 days after reconstitution, or until the expiration date marked on the vial.

3. FT4 calibrators

Seven vials of lyophilized processed human serum, labeled A though G. At least 30 minutes before use, reconstitute the zero calibrator A with 2.0 ml distilled water, and each of the remaining calibrators B through G with 1.0 ml distilled water. Use volumetric pipettes and mix by gentle swirling. Store at 2-8 °C for 30 days after reconstitution. The reconstituted calibrators have lot-specific FT4 values of

approximately 0, 0.1, 0.5, 1.3, 2.2, 4.8 and 10 ng/dL of thyroxine; equivalently approximately: 0, 1.3, 6.4, 16.7, 28.3, 61.8, 28.3, 61.8, and 128.7 pmol/L. Refer to the labels for exact values in ng/dL.

**FT3 radioimmunoassay by DPC solid-phase RIA**

1. FT3 Ab-coated tubes

Polypropylene tubes coated with antibodies to triiodothyronine and packaged in zip-lock plastic bags. Store refrigerated and protected from moisture, carefully resealing the bags after opening. Stable at 2-8° C until the expiration date marked on the bag. Color: aquamarine.

2. $^{125}$I FT3

Lyophilized tracer, consisting of an iodinated T3 analog. Reconstitute each vial by adding a measured 110 ml distilled water. Let stand for 10 minutes. Then mix by gentle inversion. Stable at 2-8°C for 30 days after reconstitution, or until the expiration date marked on the vial.

4. FT3 calibrators

Seven vials of lyophilized processed human serum, labeled A though G. At least 30 minutes before use, reconstitute the zero calibrator A with 2.0 ml distilled water, and each of the remaining calibrators B through G with 1.0 ml distilled water. Use volumetric pipettes and mix by gentle swirling. Store at 2-8 °C for 30 days after reconstitution. The reconstituted calibrators have lot-specific FT3 values of approximately 0, 0.5, 1.6, 3.5, 7.0, 21 and 42 pg/mL of FT3; equivalently approximately: 0, 0.77, 2.5, 5.4, 11, 32 and 65 pmol/L. To convert to pmol/L, multiply by 1.

**Urinary iodine**

1. Ammonium persulfate solution:

1.0 M $(NH_4)_2S_2O_8$ : 228.2 gm of $(NH_4)_2SO_8$ in 1 L of deionized water.

2. Arsenous acid solution: $AS_2O_3$ with 2.5% NaCl in 400 ml of 5 $NH_2SO_4$ and adjusted volume to 2 L with deionized water.

3. Ceric ammonium sulfate solution (CAS): 2.4% ammonium sulfate in 1L of 3.5 $NH_2SO_4$.

4. Potassium iodate standard ($KIO_3$):

Stock A: 0.168 gm of $KIO_3$ in deionized water and make to 1L. This solution is equivalent to 100 mg/ml. Store in 4°C and stable for months.

Stock B: 0.5 ml of stock A in deionized water and make to 1L. This solution is equivalent to 0.5 μg/ml. Store in 4°C and stable for months.

Working standard iodine: 0, 10, 20, 40, 60 and 80 μl of stock B were diluted to 250 μl with deionized water. The final concentrations of iodine were 0, 20, 40, 60 and 80,120 and 160 μg/L respectively.

5. Control urine of low (26.15-28.09 μg/L), medium (53.18-55.12 μg/L) and high (104.3-108.3 μg/L).

# METHODS

## Specimen collection

### Serum

350 normal pregnant women referred to prenatal clinic of Jakkarat hospital were each examined by each examiners and their thyroid gland size will be determined according to WHO goiter classification as Table 1. Patient's name, gestation age, and the number of previous pregnancies were recorded. Subjects with a previous history of thyroid disease or those taking thyroid related medications and high risk diseases such as HIV, HBV, and Syphilis etc were excluded. Routine blood sample was collected for serum TSH determination using in-house immunoradiometric assay (IRMA). Serum FT4 and FT3 were also measured by using DPC solid-phase RIA. The pregnant women need not be fasting, and no special preparations are necessary. Collect blood by venipuncture into plain tubes, and separate the serum from the cells by centrifugation. Heparin has been reported to have in vivo and in vitro effects on free thyroid hormones. Lipemic, icteric or grossly contaminated samples may give erroneous results. The use of an ultracentrifuge is recommended to clear lipemic samples. Store at 2-8°C for 2 days, or for up to 2 months frozen at -20°C. Before assay, allow the samples to come to room temperature (15-28°C) and mix by gentle swirling or inversion. Aliquot, if necessary, to avoid repeated thawing and freezing. Do not attempt to thaw frozen specimens by heating them in a waterbath.

### Urine

Urine iodine sample was collected into plastic cup and transferred approximately 5 ml of urine into a clean, dry plastic tube and capped tightly. Tube was labeled with a code number using a permanent marker. The urine samples were kept in refrigerator at -20°C. When sending to Nuclear medicine laboratory the samples were packed into sealable plastic bags and organized into foam boxes with pre-frozen refrigerant gel packs. Urinary iodine excretions were investigated by modified ammonium persulfate method.

**In-house immunoradiometric assay (IRMA) of TSH in serum using monoclonal anti-TSH.**

1. Prepare and label laboratory test tubes in duplicate tubes for total $^{125}$I-anti TSH count (TC); standard TSH of 0, 0.25, 0.5, 1.5, 3.0, 7.5, 3.0, 60 mU/L; control serum of low, median, high; and serum samples.

2. 100 µl of the standards and serum samples were pipetted into standard and sample tubes and then 300 µl of assay buffer was pipetted into each tube (except TC tubes).

3. 50 µl of $^{125}$I-anti TSH was pipetted into each tube and 50 µl of anti-TSH solid phase was added during mixing (except TC tubes).

4. The final mixture tubes were mixed tubes by vortex mixer and rotated on rotator at room temperature over night.

5. The solid phase was washed twice by adding 1 ml of wash buffer, mixed on vortex mixer, precipitated in refrigerated centrifuge, and decanted the supernatant.

6. The radioactivity of precipitate in each tube was counted for 150 seconds by automatic gamma counter.

7. A standard curve was constructed by RIA-Cal-program (plotting cpm versus the standard TSH concentration). Finally the corresponding TSH concentration (mIU/L) of each sample was read on the standard curve.

**FT4 DPC solid-phase radioimmunoassay procedure**

All components must be at room temperature (15-28°C) before use.

1. Plain tube: Label 4 plain (uncoated) 12x75 mm polypropylene tubes T (total counts) and NSB (non specific binding) in duplicate. Because nonspecific binding in the COAT-A-COUNT procedure is characteristically low, the NSB tubes may safely be omitted without compromising accuracy or quality control.

Coated tubes: Label 14 FT4 Ab-Coated Tubes A (maximum binding) and B through G in duplicate. Label additional antibody-coated tubes, also in duplicate, for control and patient samples.

2. Pipette 50 µl of the zero calibrator A into the NSB and A tubes, and 50 µl of each remaining calibrator, control and patient sample into the tubes prepared. Pipette directly to the bottom.

3. Add 1.0 mL of $^{125}$I FT4 to every tube. Vortex.

4. Incubate for 60 hours at 37°C. Use a waterbath; neither an oven nor a heat block is suitable.

5. Decant thoroughly. Removing all visible moisture will greatly enhance precision. Using a foam decanting rack, decant the contents of all tubes (except the T tube) and allow them to drain for 2 or 3 minutes. Then strike the tubes sharply on absorbent paper to shake off all residual droplets.

6. Count for 1 minute in a gamma counter

7. Calculate result by RIA-CAL Program.

**FT3 DPC solid-phase radioimmunoassay procedure**

All components must be at room temperature (15-28°C) before use.

1. Plain tube: Label 4 plain (uncoated) 12x75 mm polypropylene tubes T (total counts) and NSB (non specific binding) in duplicate. Because nonspecific binding in the COAT-A-COUNT procedure is characteristically low, the NSB tubes may safely be omitted without compromising accuracy or quality control.

Coated tubes: Label 14 FT3 Ab-Coated Tubes A (maximum binding) and B through G in duplicate. Label additional antibody-coated tubes, also in duplicate, for control and patient samples.

2. Pipette 100 µl of the zero calibrator A into the NSB and A tubes, and 100 µl of each remaining calibrator, control and patient sample into the tubes prepared. Pipette directly to the bottom.

3. Add 1.0 mL of $^{125}$I FT3 to every tube. Vortex.

4. Incubate for 3 hours at 37°C. Use a waterbath; neither an oven nor a heat block is suitable.

5. Decant thoroughly. Removing all visible moisture will greatly enhance precision. Using a foam decanting rack, decant the contents of all tubes (except the T tube) and allow them to drain for 2 or 3 minutes. Then strike the tubes sharply on absorbent paper to shake off all residual droplets.

6. Count for 1 minute in a gamma counter.

7. Calculate result by RIA-CAL Program.

**Urinary iodine excretion measurement by modified ammonium persulfate method**

The measurement of urine iodine was performed as follow:

Urine iodine was analyzed by modified ammonium persulfate method. It is necessary to mark all test tubes used at the 1 ml mark so all solution can be diluted to 1 ml. Urine samples were mixed to suspend sediment and pipetted 250 µl each sample into 13x100 mm test tube. The duplicate tube were prepared for standard by pipetting 0, 10, 20, 40, 60, and 80 µl of solution B into test tubes and adding 250, 240, 230, 210,190 and 170 µl deionized water to these tubes respectively to give a volume of 250 µl in each tube. This gives a standard curve ranging from 0, 20, 40, 80, 120 and 160 µl/L. 1 ml of 1.0 M ammonium persulfate was added and mixed gently. All tubes were heated for 25 minutes at 110°C in a fume hood with a perchloric acid trap and cooled tube to the room temperature and diluted to the 1 ml mark on the test tubes. 3.5 ml of arsenic acid solution was added, mixed and standard for about 10 minutes. 700 µl of ceric ammonium sulfate solution was added to each tube at 30 seconds time intervals and mix each tube after the addition. Exactly 30 minutes after the addition of ceric ammonium sulfate to the first tube, read its absorbance at 420 nm, and read successive tube at 30 second time intervals.

A standard curves was constructed by plotting log (absorbance at 420 nm) versus the standard iodine concentration. Finally, the samples were read off the corresponding iodine concentration (µl/L).

