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Anti Thyroid Antibodies Among Palestinian Women Suffering from Recurrent Abortion in Gaza Strip

الأجسام المضادة للغدة الدرقية لدى المرأة الفلسطينية التي تعاني من الإجهاض المتكرر في قطاع غزة

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بسم الله الرحمن الرحيم

﴿يرفع الله الذين آمنوا منكم والذين أوتوا العلم درجات﴾

(المجادلة: 11)

صدق الله العظيم

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Signature	*Name*	*Date*
Sami	**Sami S. AL-Derawi**	**Agust-2009**

Dedication

To the pure spirit of my mother

To my wife Manal who supported me whole heartedly

To my sons Said & Ibrahim

To my daughters Ola, Haya, May & Marah

To all my friends who spare no effort to help

To all of them I dedicate this work

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Abstract

Anti Thyroid Antibodies Among Palestinian Women Suffering From Recurrent Abortion In Gaza Strip

Recurrent pregnancy loss (RPL) is one of the most frustrating and difficult areas in reproductive medicine because the etiology is often unknown. Autoimmunity has been directly associated in the etiology of several reproductive conditions including unexplained infertility and recurrent pregnancy loss. This study focused on anti-thyroid autoantibodies and thyroid hormones as immunological and hormonal causative factors for RPL. Because the human fetal thyroid does not secrete thyroid hormones until approximately 16 weeks of gestation, the fetus is dependent until that time on a supply from the maternal circulation. The presence of thyroid autoantibodies among pregnant women may create very low thyroid hormones level which may be associated with an increased risk of miscarriages.

Aim: To investigate the relationship between anti-thyroid autoantibodies (TG-abs and TPO-abs) and recurrent pregnancy loss among women in Gaza strip, and to characterize thyroid hormones level in these spontaneous recurrent aborter women.

Methods: The study was case control study where two hundred women 18-30 years old who reside in Gaza strip were examined: 100 healthy non pregnant women with history of RPL during the first trimester as cases , and 100 healthy multigravida without any pervious abortion as a control group. For all women in the two groups serum concentration for anti TG-abs, TPO-abs, thyroid stimulating hormone (TSH) and free thyroxine hormone (FT4) were measured using ELISA and MEIA.

Results: There was a statistically significant relation between recurrent pregnancy loss and anti TPO autoantibodies ($\mathbf{X^2}$= 10.039, ***P***= 0.002), where the majority of positive results were in cases as follow: 23.0% of cases were positive for TPO antibodies compared to 7.0% of control group. Also there was a statistically significant relation between recurrent pregnancy loss and anti TG autoantibodies ($\mathbf{X^2}$= 11.317, ***P***= 0.001), where the majority of positive results were in cases as follow: 21% of cases were positive for TG antibodies compared to 5% of control group. The results showed statistically

significant relationship between anti-TPO Abs and anti-TG Abs with TSH level ($\mathbf{X^2}$=9.968, **P**=0.002**,** and $\mathbf{X^2}$=5.977, **P**= 0.034**,** respectively). Free T4 levels were normal in the two groups. On other hand, there was no statistically significant relationship between thyroid antibodies and Toxoplasma, Cardiolipin and CMV antibodies among women in Gaza Strip.

Conclusion: Recurrent pregnancy loss has a statistically significant relation with anti-thyroid autoantibodies (TG-abs and TPO-abs), where 32% of cases were positive for one or both (anti TPO antibodies and/or anti TG antibodies), while the positivity in control where 9.0%.

Keywords: Recurrent pregnancy loss, Gaza strip, anti-thyroid autoantibodies.

مستخلص

الأجسام المضادة للغدة الدرقية لدى المرأة الفلسطينية التي تعاني من الإجهاض المتكرر في قطاع غزة

يعتبر الإجهاض المتكرر من أشد الأشياء المحبطة والصعبة في مجال الطب الإنجابي لأن السبب في كثير من الأحيان يكون غير معروف. وتعتبر المناعة الذاتية من احد العوامل المرتبطة مباشرة مع أسباب العقم الغامضة وحدوث الإجهاض المتكرر، بصورة خاصة ركزت هذه الدراسة على الأجسام المضادة للغدة الدرقية بدورها المناعي والهرموني وتأثيرها على وظيفة هذه الغدة لتكون كأحد العوامل المسببة لحدوث إجهاض الحمل المتكرر.
إن النمو الطبيعي لمخ جنين الإنسان يخضع لإمداد منتظم لهرمونات الغدة الدرقية ،لان الكثير أو القليل منها يمكن إن تعوق النمو العصبي،حيث إن الغدة الدرقية للجنين البشري تبدأ بإفراز هرمونات الغدة الدرقية بعد حوالي 16 أسبوعا من الحمل، ويعتمد الجنين حتى ذلك الوقت على الإمدادات من الأمهات. إن وجود الأجسام المضادة للغدة الدرقية في النساء الحوامل قد يؤدي إلى خفض مستوى هرمونات الغدة الدرقية مما يكون له تأثير على صحة الجنين وحدوث الإجهاض.

الهدف: دراسة دور الأجسام المضادة للغدة الدرقية خاصة الأجسام المضادة للغلوبين الثيرويدي (.TG abs) والأجسام المضادة لإنزيم بايروكسيداز الثيرويدي (.TPO abs) ووصف الحالة الوظيفية لهرمون الثيروكسين(T4) وهرمون ثلاثي ثايرونين(T3) عند المرأة الفلسطينية التي تعانى من الإجهاض المتكرر في قطاع غزة.

الطرق: تم دراسة عينة مكونة من 200 امرأة ومقسمة إلى مجموعتين، المجموعة الأولى مكونة من 100 امرأة غير حامل تعاني من الإجهاض المتكرر، والمجموعة الثانية مكونة من 100 امرأة منجبة بدون أي إجهاض سابق كعينة ضابطة. وقد تم فحص الأجسام المضادة (.TPO abs) و(.TG abs) باستخدام تقنية MEIA وقياس معدل هرموني TSH ، FT4 بتقنية ELISA.

النتائج: أظهرت نتائج الدراسة وجود علاقة ذات دلالة إحصائية بين الإجهاض المتكرر (RPL) و الأجسام المضادة للغدة الدرقية، حيث بينت النتائج أن 32% من الحالات التي تعاني من الإجهاض المتكرر لديها أجسام مضادة إما TG-abs و/ أو TPO-abs مقارنة مع المجموعة الضابطة حيث إن 9% لديها أجسام مضادة للغدة الدرقية، كما بينت النتائج وجود علاقة ذات دلالة إحصائية بين الاجهاض المتكرر (RPL) و مستوى هرمون TSH ، كذلك أشارت النتائج إلى عدم وجود علاقة ذات دلالة إحصائية بين تواجد الأجسام المضادة للغدة الدرقية و الأجسام المضادة لكل من CMV, Toxoplasma، CL, وأيضا عدم وجود علاقة بين تواجد الأجسام المضادة للغدة الدرقية والعمر.

الخلاصة: هناك علاقة ذات دلالة إحصائية بين الإجهاض المتكرر (RPL) وتواجد الأجسام المضادة للغدة الدرقية لدى المرأة الفلسطينية التي تعاني من الإجهاض المتكرر في قطاع غزة.

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Abbreviations

AH	Autoimmune Hypothyroidism
AITD	Auto Immune Thyroid Disease
Anti-β_2GP1 abs	Anti-beta 2 glycoprotein 1
ACL	Anti Cardiolipin
Anti-PS abs	Anti phosphatidyl-serine
ART	Assisted reproductive technique
Anti-TG abs	Anti thyroglobulin
BMI	Body Mass Index
CMV	Cytomegalo virus
DZ	Dizygotic
EIA	Enzyme immunoassay
ELISA	Enzyme linked Immunosorpent Assay
FasL	Fas ligand
FSH	Follicle-Stimulating Hormone
GD	Graves' Disease
HCG	Human Chorionic Gonadotropin
HLA	Human Leukocyte Antigen system
HT	Hashimoto's thyroiditis
LH	Luteinizing Hormone
MCT8	Monocarboxylate Transporter 8
MEIA	Microparticle Enzyme Immuno Assay
MZ	Monozygotic
NIS	Na+ / i- symporter
NK	Natural killer
PCOS	Polycystic ovary syndrome
PRL	Prolactin
PTH	Parathyroid Hormone
RPL	Recurrent pregnancy loss
T4	Thyroxine
T3	Triiodothyronine
TAO	Thyroid Associated Orbitopathy
TBG	Thyroxine-binding globulin
TG	Thyroglobulin
TH1	T-helper subset 1
TH2	T-helper subset 2
TPO	Thyroid Peroxidase
TSAbs	Thyroid stimulating antibodies
TSH	Thyroid stimulating hormone
TSHB	Thyroid-stimulating hormone β (beta) subunit

CHAPTER ONE INTRODUCTION

1.1 Overview

Pregnancy loss is one of the most common obstetrical complications. It has been shown that the total rate of spontaneous pregnancy loss is 31.0% **(1).** The majority of pregnancy losses are random or isolated incidences that in many cases are related to genetic abnormalities. Although approximately 25% of all recognized pregnancies result in miscarriage, less than 5% of women experience two consecutive miscarriages, and only 1% experience three or more **(2).** Recurrent pregnancy loss (RPL) is a devastating problem for both the patient and the treating physician. RPL classically refers to the occurrence of three or more consecutive losses of clinically recognized pregnancies prior viability (20th week of gestation) **(3).**

Women with RPL at first trimester were classified into three separate groups, as primary, secondary and tertiary aborters. Primary aborters are women with no previous live birth, secondary aborters if there was a live birth followed by pregnancy losses, and tertiary aborters are women who had pregnancy losses followed by a live birth **(4).** RPL is a heterogeneous condition that has many possible causes including genetic, hormonal, metabolic, uterine anatomical, infectious, environmental, occupational and personal habits, thrombophilia, or immune disorders. The risk of miscarriage is increased with maternal age, and influenced by past obstetrical history **(5).** The causes of RPL are unexplained in up to 60% of studied couples **(6).**

Autoimmunity has been directly associated in the etiology of several reproductive conditions including unexplained infertility and recurrent pregnancy loss. The thyroid gland undergoes many changes during pregnancy and understanding of these changes is essential for the effective management of thyroid diseases in pregnancy **(7).**

Thyroid disorders are 4-5 folds more frequent in women than in men, particularly during pregnancy **(8).** Disorders of thyroid gland are frequently caused by autoimmune reaction.

Autoimmune thyroid diseases are characterized by the presence of antithyroid antibodies, specifically anti thyroglobulin (TG-abs), and anti thyroid peroxidase (TPO -abs).