# CHAPTER 5
# RESULTS

The study population consisted of 350 pregnant women (median age: 25 year, range: 15-45 years) at Jakkarat Hospital, Nakornrajasima province, Thailand. No subject was taking thyroid medication or iodine supplements other than in their iodated salt (1:20,000) intake. The subjects consented to the collection of blood and urine samples. Serum TSH, FT4, FT3 and urinary iodine concentration were determined.

**Table 2.** The median values and interquatile ranges of thyroid stimulating hormone (TSH), free thyroxine (FT4), free triiodothyronine (FT3) and urinary iodine concentration in pregnant women at Jakkarat Hospital, Nakornrajasima province, Thailand.

| Variable | Trimester (Median) | | |
|---|---|---|---|
| | First (n = 190) | Second (n = 87) | Third (n = 73) |
| **Serum :** | | | |
| **TSH (0.26-4.0 mIU/L)** | 0.91(0.46 - 1.31) | 1.20 (0.88 - 1.84) | 2.02 (1.34 - 4.45) |
| **FT4 (0.8-2.1 ng/dl)** | 1.32(1.20 - 1.60) | 1.25(1.09 - 1.42) | 1.15(1.03 - 1.33) |
| **FT3 (1.4-4.7 pg/ml)** | 3.42(2.80 - 4.10) | 3.17(2.30 - 4.00) | 2.27(1.05 - 3.08) |
| **Urine :** | | | |
| **UI (90-230 μg/L)** | 297.11(119.80-358.00) | 396.62(128.16-481.96) | 375.44(181.58-616.86) |

Serum and urinary iodine findings are given in Table 2. During pregnancy, high median urinary iodine excretion was found in all of various trimesters while serum TSH, FT4 and FT3 were within normal range. The median and interquatile of TSH concentration in the first trimester of pregnant women was 0.91(0.46 - 1.31) mIU/L and this had increased to the second trimester of 1.20 (0.88 - 1.84) mIU/L. The level of serum TSH in the third trimester of 2.02 (1.34 - 4.45) mIU/L was also higher than the second trimester (Fig. 6).

In contrary, both median FT4 and FT3 concentration were decreased from the first to the second and the third trimester of pregnancy. The FT4 values was 1.32(1.20 - 1.60), 1.25(1.09 - 1.42) and 1.15(1.03 - 1.33) ng/dl while FT3 was 3.42(2.80 - 4.10), 3.17(2.30 - 4.00) and 2.27(1.05 - 3.08) pg/ml, respectively (Fig. 7 and 8).

Median urine iodine concentration throughout pregnancy is shown to be higher than reference range. The level of median UI in the first, second and third trimester were 297.11(119.80 – 358.00), 396.62(128.16 - 481.96) and 375.44(181.58 - 616.86) µg/L, respectively.

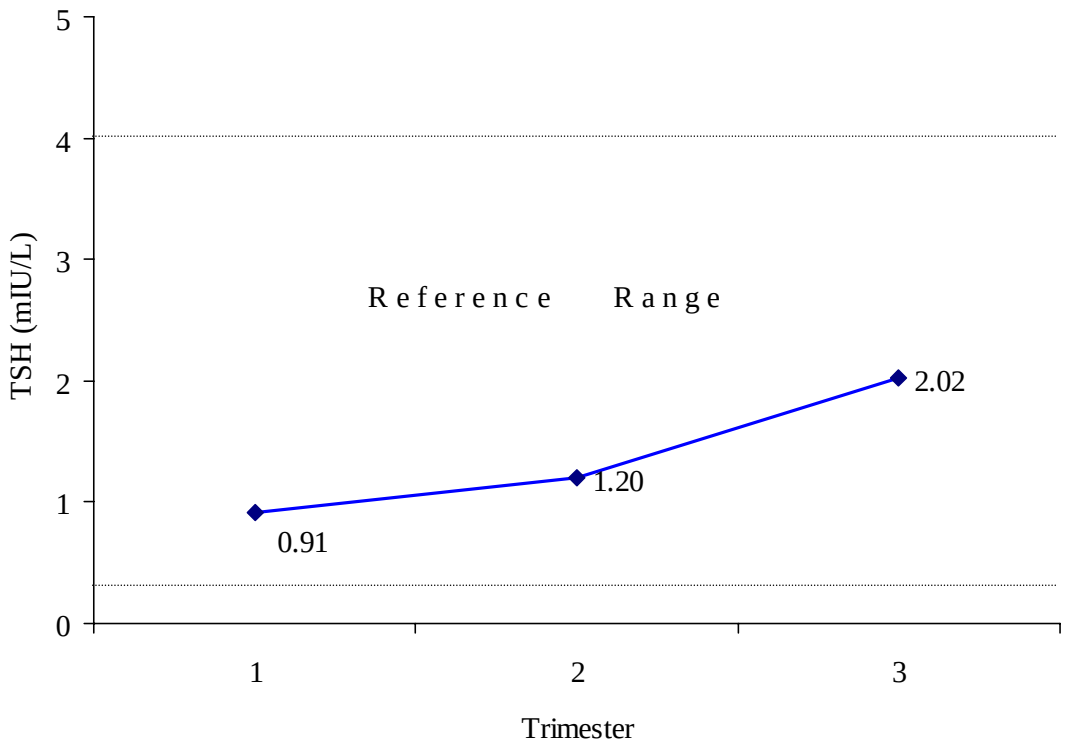



**Fig. 6** Median values of TSH (mIU/L) changes in various trimesters of pregnant women at Jakkarat Hospital.

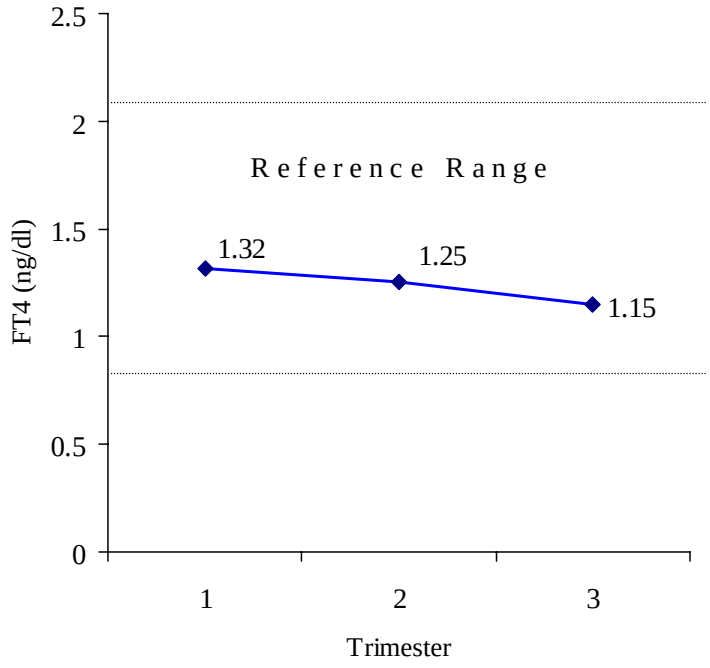



**Fig. 7** Median values of FT4 (ng/dl) changes in various trimesters of pregnant women at Jakkarat Hospital.

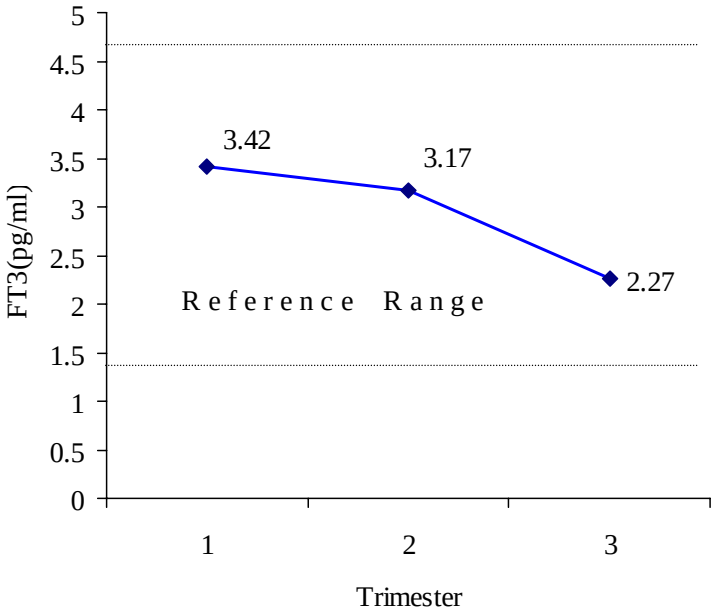



**Fig. 8** Median values of FT3 (pg/ml) changes in various trimesters of pregnant women at Jakkarat Hospital.

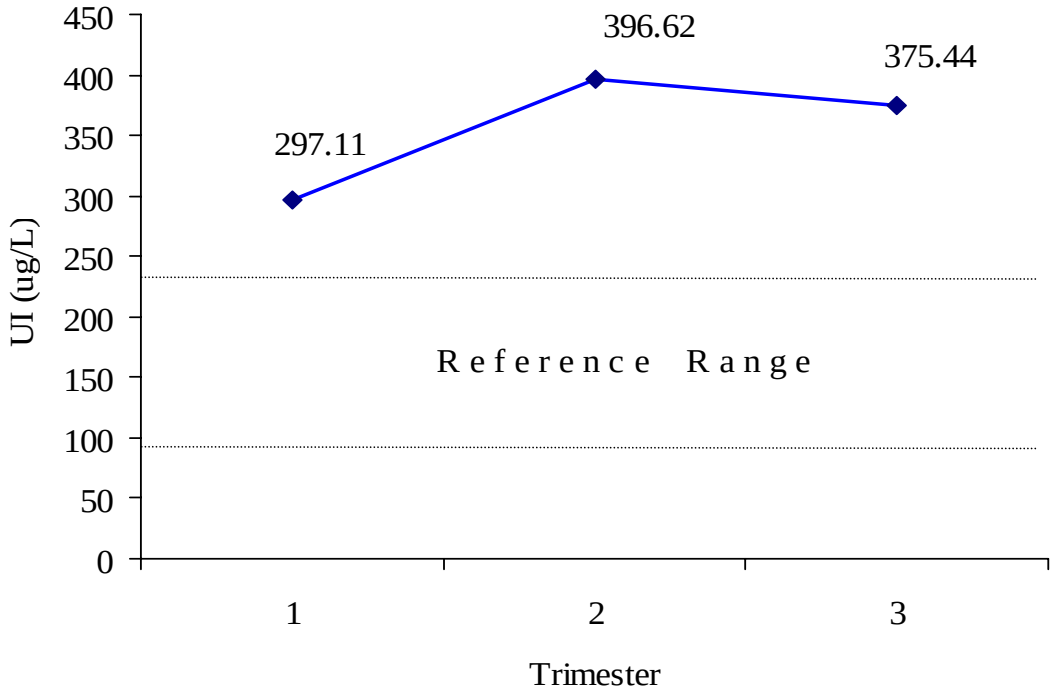



**Fig. 9** Median values of urinary iodine excretion (μg/L) changes in various trimesters of pregnant women at Jakkarat Hospital.