Thyroid peroxidase (TPO) is a glycoprotein with molecular weight of 100-107 KD present on the thyroid cell surface and is important antigenic target in autoimmune thyroid disease. It is responsible for the iodination of tyrosine residues on thyroglobulin (TG) and the intramolecular coupling reaction of iodinated tyrosine leading to the formation of thyroxine (T4) and triiodiothyronine (T3) **(9).**

Thyroglobulin (TG) is a large glycoprotein dimer (each subunit is 330KD) which is synthesized and secreted by the cell membrane. It is iodinated on tyrosine residues by thyroid peroxidase. Basal production of thyroid hormones results from pinocytosis of iodinated TG and hydrolysis by lysosomal enzymes. Post-translation modifications, together with the degree of TG iodination, are important determinants of the immunogenicity of the TG molecule **(10).** Thyroid replacement therapy appears to be effective in preventing a new miscarriage in women with history of RPL, who have positive thyroid antibodies or mild thyroid abnormalities **(11).**

This study focused on anti-thyroid autoantibodies and thyroid hormones as immunological and hormonal causative factors for RPL. Normal development of the human brain requires a regulated supply of thyroid hormones, either too much or too little thyroid hormones can impair neurological development. Because the human fetal thyroid does not secrete thyroid hormones until approximately 16 weeks of gestation, the fetus is dependent until that time on a supply from the maternal circulation. The presence of thyroid autoantibodies in pregnant women may create very low thyroid hormones level which may be associated with an increased rate of miscarriages **(12).**

During a pregnancy the developing fetal placental unit can be considered a semi-allograft, during which allogenic paternal Human Leukocyte Antigen system (HLA) are presented to a mother **(13).** Human fetus expresses huge amounts of paternal histocompatibility antigens that affect the immune system of the mother; these immune adaptations are

impaired and make an increased prevalence of miscarriage **(14).** During pregnancy; HLA-G, corticotrophin releasing hormone, progesterone, estrogen and human chorionic gonadotropin (HCG) collectively down regulate the immune system of the mother **(15, 16, 17, 18, 19).** However, the acceptance of the fetal allograft within the uterus remains one of the major physiologic responses to pregnancy; the immune system has long been thought to be responsible for many pregnancy losses **(20).**

1.2 Aims of the study

The major objective of the study is to investigate the relationship between anti-thyroid autoantibodies (TG-abs and TPO-abs) and recurrent pregnancy loss among women in Gaza strip.

The specific objectives

Particularly to accomplish the following:

- To detect both anti-thyroid autoantibodies (TG-abs and TPO-abs) level among women with recurrent pregnancy loss during the first trimester and healthy multigravida women in Gaza Strip.
- To compare the presence of TG-abs and TPO-abs between healthy non pregnant women with history of spontaneous recurrent abortion during the first trimester and healthy multigravida women.
- To determined thyroid hormones levels in these spontaneous recurrent aborters women.

1.3 Significance

This study is the first to investigate the relationship between anti-thyroid autoantibodies (TG-abs and TPO-abs) and recurrent pregnancy loss in women in Gaza strip. It will help in detection, diagnosis and treating one of the idiopathic causes of recurrent pregnancy loss in Gaza Strip.

2.1 Recurrent pregnancy loss

Recurrent pregnancy loss (RPL) is one of the most frustrating and difficult areas in reproductive medicine because the etiology is often unknown and there are few evidence-based diagnostic and treatment strategies. Recurrent pregnancy loss is classically defined as 3 or more consecutive losses. There is some debate, however, about the definition of recurrent pregnancy loss. Some feel that 2 rather than 3 losses are sufficient to define recurrent pregnancy loss; some include only first-trimester miscarriages, whereas others include second-trimester losses **(21).**

The risk of pregnancy loss occurring after a pregnancy has been confirmed is about 15% to 20%. The vast majority of losses occur during the first trimester; thereafter, the risk is lower. In fact, about 30% to 50% of implanted embryos are lost in the first few weeks of pregnancy, but most of these very early losses are not even recognized, as they occur at the time of the expected menstruation. Chromosomal abnormalities of the embryo cause more than half of the early losses. A genetic etiology is less likely with late first-trimester or second-trimester losses. The risk of miscarriage increases with maternal age and is influenced by past obstetric history. Among women with 2 or more previous losses and no previous live births, the risk of miscarriage is about 40%. Assisted reproduction has also been associated with a slightly increased risk of miscarriage **(22).**

2.1.1 Causes of recurrent pregnancy loss

Potential causes of recurrent pregnancy loss include: anatomic defects, genetic problems, hormonal abnormalities, immunologic factors, hematological problems, infectious agents or environmental effects. Clear etiology cannot be identified in about 50% to 75% of couples with recurrent pregnancy losses **(23).**

A. Anatomic defects

The uterine cavity can be distorted by congenital and acquired abnormalities. Some of the congenital defects are associated with pregnancy loss, but others are not. For example, an arcuate uterus is usually associated with normal progression of a pregnancy, whereas a septate uterus is a frequent cause for loss. Anatomic defects can be identified in about 3% to 5% of women with recurrent losses **(24).**

B. Genetic/chromosomal causes

A chromosome analysis performed from the parents' blood identifies an inherited genetic cause in less than 5% of couples. Translocation (when part of one chromosome is attached to another chromosome) is the most common inherited chromosome abnormality. Although a parent who carries a translocation is frequently normal, their embryo may receive too much or too little genetic material. When this occurs, a miscarriage usually occurs. Couples with translocations or other specific chromosome defects may benefit from pre-implantation genetic diagnosis in conjunction with in vitro fertilization. In contrast to the uncommon finding of an inherited genetic cause, many early miscarriages are due to the random (by chance) occurrence of a chromosomal abnormality in the embryo. In fact, 60% or more of early miscarriages may be caused by a random chromosomal abnormality, usually a missing or duplicated chromosome. The chance of a miscarriage increases as a woman ages. After age 40, more than one-third of all pregnancies end in miscarriage. Most of these embryos have an abnormal number of chromosomes **(25).**

C. Hormonal problems

Various hormonal problems can disrupt the implantation process and the development of the early embryo. Severe thyroid dysfunction (both hypothyroidism and hyperthyroidism) can disrupt the process of follicle development. Unless it leads to hypothyroidism, an autoimmune response against the thyroid (ie, with production of

antithyroglobulin and antithyroid peroxidase antibodies) has not been shown to be associated with recurrent pregnancy loss **(26).**

Hyperprolactinemia has often been diagnosed among women with recurrent pregnancy loss. In these cases, the luteal phase is shortened, and patients usually complain about spotting for days before the onset of menses. Prolactin suppresses follicular stimulating hormone (FSH) and leutinizing hormone (LH) release from the pituitary and, in severe cases, can lead to amenorrhea. In milder cases, the hormonal support of the luteal phase is reduced probably by altering the secretory pattern of LH. Prolactin also plays a role in the local regulation of steroid production. Prolactin may interfere with the normal activity of the developing placenta and therefore could affect the progress of pregnancy **(27).**

Higher rates of spontaneous abortions are observed among women with polycystic ovary syndrome (PCOS). This may be due to hyperandrogenemia, hypersecretion of LH, or insulin resistance. High levels of androgens have been shown to interfere with normal endometrial development. They alter the production of certain growth factors and may be responsible for pregnancy failure. LH stimulates ovarian androgen synthesis; therefore, LH hypersecretion is likely to interfere with early pregnancy via hyperandrogenism **(28).**

D. Immunological causes

The implanting embryo inherits its antigens from both the mother and the father. The paternal antigens are identified as foreign by the maternal immune system. In order to prevent the rejection of the pregnancy, this immune response needs to be modulated. It has been proposed that in otherwise unexplained pregnancy losses, dysregulation of the immune system could be responsible for the failure. For example, trophoblasts do not express major histocompatibility complex class II antigens (MHC II) on their surface; these cells are thus hidden from the maternal immune system. There have been reports, however, that trophoblasts from women with recurrent pregnancy loss aberrantly express MHC II antigens, and it may be that an immune response is mounted against these trophoblasts **(29).**

An alternative explanation suggests that blocking antibodies need to be present that hide the paternal antigens from the maternal immune system, allowing successful implantation and progress of pregnancy. However, successful pregnancies have been reported among women without blocking antibodies and also among women with agammaglobulinemia **(30).**

Natural killer cells can be found in large numbers in the uterus. Elevated levels of natural killer cells have been found in peripheral blood among women with reproductive failure. These cell populations differ from the natural killer cells of the endometrium. However, reports about uterine natural killer cells are contradictory. Their exact role in pregnancy loss is unknown. Natural killer cells are thought to alter the humoral response to pregnancy and induce the Th1 dominance that would lead to pregnancy loss **(31).**

Progesterone plays an important role during implantation and pregnancy. Progesterone is required to induce secretory changes that prepare the endometrium for the arriving embryo. However, progesterone also has an immunomodulatory effect. It stimulates the production of progesterone-induced blocking factor that suppresses natural killer cell activity and increases the Th-2 response. This suppressive effect favors implantation and the progress of early development **(32).**

E. Hematological factors

Intensive angiogenesis, coagulation, and fibrinolysis accompany implantation. Coagulation and fibrinolysis are simultaneously ongoing processes in the body. A healthy balance will prevent both thrombosis and bleeding. If the balance is tipped, however, clots may form or spontaneous bleeding may happen. There are certain inherited and acquired conditions that favor thrombin production. If the balance is disrupted during pregnancy, occlusion of the placental vessels may result, which could lead to spontaneous abortion. Congenital defects in the coagulation pathway will result in increased thrombin formation. The most common defects are mutation in factor V Leiden, a mutation that results in resistance to activated protein C, which is supposed to slow down thrombin formation;

prothrombin gene mutation; and hyperhomocysteinemia. Protein C, protein S, and antithrombin III deficiency are less frequent causes **(33).**

Autoantibodies to phospholipids (eg, phosphatidylserine, cardiolipin, lupus anticoagulant) are responsible for the so-called antiphospholipid syndrome. The diagnosis can be established when there is an adverse obstetric outcome (eg, pregnancy loss, fetal demise, severe toxemia, intrauterine growth restriction) and one of the antibodies (IgG, IgM) is detected **(34).**

The patient with recurrent pregnancy loss, especially if she has a history of adverse pregnancy outcome in later trimesters, should be evaluated for activated protein C resistance, factor V Leiden mutation, prothrombin gene defect, hyperhomocysteinemia, and antiphospholipid antibodies. If the results are negative, tests for the less common causes (protein C/protein S and antithrombin III deficiency) should be performed **(35).**

F. Infectious agents

The role of viral and bacterial infections in the pathogenesis of recurrent abortion is controversial. TORCH, as an acronym, stands for Toxoplasmosis, Other [*T. pallidum*, Varicella-zoster virus (VZV), Parvovirus B19], Rubellavirus, Cytomegalovirus (CMV), and Herpes Simplex Virus (HSV). The usual way in which the fetus is infected is by transplacental spread after maternal infection in which the organism circulates in the mother's blood. These infections, acquired in utero, can be severe enough to cause fetal loss or can result in intrauterine growth restriction, prematurity, or chronic postnatal infection. In most cases the maternal illness is mild but the impact on the developing fetus is more severe. The degree of severity is dependent on the gestational age of the fetus when infected, the virulence of the organism, the damage to the placenta, and the severity of maternal disease **(36).**