**Table 3.** The mean ± SD values of thyroid stimulating hormone (TSH), free thyroxine (FT4), free triiodothyronine (FT3) and urinary iodine concentration in pregnant women at Jakkarat Hospital.

| Variable | Trimester (Mean ± SD) | | |
|---|---|---|---|
| | **First (n = 190)** | **Second (n = 87)** | **Third (n = 73)** |
| **Serum:** | | | |
| **TSH (mIU/L)** | 1.04 ± 0.81 | 1.48 ± 0.87 | 2.93 ± 2.35 |
| **FT4 (ng/dl)** | 1.40 ± 0.30 | 1.30 ± 0.27 | 1.20 ± 1.26 |
| **FT3 (pg/ml)** | 3.38 ± 0.96 | 3.30 ± 1.26 | 2.21 ± 1.26 |
| **Urine:** | | | |
| **UI (μg/L)** | 287.40 ± 317.61 | 406.56 ± 511.85 | 540.11 ± 638.75 |

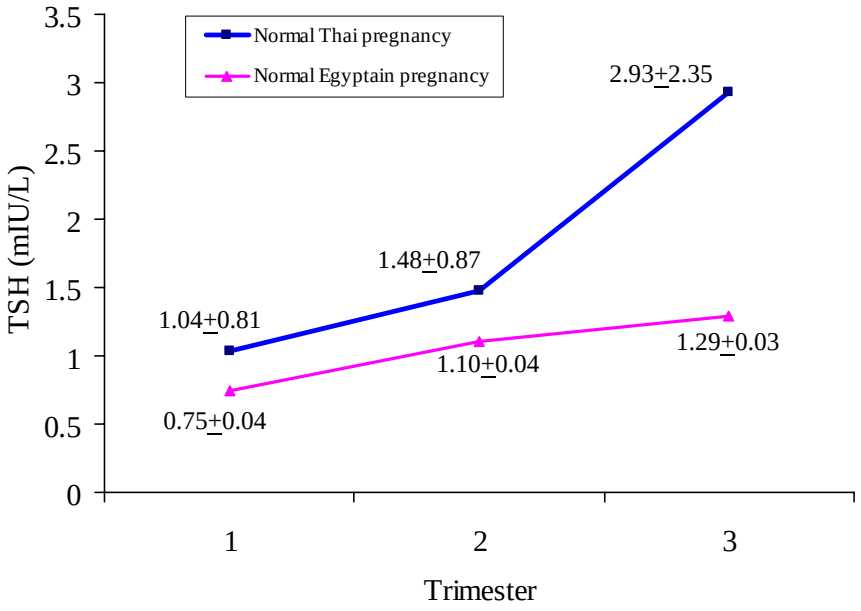



**Fig. 10** Mean ± SD values of TSH changes in various trimesters of Thai pregnant women compared with normal Egyptian pregnancy.

In Jakkarat Hospital, the mean ± SD of TSH values by immunoradiometric assay (IRMA) in the third trimester was the highest level (2.93 ± 2.35 mIU/L), while the second trimester was 1.48 ± 0.87 mIU/L which higher than the first trimester (1.04 ± 0.81mIU/L).

Comparison of the mean ± SD of TSH levels between Thai and Egyptian pregnant women is shown in Fig. 10 and Table 4. For all trimesters, the level of TSH in Thai pregnancy was higher than Egyptian pregnancy.

**Table 4.** Comparison of the mean ± SD of TSH levels between Thai and Egyptian pregnancy.

| | **Serum TSH (mean ± SD)** | |
|---|---|---|
| | **Thai** | **Egyptian** |
| **First trimester** | 1.04 ± 0.81 | 0.75 ± 0.04 |
| **Second trimester** | 1.48 ± 0.87 | 1.10 ± 0.04 |
| **Third trimester** | 2.93 ± 2.35 | 1.29 ± 0.03 |

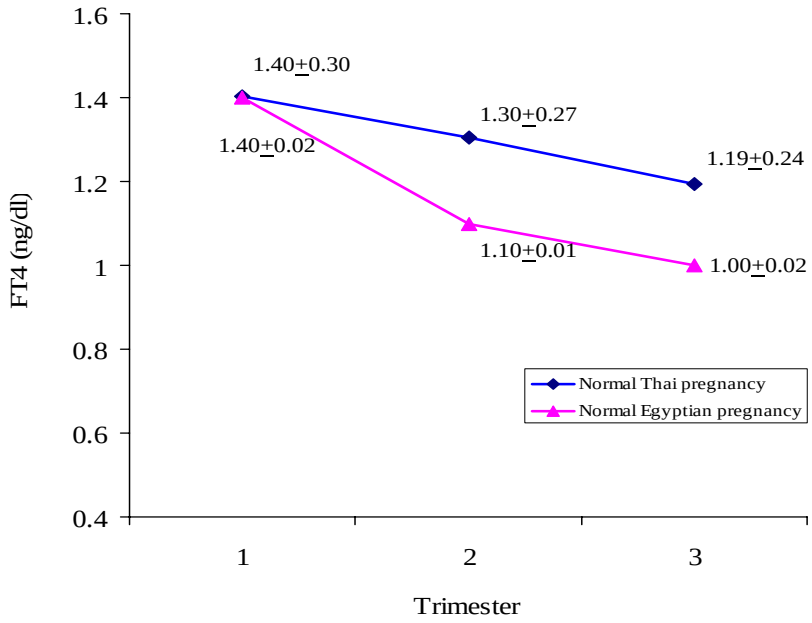



**Fig. 11** Mean ± SD values of FT4 changes in various trimesters of Thai pregnant women compared with normal Egyptian pregnancy.

In Jakkarat hospital, the mean ± SD of FT4 values by DPC solid-phase RIA method in the first trimester was the highest level (1.40 ± 0.30 ng/dl), in the second trimester was 1.30 ± 0.27 ng/dl which higher than the third trimester (1.19 ± 0.24 ng/dl).

Comparison of the mean ± SD of FT4 levels between Thai and Egyptian pregnant women is shown in Fig. 11 and Table 5. For all trimesters, the level of FT4 in Thai pregnancy was higher than Egyptian pregnancy.

**Table 5.** Comparison of the mean ± SD of FT4 levels between Thai and Egyptian pregnancy.

| | **Serum FT4 (mean ± SD)** | |
|---|---|---|
| | **Thai** | **Egyptian** |
| **First trimester** | 1.40 ± 0.30 | 1.40 ± 0.02 |
| **Second trimester** | 1.30 ± 0.27 | 1.00 ± 0.01 |
| **Third trimester** | 1.19±0.24 | 1.00±0.02 |

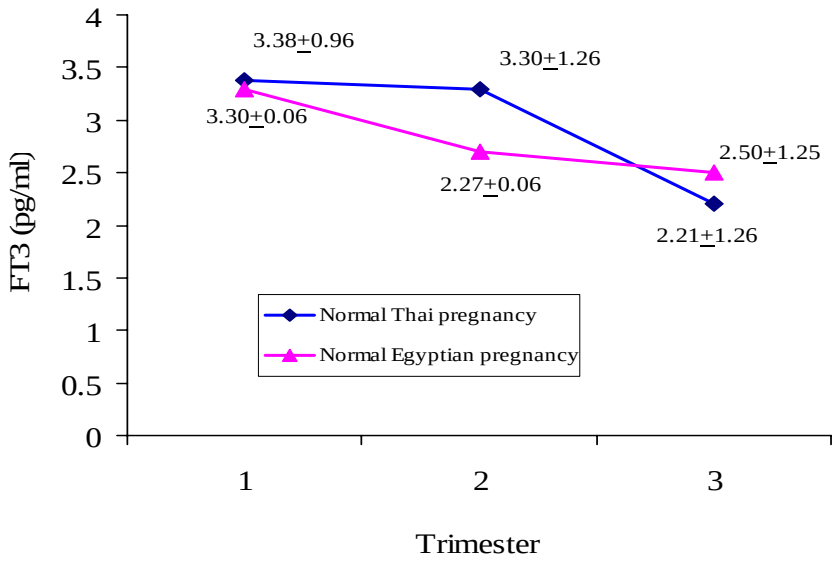



**Fig. 12** Mean ± SD values of FT3 changes in various trimesters of Thai pregnant women compared with normal Egyptian pregnancy.

In Jakkarat hospital, the mean ± SD of FT3 values by DPC solid-phase RIA method in the first trimester was the highest level (3.38 ± 0.96 pg/ml), in the second trimester was 3.30 ± 1.26 pg/ml which higher than the third trimester (2.21 ± 1.26 pg/ml).

Comparison of the mean ± SD of FT3 levels between Thai and Egyptian pregnant women is shown in Fig. 12 and Table 6. The level of FT3 in Thai pregnancy showed higher than Egyptian pregnancy in both first and second trimesters. The third trimester showed lower than Egyptian.