The pathogenetic mechanisms of these infections are unique. Because of their relatively low virulence, the organisms involved seldom lead to fetal death beyond the earliest stages

of embryogenesis. Since the fetus is essentially a graft of foreign tissue in the uterus, the placenta constitutes a protective immunologic barrier that shields the fetus from the mother's humoral and cell-mediated immune responses. This makes the fetus especially susceptible to infection during the first trimester and the perinatal period **(37).** As one of these infections, *Toxoplasma gondii* (*T. gondii*) has been estimated that up to one third of the world's population were infected by *T. gondii*. Infections in humans are usually asymptomatic but in some infected persons cervical lymphadenopathy or ocular disease may occur **(38).** However, primary infection acquired during pregnancy may result in severe damage to the fetus **(39).** Therefore, we tried to determine the prevalence of *T. gondii and CMV* infections in women with recurrent pregnancy loss in Gaza strip. The association between toxoplasma, rubella, herpes, cytomegalovirus, mycoplasma infection, and spontaneous abortion is weak. It is also very difficult to prove that microorganisms lead to repetitive pregnancy losses. In order to lend sufficient scientific support to this explanation, one would need to culture out the same microorganism with each pregnancy loss and show that antimicrobial therapy effectively reduces the subsequent risk **(40).**

G. Toxic and environment effects

Body weight is closely linked to reproductive success. Both obesity and low weight are associated with adverse pregnancy outcome. Women planning to conceive should be instructed on the benefits of a healthy diet (eg, sufficient folic acid intake) and regular exercise. A woman with repetitive losses and low (< 19 kg/m^2) or high (> 25 kg/m^2) body mass index (BMI) should not attempt pregnancy without achieving a normal body weight. Maternal smoking, caffeine and alcohol consumption, and illicit drug (eg, cocaine) use have been linked to pregnancy loss. Stress has also been shown to be associated with higher pregnancy loss rate **(41).**

The risk of pregnancy loss is higher in women with certain chronic maternal diseases (eg, liver, renal, autoimmune disease). Women suffering from such conditions should attempt pregnancy when the disease is well controlled. A consultation with a perinatologist can help the preparation for pregnancy. Some medications, such as nonsteroidal anti-inflammatory

drugs and aspirin, are linked to pregnancy loss **(42).** Teratogen drugs should be discontinued and replaced by an alternative before conception. Certain environmental effects (radiation, environmental toxin) can also influence the outcome of pregnancy **(43).**

2.2 Thyroid gland

2.2.1 Anatomy of the thyroid gland

The thyroid is one of the largest endocrine glands in the body, weighing 2-3 grams in neonates and 18-60 grams in adults, and is increased in pregnancy. This gland is found in the neck inferior to (below) the thyroid cartilage (also known as the Adam's apple in men).as show in figure (2.1) It is a butterfly-shaped organ and composed of two cone-like lobes or wings: lobus dexter (right lobe) and lobus sinister (left lobe), connected with the isthmus. The organ is situated on the anterior side of the neck, lying against and around the larynx and trachea, reaching posteriorly the oesophagus and carotid sheath. The thyroid controls how quickly the body burns energy, makes proteins, and how sensitive the body should be to other hormones. The thyroid participates in these processes by producing thyroid hormones, principally thyroxine (T4) and triiodothyronine (T3). These hormones regulate the rate of metabolism and affect the growth and rate of function of many other systems in the body. Iodine and tyrosine are used to form both T3 and T4. The thyroid also produces calcitonin hormone, which plays a role in calcium homeostasis **(44).**

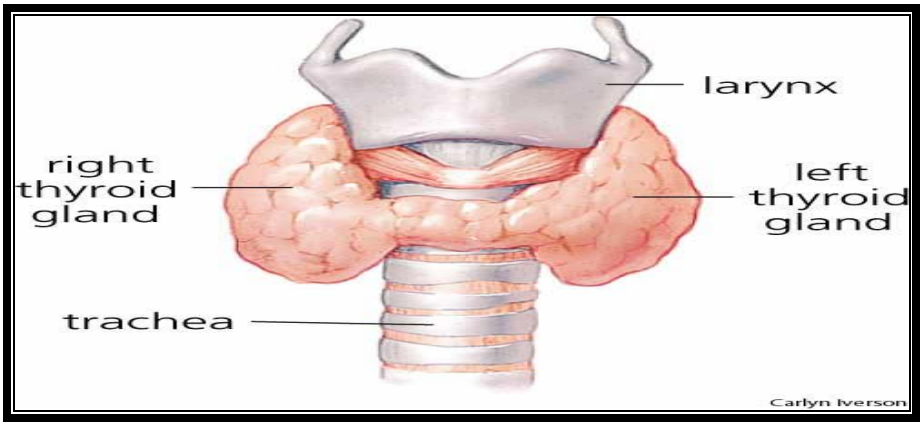

Figure 2.1: Thyroid gland (45)

2.2.2 Embryological development of thyroid gland

In the fetus, at 3-4 weeks of gestation, the thyroid gland appears as an epithelial proliferation in the floor of the pharynx at the base of the tongue between the tuberculum impar and the copula linguae at a point latter indicated by the foramen cecum. Subsequently the thyroid descends in front of the pharyngeal gut as a bilobed diverticulum through the thyroglossal duct. Over the next few weeks, it migrates to the base of the neck. During migration, the thyroid remains connected to the tongue by a narrow canal, the thyroglossal duct. The fetus starts making its own thyroid-stimulating hormone (TSH) by week 8, and the follicles of the thyroid begin to make colloid and thyroxine by the 10th week **(46).**

2.2.3 Physiology of thyroid gland

The primary function of the thyroid is production of the hormones thyroxine (T4), triiodothyronine (T3), and calcitonin. Up to 80% of the T4 is converted to T3 by peripheral organs such as the liver, kidney and spleen. T3 is about ten times more active than T4 **(47).**

2.3 The thyroid hormones

The thyroid contains two hormones, L-thyroxine (tetraiodothyronine, T_4) and L-triiodothyronine (T_3) . The thyroid hormones are the only iodine-containing compounds with established physiologic significance in vertebrates. The production of thyroid hormones is based on the organization of thyroid epithelial cells in functional units, the thyroid follicles. A single layer of polarized forms the envelope of a spherical structure with an internal compartment, the follicle lumen. Thyroid hormone synthesis is dependent on the cell polarity that conditions the targeting of specific membrane protein, either on the external side of the follicle (facing the blood capillaries) or on the internal side (at the cell-lumen boundary) and on the tightness of the follicle lumen that allows the gathering of substrates and the storage of products of the reactions**(48).** Thyroid hormone secretion relies on the existence of stores of pre-synthesized hormones in the follicle lumen and cell polarity-dependent transport and handling processes leading to the delivery of hormones into the blood stream. After concentrating iodide, the thyroid rapidly oxidizes it and binds it

to tyrosyl residues in TG followed by coupling of iodotyrosines to form T_4 and T_3. The process requires the presence of iodide, a peroxidase (TPO), a supply of H_2O_2, and an iodine acceptor protein (TG) **(49).**

2.3.1 Thyroxine (T4)

Thyroxine**(**T_4), or 3,5,3',5'-tetraiodothyronine a form of thyroid hormones is the major hormone secreted by the follicular cells of the thyroid gland. Thyroxine is synthesized via the iodination and covalent bonding of the phenyl portions of tyrosine residues found in an initial peptide, thyroglobulin, which is secreted into thyroid granules. These iodinated diphenyl compounds are cleaved from their peptide backbone upon being stimulated by thyroid stimulating hormone. T_4 is transported in blood, with 99.95% of the secreted T_4 being protein bound, principally to thyroxine-binding globulin (TBG), and, to a lesser extent, to transthyretin and serum albumin. T_4 is involved in controlling the rate of metabolic processes in the body and influencing physical development. Administration of thyroxine has been shown to significantly increase the concentration of nerve growth factor in the brains of adult mice.Thyroxine is a prohormone and a reservoir for the active thyroid hormone triiodothyronine (T_3) which is about four times more potent. T_4 is converted in the tissues by deiodinases to T_3. The "D" isomer is called "Dextrothyroxine and is used as a lipid modifying agent. The half-life of thyroxine once released into the blood circulatory system is about 1 week **(50).**

2.3.2 Triiodothyronine (T3)

Triiodothyronine(T3), is a thyroid hormone. Thyroid-stimulating hormone (TSH) activates the production of thyroxine (T_4) and T_3. This process is under regulation. In the thyroid, T_4 is converted to T_3. TSH is inhibited mainly by T_3. The thyroid gland releases greater amounts of T_4 than T_3, so plasma concentrations of T_4 are 40-fold higher than those of T_3.

Most of the circulating T_3 is formed peripherally by deiodination of T_4 (85%), a process that involves the removal of iodine from carbon 5 on the outer ring of T_4. Thus, T_4 acts as prohormone for T_3. This thyroid hormone is similar to thyroxine but with one fewer iodine atoms per molecule. In addition, T_3 exhibits greater activity and is produced in smaller quantity. It is the most powerful thyroid hormone, and affects almost every process in the body, including body temperature, growth, and heart rate. The biological half-life is 2.5 days **(50).**

2.3.3 Calcitonin

An additional hormone produced by the thyroid contributes to the regulation of blood calcium levels. Parafollicular cells produce calcitonin in response to hypercalcemia. Calcitonin stimulates movement of calcium into bone, in opposition to the effects of parathyroid hormone (PTH). However, calcitonin seems far less essential than PTH, as calcium metabolism remains clinically normal after removal of the thyroid, but not the parathyroids **(49).**

2.4 Synthesis of thyroid hormones

Thyroxine (T4) is synthesised by the follicular cells from free tyrosine and on the tyrosine residues of the protein called thyroglobulin (TG). Iodine is captured with the "iodine trap" by the hydrogen peroxide generated by the enzyme thyroid peroxidase (TPO) and linked to the 3' and 5' sites of the benzene ring of the tyrosine residues on TG, and on free tyrosine as shown in (Figure 2.2).