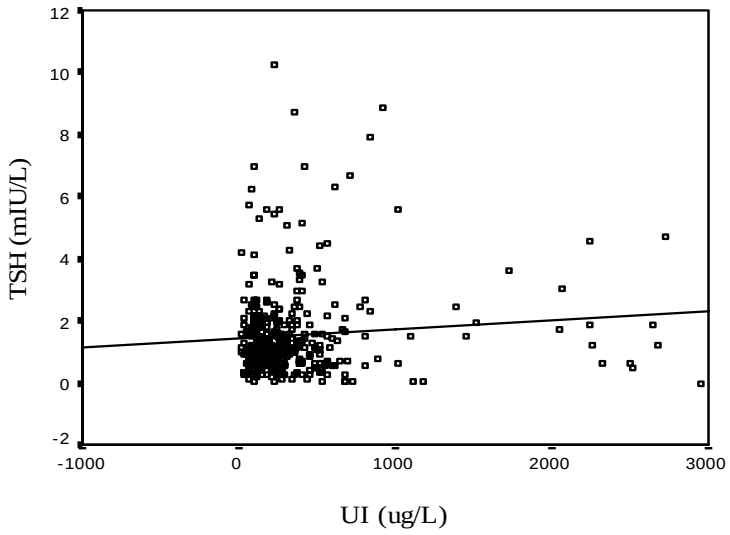
**Table 6.** Comparison of the mean ± SD of FT3 levels between Thai and Egyptian pregnancy.

| | **Serum FT3 (mean ± SD)** | |
|---|---|---|
| | **Thai** | **Egyptian** |
| **First trimester** | 3.38 ± 0.96 | 3.30 ± 0.06 |
| **Second trimester** | 3.30 ± 1.26 | 2.27 ± 0.06 |
| **Third trimester** | 2.21 ± 1.26 | 2.50 ± 1.25 |

**Table 7.** The relationship between urine iodine (UI) and serum TSH, FT4 and FT3 in 350 normal pregnant women at various trimester in Jakkarat Hospital.

| Parameters | UI | |
|---|---|---|
| | r | P |
| TSH | 0.045 | >0.05 |
| FT4 | -0.192 | < 0.05 |
| FT3 | -0.177 | <0.05 |

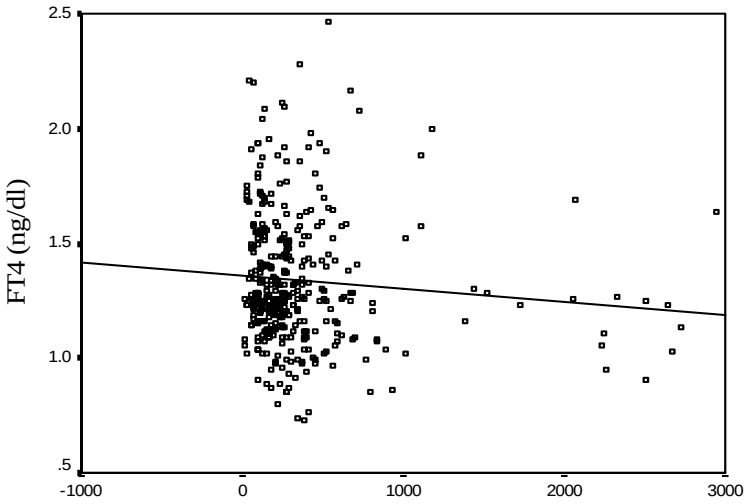
Table 7. showed the correlation coefficient (r) between parameters of thyroid function in the pregnant women. The highest correlation between FT4 and UI was -0.192, P< 0.05 and the regression line was Y = 1.356- (5.700E-05)X as shown in Fig. 14. The correlation between UI and TSH, FT3 were 0.045 (P>0.05) and -0.177 (P<0.05) while the regression line were Y = 0.000X + 1.429 and Y =0.000X + 3.259 (Fig.13 and 15).




Y = 0.000X + 1.429

r = 0.045, n = 350

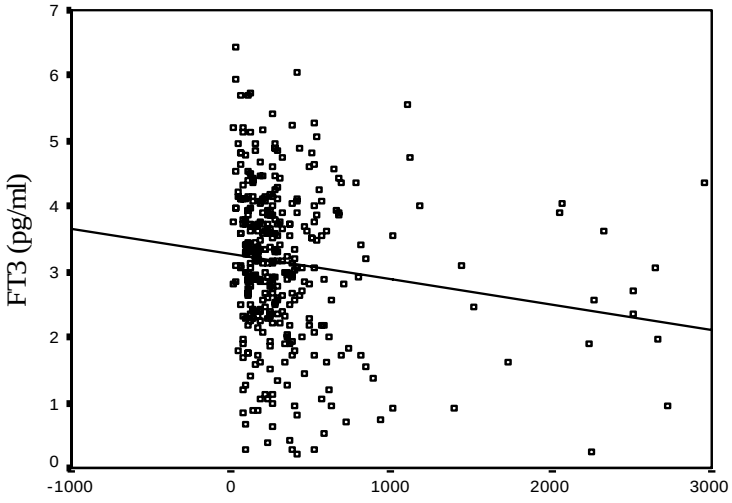
**Fig. 13** Correlation coefficient (r) between TSH and UI in various trimesters.




Y = 1.356- (5.700E-05)X

r = -0.192, n = 350

**Fig. 14** Correlation coefficient (r) between FT4 and UI in various trimesters.




Y =0.000X + 3.259

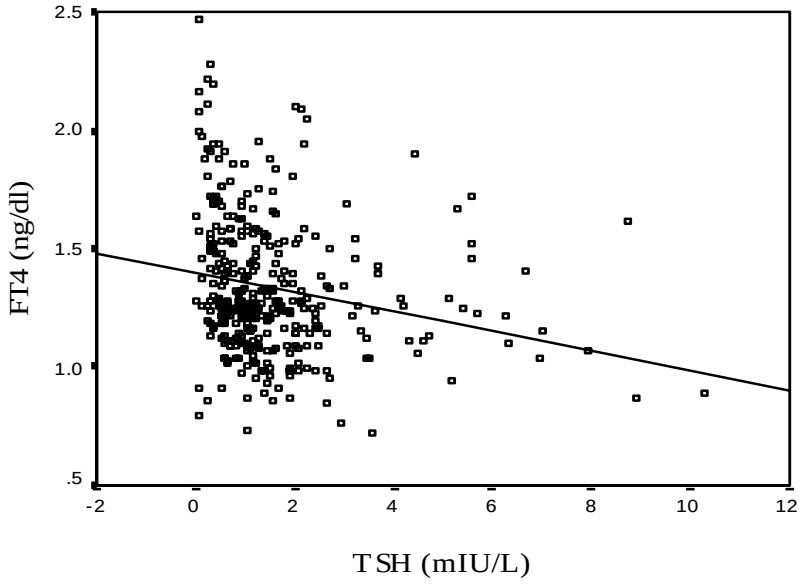
r = -0.177, n = 350

**Fig. 15** Correlation coefficient (r) between FT3 and UI in various trimesters.

**Table 8.** The relationship between TSH and FT4, FT3 in 350 normal pregnant women at various trimesters in Jakkarat Hospital.

| Parameters | TSH | |
|---|---|---|
| | r | P |
| FT4 | -0.280 | < 0.01 |
| FT3 | -0.258 | <0.01 |

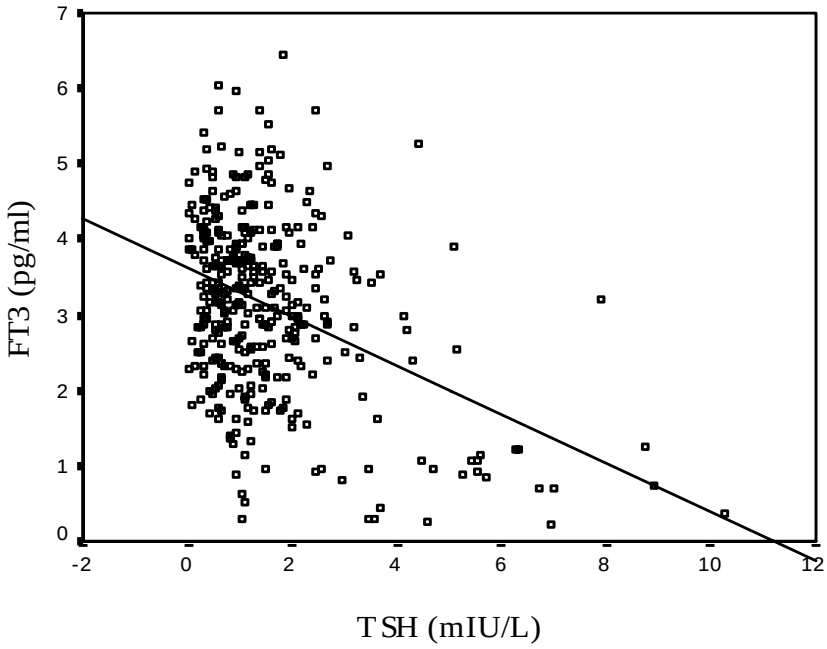
The highest relationship between FT4 and TSH was found (r = -0.280, P< 0.01) in Table 8. The regression line was Y = 1. 398 - 0.041X (Fig.16). For serum TSH and FT3 levels, the correlation and regression line were -0.258, P< 0.01 and Y = 3.612 - 0.322X (Fig. 17).




Y = 1.398 - 0.041X

r = -0.280, n = 350

**Fig. 16** Correlation coefficient (r) between FT4 and TSH in various trimesters.




Y = 3.612 - 0.322X

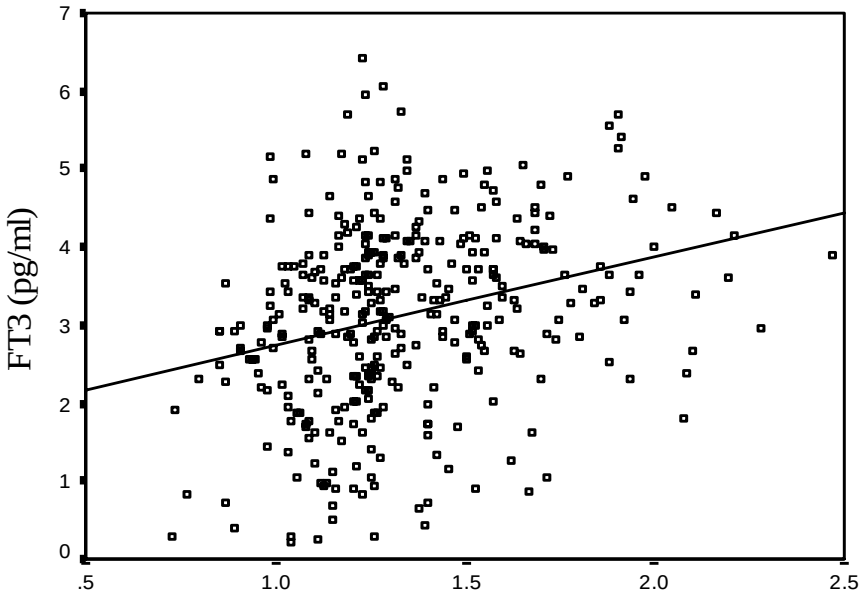
r = -0.258, n = 350

**Fig. 17** Correlation coefficient (r) between FT3 and TSH in various trimesters.

**Table 9.** The relationship between FT4 and FT3 in 350 normal pregnant women at various trimesters in Jakkarat Hospital.

| Parameters | FT4 | |
|---|---|---|
| | r | P |
| FT3 | 0.298 | <0.01 |

Table 9. showed significant positive correlation between the FT4 and FT3 ($P<0.01$). The regression line between TSH and FT4 was $Y = 1.138X + 1.598$ (Fig. 18)




Y = 1.138X +1.598

r = 0.298, n = 350

**Fig. 18** Correlation coefficient (r) between FT3 and FT4 in various trimesters.

**Table 10.** Correlation coefficient (r) between parameters of thyroid function in 350 normal pregnant women at various trimesters in Jakkarat Hospital.

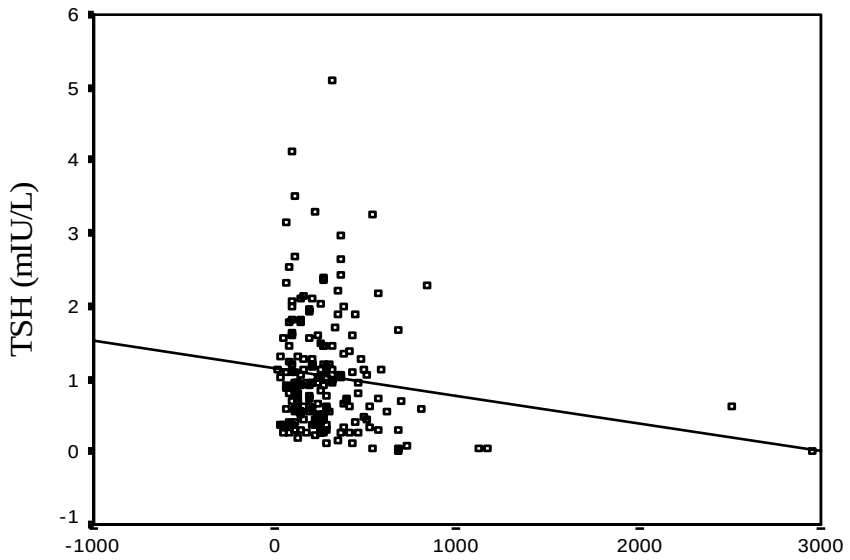
| Parameters | TSH | FT4 | FT3 | UI |
|---|---|---|---|---|
| TSH | | -0.280 [c] | -0.258 [c] | 0.045 [a] |
| FT4 | -0.280 [c] | | 0.298 [c] | -0.129 [b] |
| FT3 | -0.258 [c] | 0.298 [c] | | -0.177 [b] |
| UI | 0.045 [a] | -0.129 [b] | -.0177 [b] | |

The correlation between parameters of thyroid function and urinary iodine in the various trimesters are shown in Table 10. The highest correlation between FT4 and FT3 was found (r = 0.298, n = 350, Y = 1.138X +1.598) as shown in the underline (boxed area). When their statistical significance were [a]P>0.05, [b]P<0.05 and [c]P<0.01

**Table 11.** The relationship between urine iodine (UI) and serum TSH, FT4 and FT3 in the first trimester of pregnant women at Jakkarat Hospital (n = 190).

| Parameters | UI | |
|---|---|---|
| | r | P |
| TSH | -0.114 | < 0.05 |
| FT4 | 0.008 | >0.05 |
| FT3 | -0.122 | <0.05 |

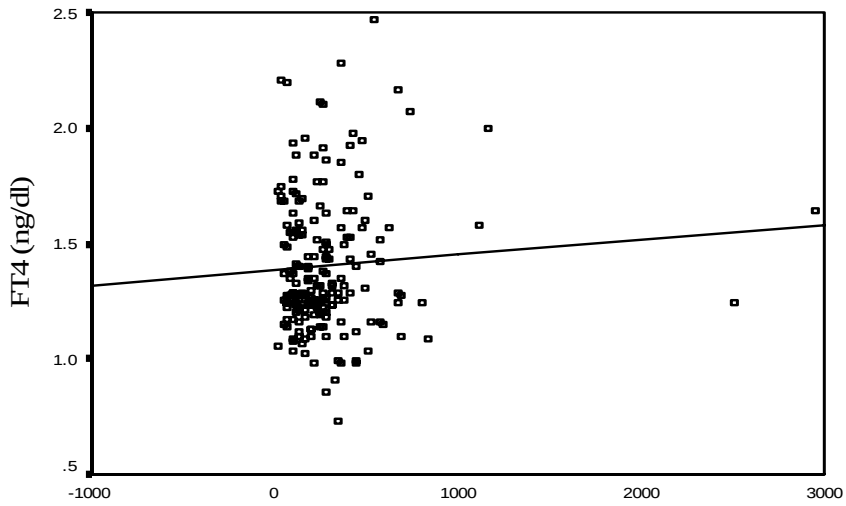
Table 11. showed the correlation coefficient (r) between parameters of thyroid function in the pregnant women. The highest correlation between FT3 and UI was found (-0.122, P<0.05) and the regression line was Y = 0.000X + 3.411 as shown in Fig. 21. The correlation between UI and TSH, FT4 were -0.114 (P<0.05) and 0.008 (P>0.05) while the regression line were Y = 0.000X + 1.144 and Y = (6.655E-05)X + 1.383 (Fig. 19 and 20).




Y = 0.000X + 1.144

r = -0.114, n = 190

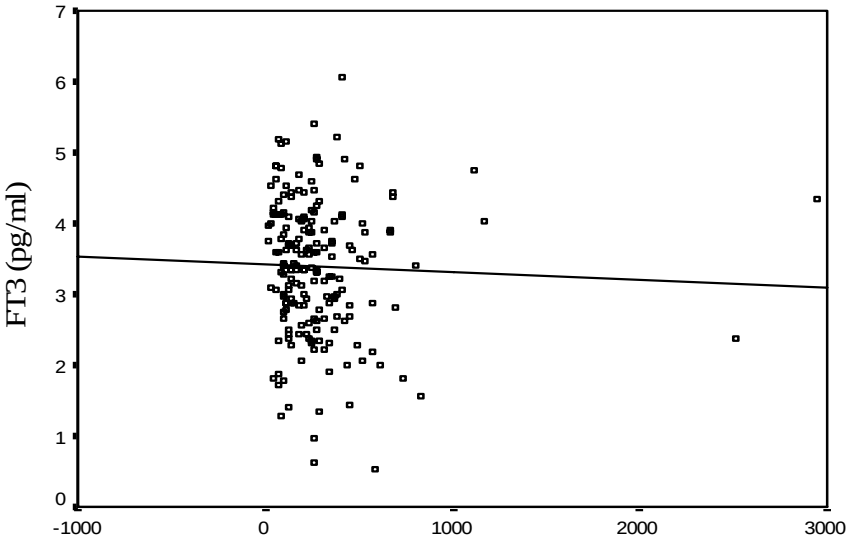
**Fig. 19** Correlation coefficient (r) between TSH and UI in the first trimester.




Y = (6.655E-05)X + 1.383

r = 0.008, n = 190

**Fig. 20** Correlation coefficient (r) between FT4 and UI in the first trimester.




Y =0.000X + 3.411

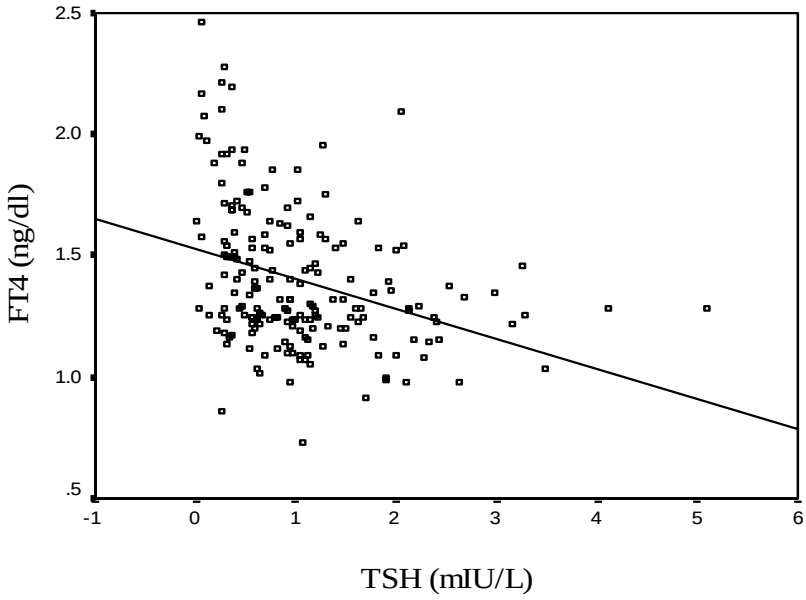
r = -0.122, n = 190

**Fig. 21** Correlation coefficient (r) between FT3 and UI in the first trimester.

**Table 12.** The relationship between serum TSH and FT4, FT3 in the first trimester of pregnant women at Jakkarat Hospital (n = 190).

| Parameters | TSH | |
|---|---|---|
| | r | P |
| FT4 | -0.378 | < 0.01 |
| FT3 | -0.124 | <0.05 |

In Table 12., the highest relationship between FT4 and TSH was found ( r = -0.378, P< 0.01). The regression line was Y = 1.531-0.125X (Fig. 22). The correlation coefficient (r) and regression line between TSH and FT3 were -0.124 and Y = 3.512-0.128X (Fig. 23).