Upon stimulation by the thyroid-stimulating hormone (TSH), the follicular cells reabsorb TG and proteolytically cleave the iodinated tyrosines from TG, forming T4 and T3 (in T3, one iodine is absent compared to T4), and releasing them into the blood. Deiodinase enzymes convert T4 to T3. Thyroid hormone that is secreted from the gland is about 90% T4 and about 10% T3 **(51).**

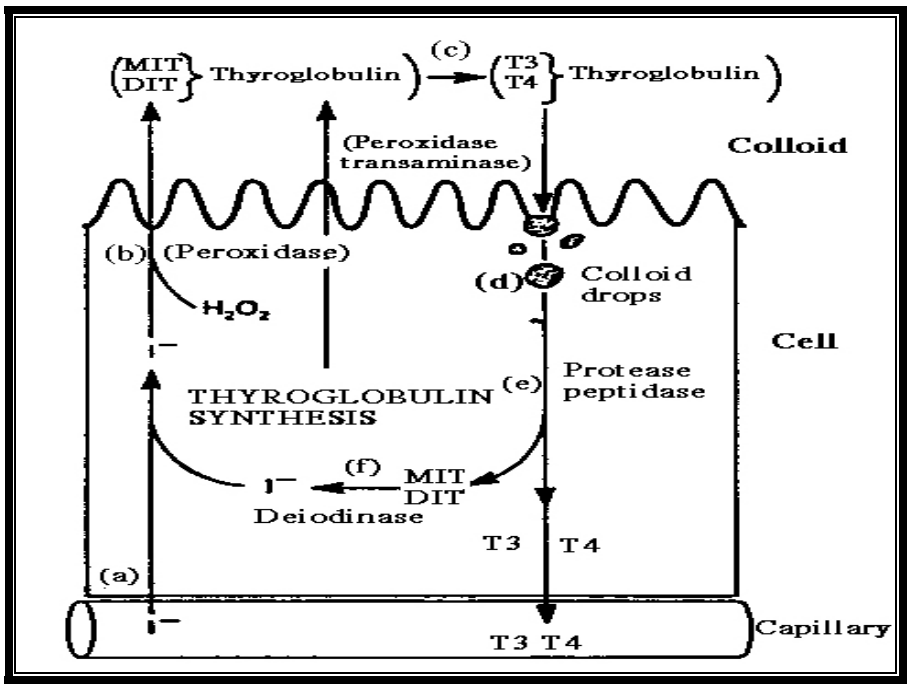

Figure 2.2: Synthesis of thyroid hormones (52)

Cells of the brain are a major target for the thyroid hormones T3 and T4. Thyroid hormones play a particularly crucial role in brain maturation during fetal development A transport protein called organic anion transporter (OATP1C1) has been identified that seems to be important for T4 transport across the blood brain barrier. A second transport protein called monocarboxylate transporter 8 (MCT8) is important for T3 transport across brain cell membranes **(53).**

In the blood, T4 and T3 are partially bound to thyroxine-binding globulin, transthyretin and albumin. Only a very small fraction of the circulating hormone is free (unbound) - T4 0.03% and T3 0.3%. Only the free fraction has hormonal activity. As with the steroid hormones and retinoic acid, thyroid hormones cross the cell membrane and bind to intracellular receptors (α1, α2, β1 and β2), which act alone, in pairs or together with the retinoid X-receptor as transcription factors to modulate DNA transcription **(54).**

2.5 Regulation of thyroid hormones

The production of T4 and T3 is regulated by thyroid-stimulating hormone (TSH), released by the anterior pituitary (that is in turn released as a result of TRH released by the hypothalamus). As shown in (Figure 2.3), the thyroid and thyrotropes form a negative feedback loop: TSH production is suppressed when the T4 levels are high, and vice versa. The TSH production itself is modulated by thyrotropin-releasing hormone (TRH), which is produced by the hypothalamus and secreted at an increased rate in situations such as cold (in which an accelerated metabolism would generate more heat). TSH production is blunted by somatostatin (SRIH), rising levels of glucocorticoids and sex hormones (estrogen and testosterone), and excessively high blood iodide concentration **(55).**

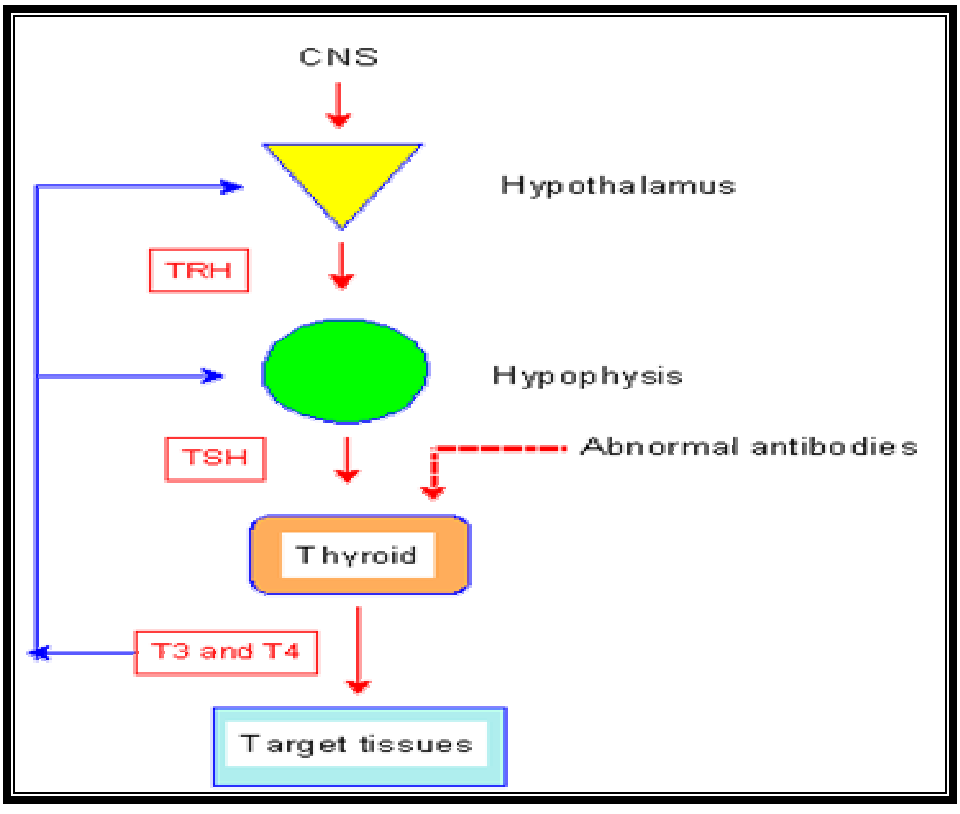

Figure 2.3: Regulation of T3 and T4 secretion (56).

2.6 Thyroid stimulating hormone (TSH)

TSH stimulates the thyroid gland to secrete the hormones thyroxine (T4) and triiodothyronine (T3). TSH is a glycoprotein which consists of two subunits, the alpha and the beta subunit. The α (alpha) subunit is identical to that of human chorionic gonadotropin (HCG), luteinizing hormone (LH), follicle-stimulating hormone (FSH). The β (beta) subunit (TSHB) is unique to TSH, and therefore determines its function. TSH production is controlled by a Thyrotropin Releasing Hormone, (TRH), which is manufactured in the hypothalamus and transported to the anterior pituitary gland via the superior hypophyseal artery, where it increases TSH production and release. Somatostatin is also produced by the hypothalamus, and has an opposite effect on the pituitary production of TSH, decreasing or inhibiting its release. The level of thyroid hormones (T3 and T4) in the blood have an effect on the pituitary release of TSH; When the levels of T3 and T4 are low, the production of TSH is increased, and conversely, when levels of T3 and T4 are high, then TSH production is decreased. This effect creates a regulatory negative feedback loop. TSH levels are tested in the blood of patients suspected of suffering from excess (hyperthyroidism), or deficiency (hypothyroidism) of thyroid hormone. Generally, a normal range for TSH for adults is between 0.5 and 5.0 mIU/mL **(57).**

2.7 Thyroid diseases

2.7.1 Hypothyroidism

Hypothyroidism is almost due to disease within thyroid gland that causes a decrease in the production of thyroid hormone. The most common cause of this disorder is autoimmune thyroid disease. There is a genetic predisposition to autoimmune thyroiditis although the mode of inheritance has not yet been defined **(58).** Several environmental factors can trigger the onset of the disease. The other is an idiopathic thyroid degeneration and atrophy. Hypothyroidism is a common disease which slowly progressive, typically occurring in older ages anyway not until 2 years of age, women are more predisposed than men **(59).** Hypothyroidism during fetal development or early infancy results in cretinism

(congenital hypothyridism) which causes respiratory difficulties, persistent jaundice, and hoarse crying, stunted growth (dwarfism), bone and muscle dystrophy, and mental deficiency in older children, and the incidence is 3 times more in girls than in boys. Infants not treated within the first 3 months or children within two years suffer irreversible mental retardation **(60)**.

2.7.1.1 Symptoms of hypothyroidism

The signs of hypothyroidism are depending on the organ which is affected. As thyroid begins to fail, slight enlargement of thyroid gland (goiter), appearing as a lump or swelling, then the patient may begin to feel tired. Skin, hair, and fingernails also grow more slowly, they become thickened, dry, and brittle. Some hair loss may be noticed. Then, as hypothyroidism becomes more severe, changes may occur in the tissues beneath skin that lead to a characteristic puffy, swollen appearance known as myxedema. This is often particularly apparent around face and eyes **(61).**

Circulation is affected and heart rate slows. Since intestinal activity slows down, patient may become constipated. A few pounds of weight gain may occur. Muscles may become painful with leg cramps. Nervous system may be affected in several ways. Some memory loss may noticed, decreased ability to think, and depression. Some patients suffer loss of balance and difficulty in walking. In women, changes in reproductive system may cause longer, heavier, and more frequent menstruation. Their ovaries may stop producing an egg each month, and, if so, it may be difficult to get pregnant. So in hypothyroid, many of the affected bodily functions simply slow down **(62).**

2.7.1.2 Diagnosis of hypothyroidism

In addition to a complete medical history and medical examination, diagnostic procedures for hypothyroidism may include many laboratory measurements tests. T4 level alone is not reliable as T4 fluctuates throughout the day and there are two distinct fractions of T4 in the blood. Free T4 and T4 bound to protein. The bound form is not possible to

measure. T4 level can be determined by a blood test. TSH level is a good concomitant factor to be measured. It is not reliable to measure alone but in a connection with free T4 measurement it increases the reliability of the test near to perfection **(63).**

2.7.2 Hyperthyroidism

Hyperthyroidism is present when the thyroid is producing too much thyroid hormone. In the early stages of this disorder, a person may have virtually no symptoms but laboratory tests may show a suppressed (below normal) TSH. Thyroid stimulating hormone is the most sensitive test in diagnosing thyroid disorders. Every year, 350,000 people develop some kind of hyperthyroidism, and it is eight to ten times more common in women than men **(64).**

The most common cause of hyperthyroidism is Graves' disease, also called diffuse toxic goiter. This is an autoimmune disease in which immune system over-stimulates whole gland to make too much hormone **.** Thyroiditis is another type of hyperthyroidism causes temporary hyperthyroidism, usually followed with hypothyroidism. Thyroiditis is an inflammation of the thyroid gland. **(65).**

About 5% of patients with Graves' disease also have some involvement with their eyes in which the eyes may become inflamed and appear enlarged. This is described as thyroid eye disease or "exophthalmos". As the hyperthyroidism becomes more severe, the symptoms may be present evidently. Hyperthyroidism is also caused by toxic nodular goiter, a condition in which one or more nodules of the thyroid becomes overactive. The overactive nodules actually act as benign thyroid tumors. Symptoms of toxic nodular goiter do not include bulging eyes or skin problems, as in Graves' disease. The cause of toxic nodular goiter is not known **(66).**

2.7.2.1 Symptoms of hyperthyroidism

The signs of hyperthyroidism include: muscle weakness especially upper arms and thighs, shaking hands, speeding up of heartbeat from a normal rate of 70 or 80 to well over 100 beats per minute, and diarrhea exists **(67).**

In woman, menstrual cycle may change, flow may become much lighter and the interval between menstrual periods may increase. More rarely, periods may become irregular, or may cease entirely, making it more difficult to become pregnant. If pregnancy does occur, there appears to be an increased likelihood that will have a miscarriage. In a man, breasts may become slightly larger. Eye disease "exophthalmos" is one problem that occurs only in the type of hyperthyroidism that is caused by Graves' disease. **(68).**

2.7.2.2 Diagnosis of hyperthyroidism

The diagnosis of hyperthyroidism of any type includes a test for thyroid stimulating hormone (TSH) from the pituitary gland, which will be low, its manufacture and release turned off by high thyroid hormone levels. Thyroid hormone levels of thyroxin (T4) and triiodothyronine (T3) are increased and indicate the degree of hyperthyroidism. A radioactive scan or ultrasound may be needed to determine whether overactive thyroid nodules are the cause of the hyperthyroidism **(63)**.