Y =1.531-0.125X

r = -0.378, n = 190

**Fig. 22** Correlation coefficient (r) between FT4 and TSH in the first trimester.

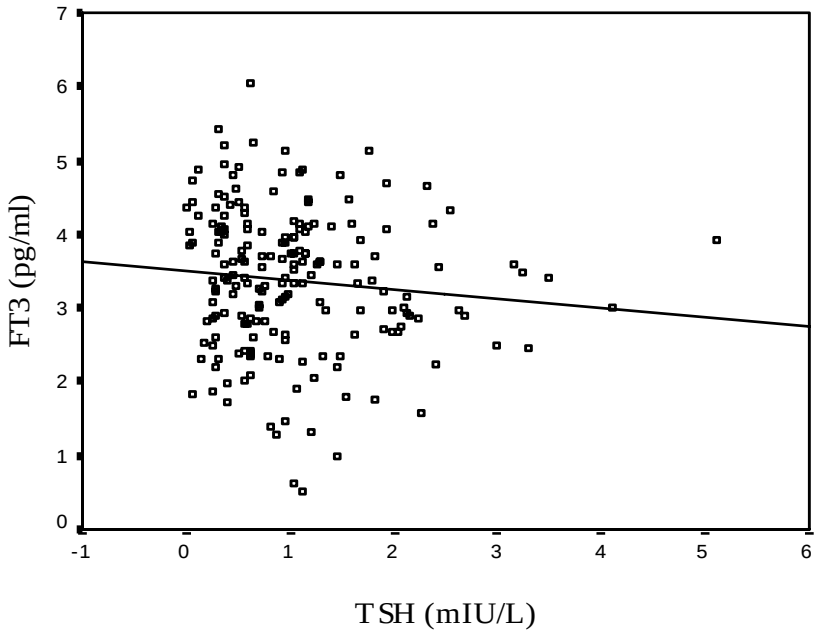
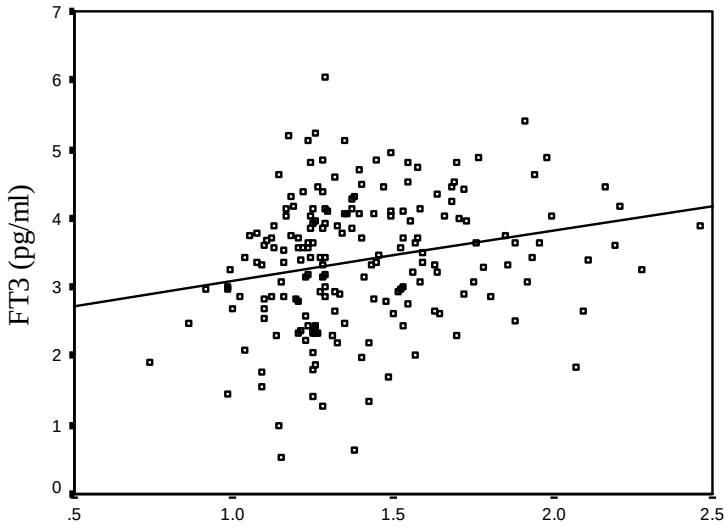



**Fig. 23** Correlation coefficient (r) between FT3 and TSH in the first trimester.

**Table 13.** The relationship between FT4 and FT3 in the first trimester of pregnant women at Jakkarat Hospital (n = 190).

| Parameters | FT4 | |
|---|---|---|
| | r | P |
| FT3 | 0.235 | <0.01 |

In Table 13., showed significant positive correlation between the FT4 and FT3 ($r = 0.235$, $P<0.01$). The regression line was $Y = 0.710X + 2.384$ (Fig. 24).




Y = 0.710X +2.384

r = 0.235, n = 190

**Fig. 24** Correlation between FT3 and TSH in the first trimester.

**Table 14.** Correlation coefficient (r) between parameters of thyroid function in the first trimester of pregnant women in Jakkarat Hospital (n =190).

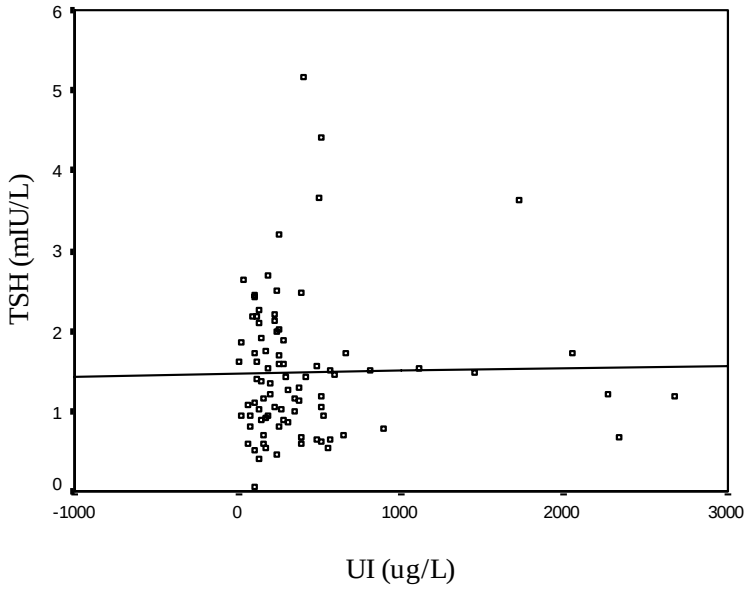
| Parameter | TSH | FT4 | FT3 | UI |
|---|---|---|---|---|
| TSH | | -0.378 [b] | -0.124 [b] | -0.114 [b] |
| FT4 | -0.378 [b] | | 0.235 [c] | 0.008 [a] |
| FT3 | -1.24 [b] | 0.235 [c] | | -0.122 [b] |
| UI | -0.114 [b] | 0.008 [a] | -0.122 [b] | |

The correlation between parameters of thyroid function and urinary iodine in the first trimester are shown in Table 14. The highest correlation between TSH and FT4 was found (r = 0.378, n = 190, Y = 1.531 – 0.125X) as shown in the underline (boxed area). When their statistical significance were [a]P>0.05, [b]P<0.05 and [c]P<0.01

**Table 15.** The relationship between urine iodine (UI) and TSH, FT4 and FT3 in the second trimester of pregnant women at Jakkarat Hospital (n = 87).

| Parameters | UI | |
|---|---|---|
| | r | P |
| TSH | -0.030 | >0.05 |
| FT4 | -0.148 | >0.05 |
| FT3 | -0.195 | <0.05 |

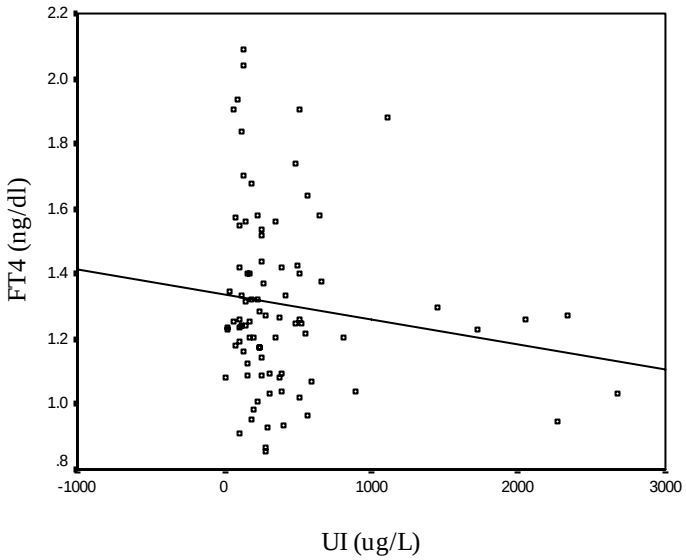
In Table 15., showed the correlation coefficient (r) between parameters of thyroid function and urinary iodine in the pregnant women. The highest correlation between FT3 and UI was found (r = -0.195, P<0.05) and the regression line was Y = 0.000X + 3.454 (n = 87) as shown in Fig. 27. The correlation between UI and TSH, FT4 were found (r = -0.030, P>0.05 and r = -0.148, P>0.05) while the regression line were Y = (2.849E-05)X + 1.467 and Y = (7.695E-05)X + 1.388 (Fig. 25 and 26).




Y = (2.849E-05)X + 1.467

r = -0.030, n = 87

**Fig. 25** Correlation coefficient (r) between TSH and UI in the second trimester.




Y = (7.695E-05)X + 1.388

r = -0.148, n = 87

**Fig. 26** Correlation coefficient (r) between FT4 and UI in the second trimester.

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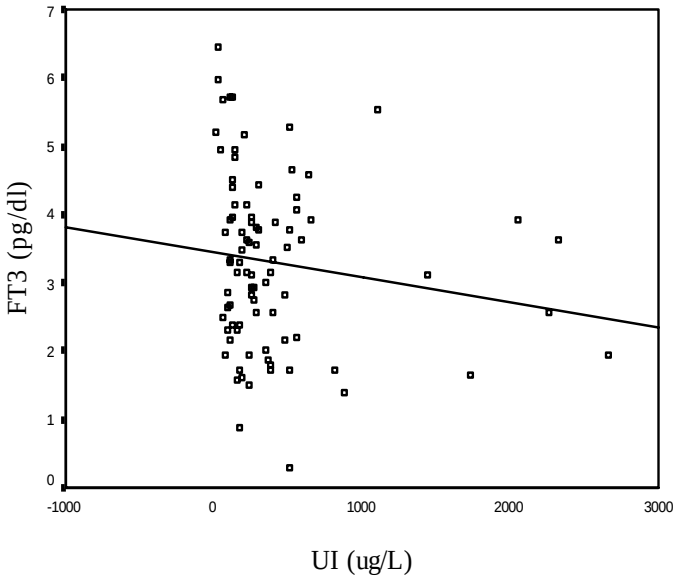



y = 0.000x + 3.454

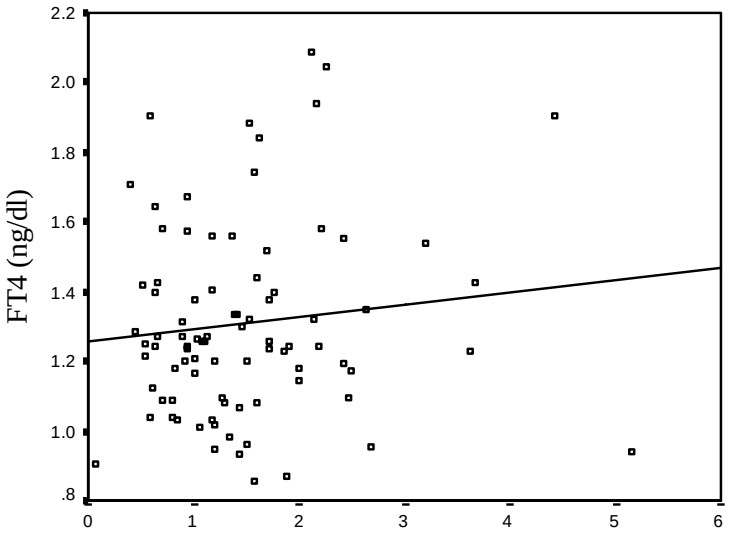
r = -0.195, n = 87

**Fig. 27** Correlation coefficient (r) between FT3 and UI in the second trimester.

**Table 16.** The relationship between TSH and FT4, FT3 in the second trimester of pregnant women at Jakkarat Hospital (n = 87).

| Parameters | TSH | |
|---|---|---|
| | r | P |
| FT4 | 0.056 | > 0.05 |
| FT3 | 0.086 | > 0.05 |

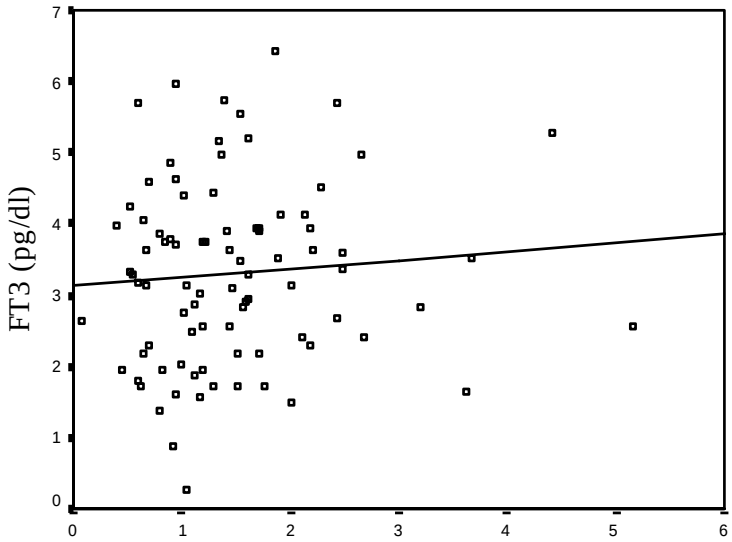
In Table 16. and Fig. 28, showed the highest relationship between FT3 and TSH was (r = 0.086, P> 0.05). The regression line was Y = 0.123X + 3.911 (Fig. 29). The correlation and regression line between TSH and FT4 were lower than FT3 and TSH (r = 0.056 and Y = 0.036X + 1.254).




Y = 0.036X + 1.254

r = 0.056, n = 87

**Fig. 28** Correlation coefficient (r) between FT4 and TSH in the second trimester.




Y = 0.123X + 3.911

r = 0.086, n = 87

**Fig. 29** Correlation coefficient (r) between FT3 and TSH in the second trimester.

**Table 17.** The relationship between FT4 and FT3 in the second trimester of pregnant women at Jakkarat Hospital (n = 87).

| Parameters | FT4 | |
|---|---|---|
| | r | P |
| FT3 | 0.191 | >0.05 |

Table17. showed non-significant positive correlation between the FT4 and FT3 (r = 0.191, P> 0.05). The regression line was Y = 0.913X + 2.108 (Fig. 30).

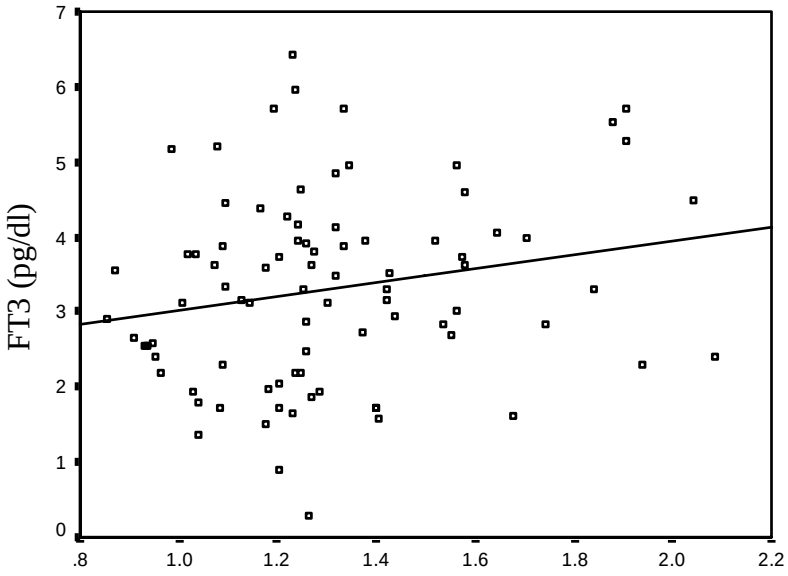



**Fig. 30** Correlation coefficient (r) between FT3 and FT4 in the second trimester.

**Table 18.** Correlation coefficient (r) between parameters of thyroid function in the second trimester of pregnant women in Jakkarat Hospital.

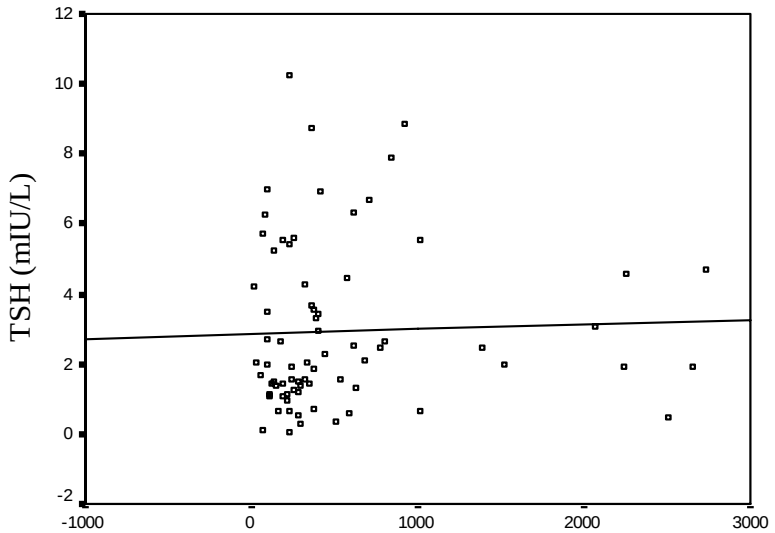
| Parameter | TSH | FT4 | FT3 | UI |
|---|---|---|---|---|
| TSH | | 0.056 [a] | 0.086 [a] | -0.030 [a] |
| FT4 | 0.056 [a] | | 0.191 [a] | -0.148 [a] |
| FT3 | 0.086 [a] | 0.191 [a] | | -0.195 [b] |
| UI | -0.030 [a] | -0.118 [a] | -0.195 [b] | |

The correlation between parameters of thyroid function and urinary iodine in the second trimester are shown in Table 18. The highest correlation between FT3 and UI was found (r = -0.195, n = 87, Y = 0.000X + 3.454) as shown in the underline (boxed area). When their statistical significance were [a]P>0.05 and [b]P<0.05

**Table 19.** The relationship between urine iodine (UI) and TSH, FT4 and FT3 in the third trimester of pregnant women at Jakkarat Hospital (n = 73).

| Parameters | UI | |
|---|---|---|
| | r | P |
| TSH | 0.129 | >0.05 |
| FT4 | -0.187 | >0.05 |
| FT3 | -0.109 | >0.05 |

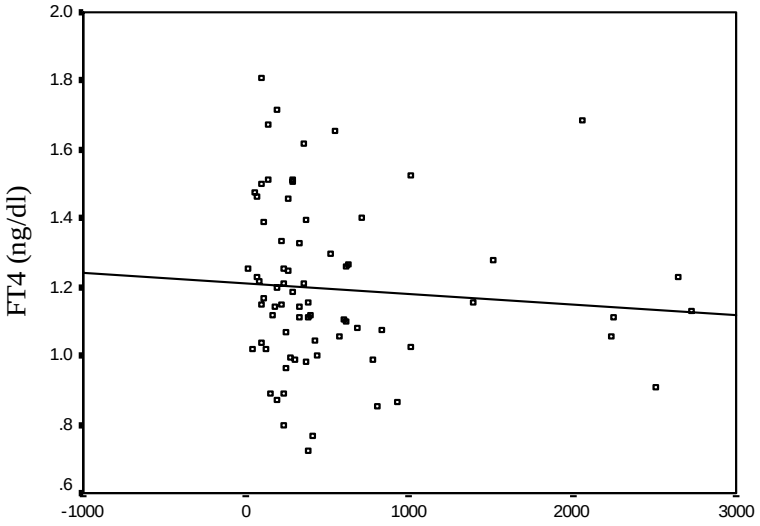
In Table 19., showed the correlation coefficient (r) between parameters of thyroid function and urinary iodine in the pregnant women. The non-significant highest correlation between FT4 and UI was found (r = -0.187, P>0.05) and the regression line was Y = 1.212 - (3.210E-05)X as shown in Fig. 32. The non-significant correlation between UI and TSH, FT3 were found (r = 0.129 and r = -0.109), while the regression line were Y = 0.000X + 2.857 and Y = 0.000X + 2.290 (Fig. 31 and 33).




Y = 0.000X + 2.857

r = 0.129, n = 73

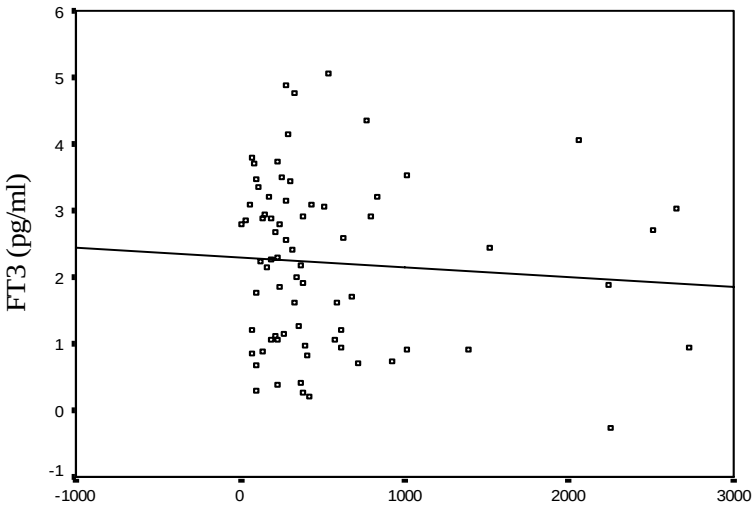
**Fig 31.** Correlation coefficient (r) between TSH and UI in the third trimester.