2.7.3 Subclinical hypothyroidism

The term subclinical hypothyroidism is used for patients who have mildly increased levels of serum thyrotropin hormone (TSH) but normal thyroid hormone (thyroxine and triiodiothyronine) levels **(69).** An increase in serum TSH concentration is an early and sensitive indicator of decreased thyroid reserve. However, it should be kept in mind that interpretation of thyroid function tests in the pediatric age range is more difficult than in adults. Although normal ranges have been defined for all age groups from birth to maturity, significant discrepancies still persist between different laboratories. It is therefore important

that each laboratory determines its own normal values and the results must always be interpreted cautiously **(70).**

It is clear that thyroxine therapy is indicated in overt hypothyroidism and uniform agreement exists that it is also indicated for patients whose TSH levels are permanently increased above 10 mIU/L. The grey zone to treat or not to treat considers patients with TSH levels between 5 – 10 mIU/L. Therapy for these milder forms is controversial. In clinical practice some doctors treat all such patients while others choose to reassess the thyroid function in 3-6 months to find out if the thyroid abnormality is transient. Subclinical hypothyroidism is one spectrum of autoimmune thyroiditis , the clinical course is variable and spontaneous remission may occur in adolescence **(71).**

Adults with subclinical hypothyroidism, especially with thyroid antibodies have been shown to result in overt hypothyroidism with a rate of 5-20 % per year. In the contrary, a very low risk for overt hypothyroidism has been shown in children and adolescent during a 5 year follow up but it has to be kept in mind that only few follow-up studies have been done in children and adolescent **(72).**

Children and adolescents with type 1 diabetes, with juvenile artritis and with epilepsy who are treated with valproate or carbamazepine are in risk for subclinical hypothyroidism and their thyroid function should be followed regularly **(73).** Early detection of subclinical hypothyroidism with treatment of thyroxine has shown to improve growth and metabolic control in type 1 diabetics **(74).** Increased TSH-levels with mildly increased thyroid hormone levels have also been found in up to 15 % of obese children and adolescents **(75).** There is, however, no need to treat these patients, hyperthyrotropinemia is reversible after weight loss **(76).**

It has been suggested recently that subclinical hypothyroidism is a cardiovascular risk factor in adults and physiological thyroxine replacement has a beneficial effect on low density lipoprotein cholesterol levels **(77).**

2.7.4 Subclinical hyperthyroidism

Subclinical hyperthyroidism is an entity that is being increasingly recognized. This may be both due to the aging of the population and the development of more sensitive

thyroid stimulating hormone (TSH). Subclinical hyperthyroidism is defined as the combination of a supressed, usually undetectable serum TSH concentration, and normal serum free T3 and T4 concentrations. The TSH value is measured by an assay with a threshold of detection that is 0,3 mU per liter or less **(78).** Patients with subclinical hyperthyroidism are usually euthyroid, but now we understand that subtle symptoms or signs of thyrotoxicosis such as malaise, tachycardia, nervousness and anxiety may be present. In elderly, atrial fibrillation may be the initial manifestation. The sensitivity of the pituitary gland to respond to minor elevations in serum or tissue T3 and T4 levels is the main pathophysiological mechanism of subclinical hyperthyroidism. Abnormal TSH levels may remain for years without clinical symptoms of overt hyperthyroidism. The rate of progression of subclinical hyperthyroidism to overt disease is at least 1 to 3 percent per year **(79).**

A suppression of TSH level may be due to nonthyroidal illness, steroid or dopamine administration, or pituitary dysfunction; so, these conditions must be excluded. According to its cause, subclinical hyperthyroidism can be classified as endogenous and exogenous. The endogenous causes of subclinical hyper-thyroidism include multinodular goiter, Graves' disease (early), solitary auto-nomous adenoma, thyroiditis and other causes of hyperthyroidism (e.g., trophoblastic tumors). The exogenous causes of subclinical hyperthyroidism include treatment with levothyroxine, exogenous iodine exposure such as recent administration of radio contrast material. A 24-hour radioactive iodine uptake (RAIU) will generally be elevated in patients with Graves' disease, multinodular goiter, and solitary autonomous nodule; but will be decreased in patients in the hyperthyroid phase of subacute, silent, or postpartum thyroiditis and in patients taking excess exogenous thyroid hormone . In clinical examination, thyroid gland may be enlarged in some patients, but in most patients it is usually normal in size **(80).**

2.8 Regulation of thyroid function during normal pregnancy

A. Increase in thyroid-binding globulin

During pregnancy, the affinities of the thyroxine-binding globulin (TBG), transthyretin, and albumin for T_4 and T_3 are not significantly altered, but the circulating concentration of TBG increases two- to three-fold, whereas the concentrations of albumin and transthyretin remain unchanged **(81).** Serum TBG increases a few weeks after conception and reaches a plateau during midgestation. The mechanism for this increase in TBG involves both an increase in hepatic synthesis of TBG and an estrogen-induced increase in sialylation, which increases the half-life of TBG from 15 min to 3 days for fully sialylated. TBG that lead to lowered free T4 concentrations, which results in elevated TSH secretion by the pituitary and, consequently, enhanced production and secretion of thyroid hormones. The net effect of elevated TBG synthesis is to force a new equilibrium between free and bound thyroid hormones and thus a significant increase in total T4 and T3 levels. The increased demand for thyroid hormones is reached by about 20 weeks of gestation and persists until term **(82).**

B. Increases in total T4 and T3

Total T_4 and total T_3 concentrations increase sharply in early pregnancy and plateau early in the second trimester at concentrations 30–100% greater than prepregnancy values. The etiology of this increase in total circulating thyroid hormones involves, primarily, increased concentrations of plasma TBG. Another proposed mechanism for this increase in total thyroid hormone concentrations is 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. Increased demand for T4 and T3 has been suggested to increase production of these hormones with, ultimately, increased concentrations in the circulation. The increase in T4 and T3 concentrations is less than would be expected by the increase in TBG as a (relative hypothyroxinemia) **(83).** On average, pregnant women had lower free hormone concentrations at term than nonpregnant women. Other studies have confirmed that serum free T4 and T3 are ~25% lower in women

at delivery than nonpregnant subjects. However, most pregnant women (>78%) remain within the same reference interval as nonpregnant women **(84).**

C. Thyroid stimulation by HCG

Human chorionic gonadotropin has mild thyrotropic activity. During the first trimester of pregnancy, when HCG is at its greatest concentration, serum TSH concentrations drop, creating the inverse image of HCG. In most pregnancies, this decrease in TSH remains within the health-related reference interval. Under pathological conditions in which HCG concentrations are markedly increased for extended periods, significant HCG-induced thyroid stimulation can occur, decreasing TSH and increasing free hormone concentrations. Members of the glycoprotein hormone family of luteinizing hormone, follicle-stimulating hormone, TSH, and HCG contain a common α-subunit and a hormone-specific ß-subunit. Because the HCG and TSH ß-subunits share 85% sequence homology in the first 114 amino acids and contain 12 cysteine residues at highly conserved positions, it is likely that their tertiary structures are very similar **(85)**

D. Increase in renal iodide clearance

In pregnancy, the renal clearance of iodide increases substantially because of an increased glomerular filtration rate. The iodide loss lowers the circulating concentrations of iodide and produces a compensatory increase in thyroidal iodide clearance. In areas of the world where iodine intake is sufficient, such as the US, the iodide losses in the urine are not clinically important. In other areas of the world, however, iodine deficiency during pregnancy can lead to hypothyroidism and goiter and poses a serious public health issue. Approximately 500 million people live in areas of overt iodine deficiency. In the nonpregnant condition, adequate iodine intake is estimated to be 100–150 µg/day. The World Health Organization recommends that during pregnancy, iodine intake be increased to at least 200 µg/day **(86).**

E. Increase in serum thyroglobulin

Thyroglobulin frequently is increased during pregnancy, reflecting the increased activity of the thyroid gland during pregnancy. The increase in thyroglobulin can be seen as early as the first trimester, but it is more pronounced in the latter part of pregnancy. Increased serum thyroglobulin concentrations are also associated with an increase in thyroid volume. Despite this, goiter, as defined by thyroid volume >23 mL, occurs in only 5–15% of women at term. This low incidence is likely attributable to adequate intake of dietary iodine **(87).**

2.9 Hyperthyroidism during pregnancy

The incidence of hyperthyroidism in pregnant women has been estimated at 0.2%. Most women have symptoms before pregnancy, but some will demonstrate symptoms for the first time during pregnancy. The most common cause is Graves disease, which accounts for 85–90% of all cases. Diagnosis of hyperthyroidism during pregnancy is important because untreated or poorly treated hyperthyroidism can lead to adverse obstetrical outcomes. These include first-trimester spontaneous abortions, high rates of still births and neonatal deaths, two- to three fold increases in the frequency of low birth weight infants, preterm delivery, fetal or neonatal hyperthyroidism, and intrauterine growth retardation. Diagnosis of Graves disease can be difficult because healthy pregnant women may exhibit tachycardia, palpitations, mild heat intolerance, emotional lability, diaphoresis, and warm, moist skin. For these reasons, diagnosis of hyperthyroidism during pregnancy needs to be made on careful clinical observations and well-conceived laboratory testing **(88).**

2.10 Hypothyroidism during pregnancy

The incidence of hypothyroidism in pregnant women has been estimated to be 0.3–0.7% . There is a known association between hypothyroidism and decreased fertility. For this reason, the frequency of hypothyroidism in pregnancy is actually lower than the 0.6–1.4% frequency in the general population. Autoimmune thyroid disease (Hashimoto

thyroiditis) and postthyroid ablation therapy are the most common causes of hypothyroidism. Hypothyroidism during pregnancy has been associated with pregnancy-induced hypertension, placenta abruptio, postpartum hemorrhage, and an increase in the frequency of low birth-weight infants **(89).**

Untreated hypothyroidism during pregnancy may cause a significant decrease in the Intelligence quotient (IQ) of children. In this study, the authors measured thyroid hormone concentrations in 25 216 pregnant women. Thyroid deficiency was undetected at the time of pregnancy in 48 of 62 women with low thyroid hormone concentrations. The IQ scores of children born to these women were, on average, seven points lower than those of children born to women with thyroid values within the appropriate reference intervals. Approximately 20% of these children had IQ levels of 85 or lower. This study suggests that TSH should be measured before or early in pregnancy to allow adequate treatment of the mother. Further research is required to determine when screening should take place, and treatment guidelines **(90).**

2.11 Autoimmune thyroid disease (AITD)

Autoimmune thyroid disease (AITD) is a common organ specific autoimmune disorder affecting mostly the middle aged women. About 2 to 4 percent of women and up to 1% of men are affected worldwide, and the prevalence rate increases with advancing age **(91).** AITD comprises a series of interrelated conditions including hyperthyroid Graves' disease (GD), Hashimoto's (goitrous) thyroiditis, atrophic autoimmune hypothyroidism, postpartum thyroiditis (PPT) and thyroid associated orbitopathy (TAO). Out of all these diseases, Hashimoto's thyroiditis (HT) and Graves' disease (GD) are the commonest type and share many features immunologically. One form of the disease may change to other as the course of the immune process progresses. Autoimmune hypothyroidism (AH) affects about 5 to 10% of middle aged and elderly women. Graves' disease (GD) is about one tenth as common as hypothyroidism affecting mostly the younger individuals **(92).** Many of these patients progress to hypothyroidism either spontaneously after treatment with antithyroid drugs or iatrogenically after radioiodine therapy or surgery. The development of

antibodies to thyroid peroxidase (TPO), thyroglobulin (TG) and thyroid stimulating hormone receptor (TSH-R) is the main hallmark of AITD **(93).**

2.11.1 Etiology

The etiology of AITD is multifactorial. Susceptibility to the disease is determined by a combination of genetic, environmental and constitutional factors.

2.11.1.1 Genetic factors

Numerous studies showed a higher frequency of AITD in family members of patients with autoimmune hypothyroidism and Graves' disease **(94).** Both types of the disease cluster together in families, provides additional support that these conditions share common etiologic and pathogenic features. The autoimmune polyglandular syndrome type 2, involves the occurrence of autoimmune thyroid dysfunction with other autoimmune diseases. (Type 1 diabetes mellitus, Addison's disease, pernicious anemia & vitiligo). Shared genetic factors are likely in this group of autoimmune disorders. Twin studies show increased concordance of GD and AH in monozygotic (MZ) twins, compared with dizygotic (DZ) twins. A recent investigation based on a population of 8,966 Danish twins has shown that concordance for GD in MZ twins was 35% compared with 3% in DZ twins **(95).**

Various techniques have been employed to identify the genes contributing to the etiology of AITD, including candidate gene analysis and whole genome screening. These studies have enabled the identification of several loci (genetic regions) that are linked with AITD, and in some of these loci putative AITD susceptibility genes have been identified. Some of these genes/loci are unique to Graves' disease (GD) and Hashimoto's thyroiditis (HT) and some are common to both diseases, indicating that there is a shared genetic susceptibility to GD and HT. The putative GD and HT susceptibility genes include both immune modifying genes (e.g. HLA, CTLA-4) and thyroid specific genes (e.g. TSHR, TG). Most likely these loci interact and their interactions may influence disease phenotype and severity **(96).** The most important susceptibility factor so far recognized is association of AITD with HLA-DR alleles. These MHC class-II genes play a critical role in the initiation of adaptive immune response. HLA-DR3 is the best-documented genetic factor for GD and AH in Caucasians **(97).** In non-white populations, GD is associated with different HLA alleles. For example,

it is associated more with HLA B35, B46, A2, and DPB1 0501 in Japanese; **(98)** A10, B8, and DQw2 in Indians**; (99)** and DR1 and DR3 in South African blacks **(100).**

2.11.1.2 Environmental Factors

A. Iodine

It has been well documented that the incidence of AITD is proportional to dietary iodine content. In Europe the prevalence of GD increases with national iodine intake programs. Iodine increases the antigenicity of TG with exacerbation of experimental thyroiditis in animals. Recent in vitro studies in NOD.H2h4 mouse have shown that high iodine doses alone may damage thyrocytes and enhances the disease progression in a dose-dependent manner **(101).**

B. Infection

No convincing evidence has indicated a role infection in AH except congenital rubella syndrome. An association has been proved between Yersinia infection and GD **(102).** Yersinia contains proteins that mimic TSH-R immunologically. Recently, retrovirus has received attention but the results are conflicting **(103).**

C. Stress

Some studies suggest an association between antecedent major life events and Graves' disease, but a causal role of stress in autoimmune process remains to be clearly established. Smoking is a minor risk factor for the development of thyroid ophthalmopathy **(104).** The female preponderance of thyroid autoimmunity is most likely due to the influence of sex steroids. Estrogen use is associated with a lower risk, and pregnancy with a higher risk for developing hyperthyroidism **(105).**

2.12 Thyroid autoantibodies

Autoantibodies causes cellular damage and alters thyroid gland function. Cellular damage occurs when sensitized T-lymphocytes and/or autoantibodies bind to thyroid cell membranes causing cell lysis and inflammatory reactions. Alterations in thyroid gland function result from the action of stimulating or blocking autoantibodies on cell

membrane receptors. Three principal thyroid autoantigens are involved in AITD. These are thyroid peroxidase (TPO), thyroglobulin (TG) and the TSH receptor **(106).**

2.12.1 Thyroid peroxidase (TPO) antibodies

TPO is the key thyroid enzyme catalyzing both the iodination and coupling reaction for the synthesis of thyroid hormone. It is membrane bound and found in the cytoplasm and in high concentration on the apical microvillar surface of thyrocytes. It is of mol wt between 100 to 105-kDa and previously was known as thyroid microsomal antigen **(107).** Multiple T & B Cell epitopes exists within the molecule and the antibody response to TPO is restricted at the level of the germ line heavy and light chain variable (V) region **(108).** Anti-TPO autoantibodies are found in over 90% of patients with autoimmune hypothyroidism and Graves' disease. Together with TG antibodies these are the predominant antibodies in AH. Anti-TPO antibodies are mainly of the IgG class with IgG1 and IgG4 subclasses in excess **(109).**

2.12.2 Thyroglobulin (TG) antibodies

TG is a 660-kDa glycoprotein composed of two identical subunits of 330 kDa each. It is secreted by the thyroid follicular cells into the follicular lumen and stored as colloid. Each TG molecule has around 100 tyrosine residues, a quarter of which are iodinated. These residues couple to form the thyroid hormones triiodothyronine (T3) and thyroxin (T4). The sequence of human TG has been determined **(110).**

Thyroglobulin autoantibodies are found in less than 60% of patients with lymphocytic thyroiditis and 30% of Graves' disease patients. They are polyclonal and mainly of lgG class with all four subclasses represented. TSH regulates the cell surface expression of TPO and TG altering the mRNA transcription of these two proteins, possibly at the gene promoter level. These effects are mimicked by auto antibodies (both blocking and stimulating) in the sera of the patients with GD **(111).**

2.12.3 Thyroid stimulating hormone receptor (TSH-R) antibodies

TSH-R is the prime autoantigen in Graves' disease and atrophic thyroiditis. It is located on the basal surface of thyroid follicular cells. In Graves' disease . thyroid stimulating antibodies (TSAbs) bind to the receptor and stimulate the thyroid cell to produce excessive amount of thyroid hormones resulting in hyperthyroidism. In patients with atrophic thyroiditis the major antibody is the TSH-R blocking antibody. After binding to the receptor this antibody blocks the binding of TSH to its receptor, thus preventing stimulation of thyroid cell. This results in diminished thyroid hormone output, atrophy of thyroid gland and the clinical state of hypothyroidism. TSH-R has 398 amino acid extra cellular domains, a 266 amino acid transmembrane domain (organized in seven loops) and an 83 amino acid intracellular domain **(112).**

2.12.4 Other antibodies

Na+/I- symporter (NIS) is the fourth major thyroid autoantigen; first demonstrated using cultured dog thyroid cells. Around a third of Graves' disease sera and 15% of Hashimoto's sera contain antibodies that inhibit NIS mediated iodide uptake in vitro. Antibodies to thyroid hormone can be found in 10% to 25% of patients with AITD and non-specific autoantibodies against DNA, tubulin and other cytoskeletal proteins can also be detected in a small proportion of patients **(113).**

2.13 The immune system of the mother and early pregnancy tolerance

Human fetus expresses huge amounts of paternal histocompatibility antigens that affect the immune system of the mother; these immune adaptations are impaired and make an increased prevalence of miscarriage **(114).** During pregnancy several mechanisms converge which collectively downregulate the immune system of the mother **(115).** First, HLA-G is a special paternally imprinted MHC-class 1 molecule expressed by the trophoblast which may serve as a ligand for the natural killer (NK) cell receptor thus deviating a NK cell attack away from the fetus **(116).** Second, under the influence of corticotrophin releasing hormone the expression of apoptotic Fas ligand (FasL) on

trophoblast and maternal decidual cells at the fetal-maternal interface is stimulated. The apoptosis of activated T lymphocytes through FasL induction is also stimulated by corti-cotrophin releasing hormone. Thus at the placental level activated maternal lymphocytes are more likely to die through apoptosis (cell suicide). Taken together these processes increase the likelihood of successful implantation and early pregnancy tolerance **(117).** progesterone, estrogen and hCG redirect maternal immunity away from the damaging T-helper subset 1 (TH1) cell-mediated immunity toward humoral T-helper subset 2 (TH2) mediated immunosuppression **(118).** However, the acceptance of the fetal allograft within the uterus remains one of the major physiologic responses to pregnancy; the immune system has long been thought to be responsible for many pregnancy losses **(119).**

2.14 Thyroid autoimmunity association with normal thyroid function

The prevalence of TAI is 5–10-fold higher among women than men, probably because of a combination of genetic factors, estrogen-related effects and chromosome X abnormalities. TAI is the most common autoimmune disorder, affecting 5–20% of women in the childbearing period. TAI is also the main cause of thyroid dysfunction, even though thyroid autoimmunity can be present without hormonal dysfunction **(120).**

Normal pregnancy leads to specific changes in the immune system to avoid rejection of the semiallogeneic fetal graft with its paternal antigens. Similar rates of pregnancy have been reported in euthyroid women with and without TAI, thus the disorder is not deemed to alter implantation of the embryo. Various studies do, however, support an association between miscarriage–single and recurrent–and the presence of nonorgan-specific autoantibodies and organ-specific autoimmunity **(121).**

As already alluded to above, the main cause of hypothyroidism during pregnancy is chronic autoimmune thyroiditis, at least in countries with adequate iodine intake in the general population. In prospective and retrospective studies of unselected pregnant women with hypothyroidism, thyroid autoantibodies can be found in 25–77% of the cases, with an average prevalence of 46%. In the studies with sufficient epidemiological information for

pregnant controls, TAI is up to five times more prevalent among pregnant women with a diagnosis of hypothyroidism. Many women with TAI frequently progress to hypothyroidism during pregnancy, despite a euthyroid status in early gestation, because the maternal thyroid requirements increase but cannot be met. Progressive hypothyroidism, therefore, often develops or worsens as gestation progresses **(122).**