Y = 1.212 - (3.210E-05)X

r = -0.187, n = 73

**Fig. 32** Correlation coefficient (r) between FT4 and UI in the third trimester.




Y = 0.000X + 2.290

r = -0.109, n =73

**Fig. 33** Correlation coefficient (r) between FT3 and UI in the third trimester.

**Table 20.** The relationship between TSH and FT4, FT3 in the third trimester of pregnancy at Jakkarat Hospital (n = 73).

| Parameters | TSH | |
|---|---|---|
| | r | P |
| FT4 | 0.025 | >0.05 |
| FT3 | -0.593 | < 0.01 |

The significant highest relationship between FT3 and TSH was -0.593, P< 0.01 (Table 20.). The regression line was Y = 3.136 - 0.316X (Fig. 35). The correlation and regression line between TSH and FT4 were 0.025, P> 0.05 and Y = 0.004X + 1.183 (Fig. 34).

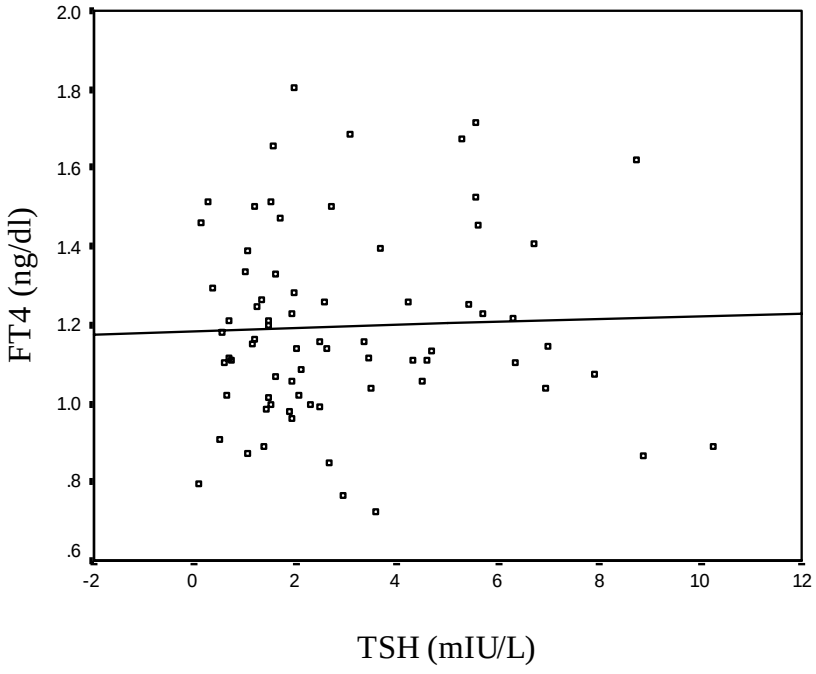
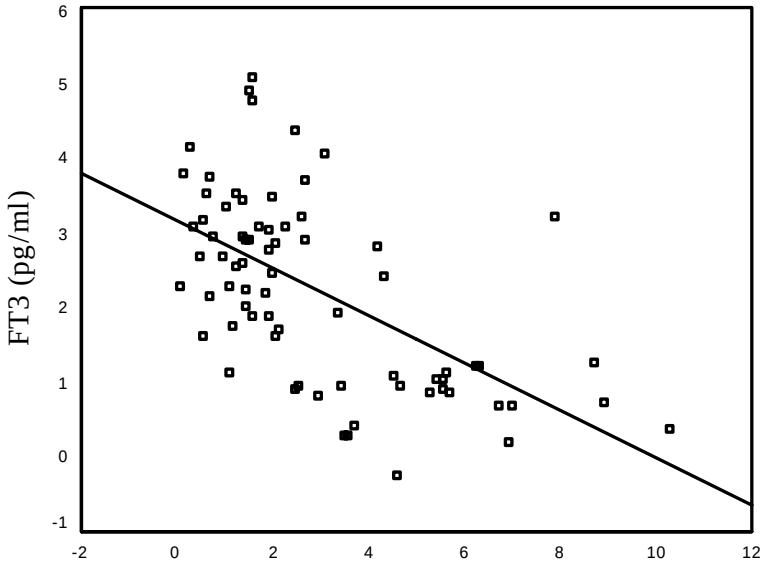



**Fig. 34** Correlation coefficient (r) between FT4 and TSH in the third trimester.




Y = 3.136 - 0.316X

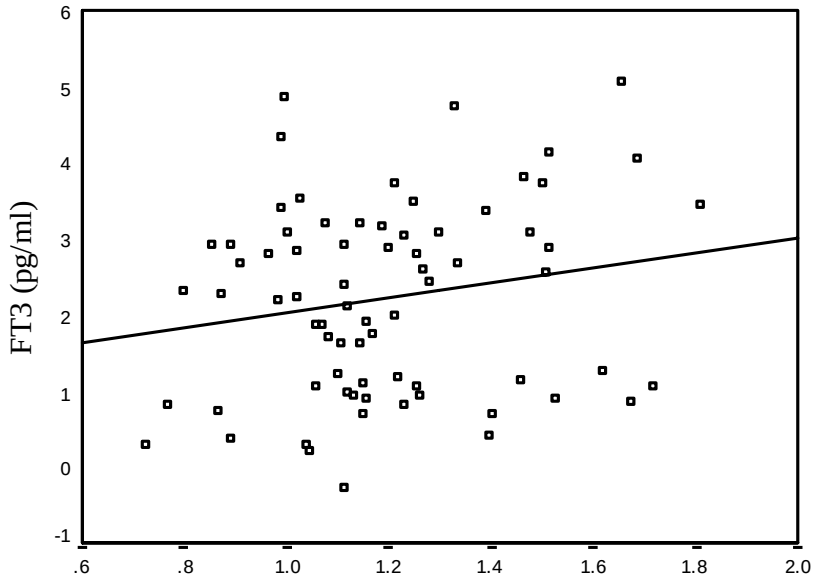
r = -0.593, n =73

**Fig. 35** Correlation between FT3 and TSH in the third trimester.

**Table 21.** The relationship between FT4 and FT3 in the third trimester of pregnant women at Jakkarat Hospital (n = 73).

| Parameters | FT4 | |
|---|---|---|
| | r | P |
| FT3 | 0.162 | >0.05 |

Table 21. showed non-significant positive correlation between the FT4 and FT3 (r = 0.162, P>0.05). The regression line was Y = 1.004X + 1.009 (Fig. 36).




Y = 1.004X + 1.009

r = 0.162, n = 73

**Fig 36.** Correlation coefficient (r) between FT3 and FT4 in the third trimester.

**Table 22.** Correlation coefficient (r) parameters of thyroid function in the third trimester of pregnant women in Jakkarat Hospital.

| Parameter | TSH | FT4 | FT3 | UI |
|---|---|---|---|---|
| TSH | | 0.025 [a] | -0.593 [b] | 0.129 [a] |
| FT4 | 0.025 [a] | | 0.162 [a] | -0.187 [a] |
| FT3 | -0.593 [b] | 0.162 [a] | | -0.109 [a] |
| UI | 0.129 [a] | -0.187 [a] | -0.109 [a] | |

The correlation between parameters of thyroid function and urinary iodine in the third trimester are shown in Table 22. The highest correlation between TSH and FT3 was found (r = -0.593, n = 73, Y = 3.136 - 0.316X) as shown in the underline (boxed area). When their statistical significance were [a]P>0.05and [b]P<0.01

# CHAPTER 6
# DISCUSSION AND CONCLUSION

Iodine deficiency disorder (IDD) is in fact worldwide problem, primarily affecting the populations of developed countries, including Thailand (51, 52, 53). IDD can cause goiter, impaired brain function and retarded development, and in the case of pregnancies can result in newborns with severe mental retardation and disability- a disorder known as cretinism (54, 55). Mental retardation from iodine deficiency is not limited to cretinism, but extends over abroad continue to mild intellectual blunting that may go unrecognized unless carefully investigated. Iodine deficiency puts virtually everyone in the affected population at some risk of brain damage (56). The main strategy for controlling IDD has been fortification of cooking salt with iodine. The world health organization recommended daily intake (RDI) of iodine is 100 μg daily for the general population and 200 μg for pregnant because of increased renal clearance and fetal transfer (57). A normal pregnancy results in a number of important physiological and hormonal changes mean that laboratory tests of thyroid function must be interpreted with caution during pregnancy. Thyroid function tests change during pregnancy due to the influence of two main hormones: human chorionic gonadotropin (hCG). The high circulating hCG levels in the first trimester may result in a slightly low TSH. Typically, the TSH in the first trimester will be normal slightly low and then remain normal throughout the duration of pregnancy. Estrogen increases the amount of thyroid hormone binding proteins in the serum which increases the total thyroid hormone levels in blood since >99% of the thyroid hormones in the blood are bound to theses proteins. However, measurements of “Free” hormone (that not bound to protein, representing the active form of the hormone) usually remain normal. The thyroid is normal function if the TSH, FT4 and FT3 are all normal throughout pregnancy (58).

In the present study followed up our subjects from the first trimester of pregnancy up to the third trimester. It was found that the median UI values of all trimesters were higher than normal references. Further more, the last trimester of pregnancy showed reduced median UI values. It may be due to the increased renal clearance of iodine which increases early in pregnancy and remains high throughout leading to increased plasma inorganic iodine.

The alterations in iodine metabolism and the thyroid function seen during pregnancies, which were characterized by increased thyroid stimulation, persisted throughout pregnancy, as evidenced by elevated TSH and reduced levels of FT3 and FT4. These findings lead us to postulate that these changes suggest further deterioration of the maternal iodine status. These findings agree with other reports regarding hypothyroxinemia during the pregnancy.

Changes in FT4 concentrations during pregnancy have often been controversial. In some studies FT4 and FT3 have been found to be decreased in pregnant women and more markedly in the third trimester whereas others have reported no change or even an increase of FT3 and FT4 with a moderate suppression of TSH. The increase in FT4 and FT3 during early pregnancy seems to be highly relevant for the fetus which produces no thyroxine during this stage of gestation.
It might be hypothesized that women with low levels of FT4 during early gestation, which seems to be without any consequences for a normal physiological pregnancy, are at risk of sub-optimal levels of FT4 for the fetus. The FT3 is clinically useful for pregnancies because serum total T3 may sometimes be misleading. The FT3 is confirming the diagnosis of T3- hypothyroidism better than total T3.

Our study suggests that measurement of urinary iodine alone gives inadequate information about the capacity of an individual to utilize on available iodine supply. The data also shows the existence of agreement patterns of thyroid response during pregnancy with Egyptian.

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