Pregnant women with hypothyroidism have a raised risk of early and late obstetric complications (namely miscarriage, anemia, gestational hypertension, placental abruption, premature delivery and postpartum hemorrhage) and of admission of the neonate to intensive care, particularly for respiratory distress syndromes. Obstetric complications are associated with overt hypothyroidism as well as subclinical hypothyroidism, and treatment with levothyroxine, with subsequent restoration of normal thyroid function, greatly reduces the frequency of these complications **(123).**

Besides obstetric complications, maternal thyroid deficiency can also adversely affect fetal development, to a degree that is dependent upon the severity and timing of onset. Various studies have indicated that undiagnosed (and hence untreated) hypothyroidism that occurs during the first half of pregnancy is associated with a risk of a poor neurodevelopmental outcomes **(124).**

2.15 Association of thyroid autoimmunity with pregnancy loss

The incidence of thyroid autoantibodies in women with recurrent fetal loss or women who miscarried appears to be increased compared with controls of reproductive age without previous abortions. There are few working hypothesis concerning the association between anti-thyroid antibodies and the increased risk for pregnancy loss. The first hypothesis suggests that women with high titers of antithyroid antibodies have underlying very mild thyroid hormones (under function). Another theory views the antithyroid antibodies as simply secondary markers of a predisposition to autoimmune disease rather than the actual cause of pregnancy loss. Approximately one-third of all pregnancies end in miscarriage. In the early 1990s it was discovered that unselected euthyroid women who present with

thyroid antibodies (thyroid peroxidase and thyroglobulin) in the first trimester of pregnancy have a two to four-fold increase in their miscarriage rates **(125).**

A screening study for 552 American women for the presence of TG- abs and TPO-abs in the first trimester of pregnancy revealed that 19.6% were positive, and the detectable levels of thyroid autoantibodies were significantly correlated with an increased rate of miscarriage. Miscarriage rate in thyroid autoantibody-positive women was 17%, compared with 8.4%, for the autoantibody negative women **(126).**

In another study in Belgium for investigation of 120 pregnant women who presented various thyroid abnormalities, such as goiter, nodularity, past history of thyroid disorder compared with control pregnant women, the miscarriage rate was increased almost 4-fold in the thyroid antibodies positive group **(127).**

To evaluate the prognostic value of antithyroid antibodies in euthyroid women with a history of RPL in first trimester on future pregnancy loss, 42 American euthyroid women with a history RPL in first trimester were evaluated for the presence TG- abs and TPO-abs before pregnancy and again as soon as the diagnosis of pregnancy was made. Thirteen of 42 women (31%) were positive for the presence of antithyroid antibodies before pregnancy. All 13 maintained positivity by the time their next pregnancy was diagnosed. Only 12 of those 42 women (29%) experienced a first trimester abortion, 8 of these 12 women (67%) were positive for one or more antithyroid antibody. In contrast, among 30 nonaborting women, only 5 of 30 (17%) exhibited thyroid antibody positivity. The detection of thyroid antibodies before conception carried an increased risk of pregnancy loss in the next pregnancy (8 of 13, 62% versus 4 of 29, and 14%) **(128).**

The Center for Reproductive Health, University of California evaluated the clinical usefulness of thyroid antibodies in determining early pregnancy outcome. Four hundred eighty-seven infertile patients that successfully conceived with assisted reproductive

techniques were tested for TG- abs and TPO-abs. There were 106 women who were antibody positive for TG- abs and TPO-abs or both, and 381 who were negative. The overall incidence of positivity was 22%. In the antibody-positive group miscarriage rate was 32% in comparison to 16% in the antibody-negative group. So thyroid antibodies proved to be a useful marker for identifying women at risk for clinical miscarriage **(129).**

To determine whether thyroid auto-antibodies could be used as a marker for detection of abortion in cases with unexplained RPL. Fifty healthy euthyroid pregnant Egyptians women with history of RPL during the first trimester were tested for TG- abs and TPO-abs; 18 (36%) were positive for either one or both antibodies, 14 (77.7%) were positive for TG-abs, 9 (50%) were positive for TPO-abs and 5 (27.7%) were positive for both antibodies. Miscarriage rate among antibody positive patients was (66.67%)(12 out of 18) while miscarriage rate among antibody negative patients was (15.63%) (5 out of 32) **(130).**

To evaluate the incidence of thyroid autoantibodies in German women with a history of RPL, a total of 22 euthyroid non-pregnant habitual aborters were analysed for TG- abs and TPO-abs; 22 nulligravida and 22 multigravida without endocrine dysfunction served as controls. Eight of the 22 women with RPL (36%) but only two of the 22 nulligravidae controls (9%) and one of 22 multigravida subjects (5%) demonstrated positive titers of TG-abs and TPO-abs or both antibodies. The incidence of thyroid antibodies in euthyroid women with RPL appears to be significantly increased compared with the controls of reproductive age without previous abortions. This finding suggests that thyroid antibodies may be a marker for autoimmune-mediated recurrent spontaneous abortions **(131).**

To investigate the relationship between thyroid autoantibodies to non-organ specific autoantibodies in German women with a history of RPL; 28 euthyroid non-pregnant habitual aborters were analysed for TG- abs and TPO-abs, and autoantibodies to thromboplastin, cardiolipin and lupus anticoagulant. Twenty eight multigravidae without previous abortions or endocrine dysfunctions served as controls, 11 of 28 women with RPL 39%, but only 2 of 28 controls 7% demonstrated positive titers of TG, TPO, or both

antibodies, 12 patients were positive for antithrombin antibodies and 3 for anticardiolipin. Only one women was lupus anticoagulant positive. No significant correlation was found between thyroid antibody positivity and positivity for antiphospholipids. This means that no correlation between the presence of thyroid autoantibodies and non-organ specific autoantibodies could be established **(132).**

In 1984 pregnant Italian women; 115 women (11.7%) were thyroid peroxidase antibody positive, these patients were divided into two groups: group A (n=57) was treated with Levothyroxine, and group B (n=58) was not treated. The 869 were thyroid peroxidase antibody negative subjects (group C) served as a normal population control group. Measuring the rate of obstetrical complications in treated and untreated groups was measured. In this study at baseline, TPO- abs (+ve) had higher TSH compared with TPO-abs (-ve); TSH remained higher in group B compared with groups A and C throughout gestation. Free T4 values were lower in group B than groups A and C after 30 wk and after parturition. Groups A and C showed a similar miscarriage rate (3.5 and 2.4%, respectively), which was lower than group B (13.8%). Group B displayed a 22.4% rate of premature deliveries, which was higher than group A (7%) and group C (8.2%). So euthyroid pregnant women who are positive for TPO-abs develop impaired thyroid function, which is associated with an increased risk of miscarriage and premature deliveries. Substitutive treatment with levothyroxine is able to lower the chance of miscarriage and premature delivery **(133).**

A screening study on 234 euthyroid Belgian women for (TPO-abs), serum TSH, and free T4 before the first Assisted Reproductive Technique (ART) cycle showed that(14%) had positive TPO-abs comparable to those of the 86% of women without antibodies in the antibody-positive group. The pregnancy rate was 53% versus 43% in the antibody-negative group. Within the group that was pregnant, the miscarriage rate was (53% and 23%) respectively. It was concluded that women with positive TPO-abs before the first ART cycle have a significantly increased risk for miscarriage **(134).**

Fifty-eight Israelian women with impaired fertility {38 women with recurrent miscarriage (RM), and 20 women with infertility, but no miscarriages} and 28 control women were screened for seven autoantibodies [antithyroglobulin (anti-TG abs), antithyroid peroxidase (anti-TPO abs), anticardiolipin (antii-CL abs), antiphosphatidyl-serine (anti-PS abs), antiprothrombin antibodies (anti-PT abs), anti-beta 2 glycoprotein 1 (anti-β_2GP1 abs), and anti-extractable nuclear antigen (anti-ENA abs)]. The analysis was performed with several panels of autoantibodies, each of which contained two or more autoantibodies. Anti-TPO was the only antibody to be associated with RM. A significant association was found between RM and autoantibodies in the anti-TG + anti-TPO + anti-ENA' or anti-TG + anti-TPO' panels. The anti-TG + anti-TPO + anti-ENA' panel was also associated with RM. When the analysis was performed only on 17 women who had secondary infertility: 10 from the 38 women with RM, and seven from the 20 women with infertility had no miscarriages. A significant association was also apparent between anti-CL and anti-PS and infertility compared with the 28 control women **(135).**

3.1 Study design

The present study is a case control study.

3.2 Target population

The study population was non pregnant women with history of recurrent pregnancy loss during the first trimester in Gaza Governorates during the spring of 2006.

3.3 Sampling and sample size

A stratified sample was used according to the number of non pregnant women with history of recurrent pregnancy loss during the first trimester in each Governorate. Therefore, the sample size of 100 women was distributed as follows: Northern (20), Gaza (35), Mid Zone (20), Khan Younis (15), and Rafah (10), in addition to the same size and same distribution as control sample.

3.4 Eligibility

3.4.1 Inclusion criteria

Healthy, non pregnant women, with a history of RPL (≥ 3 recurrent abortion) during the first trimester, (18-30) years old and who reside in Gaza strip.

3.4.2 Exclusion criteria

Women who were diagnosed or treated for thyroid dysfunction, aged less than 18 years or more than 30 years, and women, with a history of less than 3 spontaneous recurrent abortion .

3.5 Ethical considerations

The researcher obtained the necessary approval to conduct the study from Helsinki committee in the Gaza strip (Annex 1). Helsinki committee is an authorized professional body for giving permission to researchers to conduct their studies with ethical concern in the area. An official letter of request was sent to the Palestinian Ministry of Health to obtain approval to make some hormonal and immunological tests in the central laboratories of AL Remal Clinic (Annex 2). The participants were given an explanation about the purpose of

the study and assurance about the confidentiality of the information and that the participation was optional.

3.6 Questionnaire design

Before beginning the study the researcher designed an interview questionnaire with close and open-ended questions and that was according to review of the literatures related to women with history of recurrent pregnancy loss during the first trimester (Annex 3).

A face to face meeting between the researcher and subjects was the method of filling the questionnaire. The questionnaire includes several areas of questions such as personal data (e.g. age, address), number of miscarriages , gestational age before miscarriage, having a live birth, autoimmune disorder, endocrine disease, thyroid problems, receiving any medication?, and other information were reported from archive.

3.7 Pilot study

Pilot study was done before the study started to evaluate the clarity of the questionnaire and to optimize the techniques. Ten cases with recurrent pregnancy loss during the first trimester were interviewed. At the end of the pilot study, a revision and modification was done on questionnaire as necessary

3.8 Data collection

Questionnaire for Recurrent Aborters Women was used to collect the data by the researcher him self. Meeting was arranged between the researcher and recurrent aborters women to explain the purpose of the study and so on. The questionnaire was explained to the recurrent aborters women. Each hospital was visited at least 10 times to complete data collection and to collect blood samples. However, the average time for filling a questionnaire and collection of blood samples was about 10-15 minutes.

3.9 Blood sampling and processing

The time of samples collection was during 8:00- 10:00 a.m. in obstetric clinic, two days in a week for three months. Under quality control and safety procedure for sample collection, 10 ml venous blood sample have been collected from 100 women with history of recurrent pregnancy loss during the first trimester in plain vaccutainer tubes. Also, 10 ml venous blood samples were collected from 100 fertile women without history of recurrent

abortion. Serum was separated from whole blood for all specimens immediately using fine centrifugation at 3000 rpm for 15 min. Serum samples were sent to the lab. and stored at -4 to -8 ° C until analysis.

3.10 Study setting

Serum samples were stored and measured for anti TG-abs, anti TPO-abs, TSH and FT4 levels in MOH central laboratory in Gaza.

3.11 Determination of serum TSH levels

Serum TSH levels were determined using Microparticles Enzyme Immunoassay (MEIA) kits, they are close system kits for AXSYM device (full automated device) from ABBOTT Company. TSH levels were determined according to manufacturer instructions. The normal range for TSH level was 0.5-4.6 mIU/ml according to the M OH.

3.12 Determination of serum FT4 levels

Serum FT4 levels were also determined using Microparticles Enzyme Immunoassay (MEIA) kits, they are close system kits for AXSYM device (full automated device) from ABBOTT Company. FT4 levels were determined according to manufacturer instructions**.** The normal range for FT4 level was 0.8-2.0 ng /dl according to the M OH.

3.13 Determination of Anti- TPO antibodies

Anti-TPO antibodies quantitative determination was done using Enzyme Immunoassay (EIA) kits (Binding site), and Stat fax-2100 Awareness technology instrument as EIA reader according to manufacturer instructions(Binding site, Human anti TPO ORG 503).

The normal range for anti-TPO that was adopted by MOH was as:

Anti-TPO (IU/ml)	Result
< 75	Negative
75-100	Borderline
>100	Positive

3.14 Determination of Anti- TG antibodies

Anti-TG antibodies quantitative determination was done using Enzyme Immunoassay (EIA) kits (Binding site), and Stat fax-2100 Awareness technology instrument as EIA reader according to manufacturer instructions (Binding site, Human anti TG ORG 502). The normal range for anti- TG that was adopted by MOH was as:

Anti-TG (IU/ml)	Result
< 100	Negative
100-150	Borderline
>150	Positive

3.15 Statistical analysis

Data were computer analyzed using SPSS/PC (statistical package for the social science Inc., Chicago, Illinois USA, version I3.0).

Data analyses were carried out as follow

- Over viewing field questionnaire.
- Coding of questionnaire.
- Choosing data entry mode and data entry.

- Data cleaning.
- Frequency table for study variables.
- Defining and re-coding of certain variables.

The following statistical tests of significance were used :

- **Chi-square test (X^2)** to test the relationship between two qualitative categories.
- The **independent-sample t-test** procedure to compare means of two quantitative variables.
- **A NOVA**
- Graphs were plotted using Microsoft Excel program

The results in all the above mentioned procedures were accepted as statistical significant when the P-value was less than 5% (P < 0.05).

4.1 Questionnaire data analysis

The present data were collected by face to face interview from 100 RPL women (cases) and 100 multigravida women without any previous abortion (controls). Each woman of the study population filled a questionnaire, in addition to some information collected from immunology department in the MOH central laboratory. The mean age of cases was 24.3±4.1 years, while that of the control group was 23.7± 3.1 years (Fig 4.1).

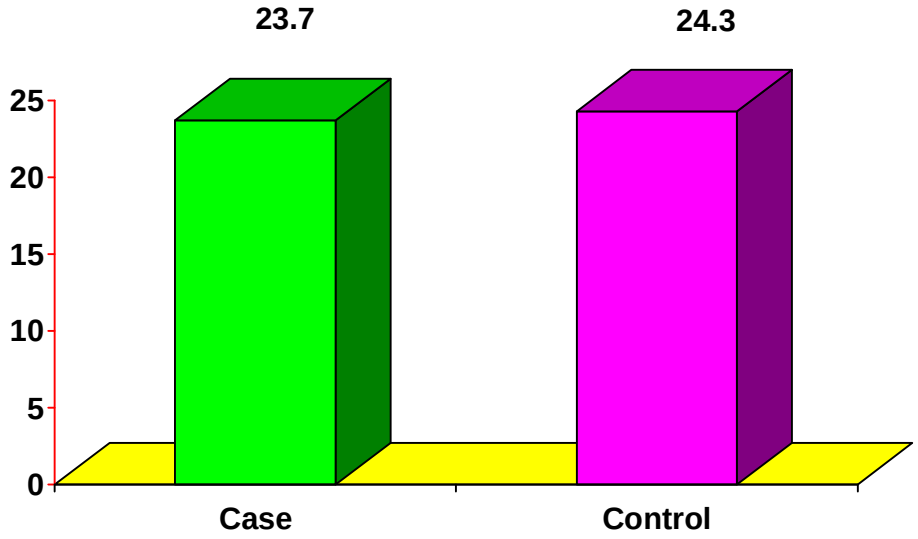

Figure 4.1: Mean age of the study population

4.1.1 Distribution of study population by the governorates of the Gaza strip

Figure 4.2 illustrates the distribution of the study population by Gaza strip governorates. Both cases (100 women) and control group (100 women) of the study population were distributed equally by various governorates as follows: North Gaza (20.0%), Gaza (35.0%), Mid Zone (20.0%), Khan Younis (15.0%) and Rafah (10.0%).

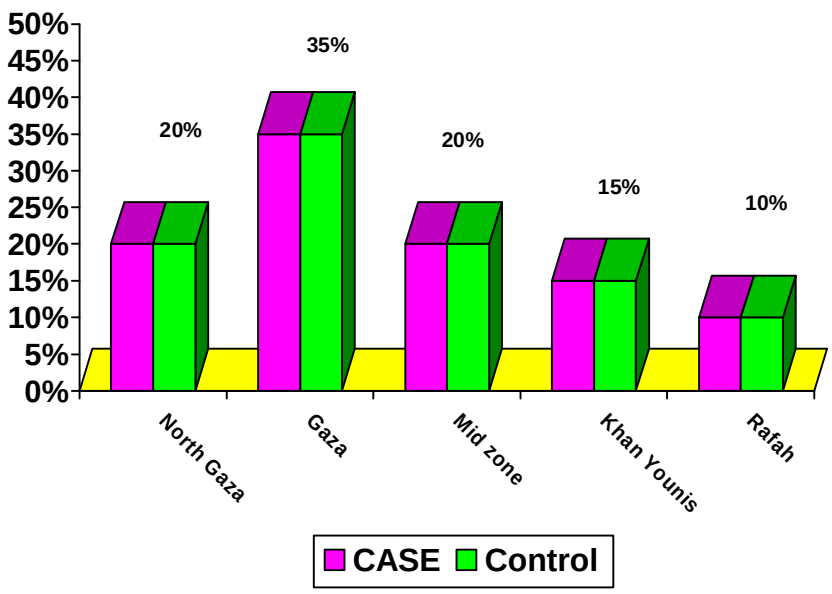

Figure 4.2: Distribution of study population by the governorates of the Gaza strip

4.1.2 Distribution of cases by miscarriage type

Women with RPL at first trimester were classified into three separate groups: primary, secondary and tertiary aborters. Tertiary aborters had been excluded according to case selective criteria, so we found that 54.0% of cases were primary aborters and 46.0% were secondary aborters (Fig 4.3).

4.1.3 Distribution of cases by previous miscarriage history

Figure 4.4 indicated that 60.0% of cases had a history of 3 spontaneous recurrent abortion and 36.0% had a history of 4 spontaneous recurrent abortion. Cases with a history of 5 and 6 spontaneous recurrent abortion were 3.0% and 1.0%, respectively**.**

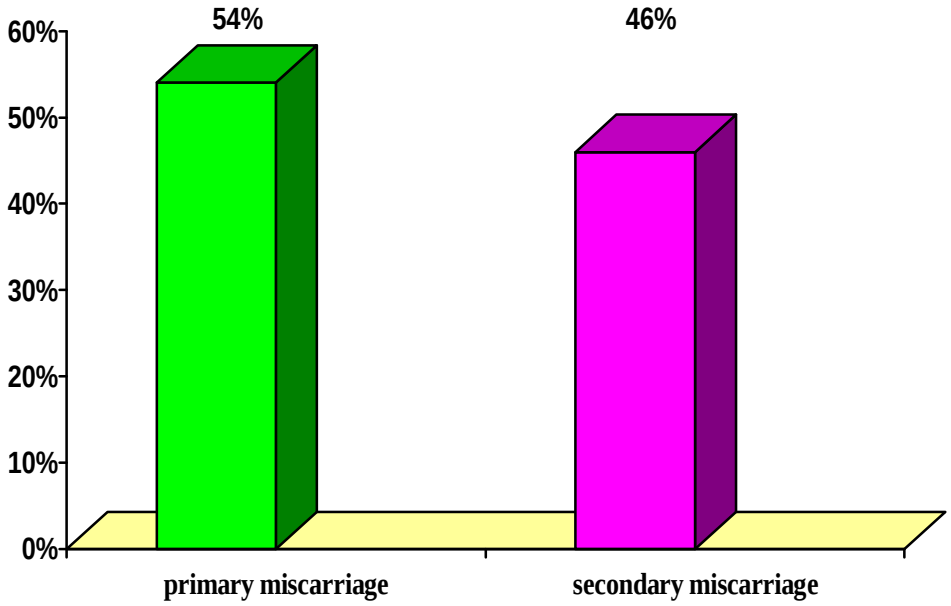

Figure 4.3: Distribution of cases by miscarriage type

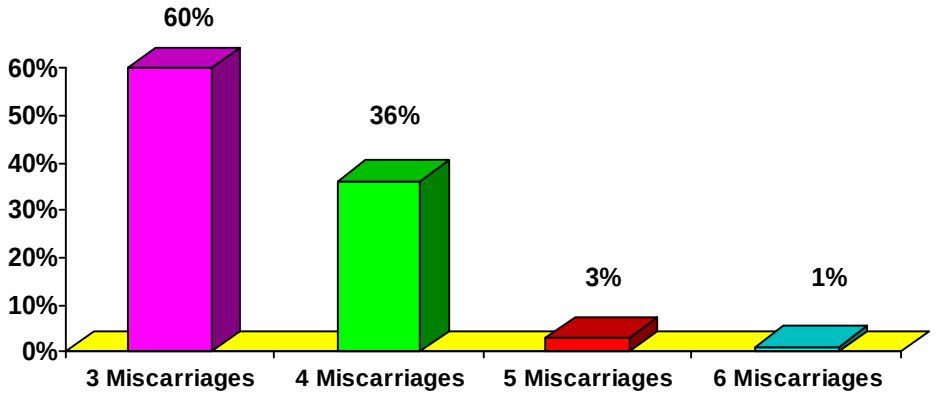

Figure 4.4: Frequency of previous miscarriages.

4.1.4 Distribution of cases by antibodies to Toxoplasma, Cytomegalo virus and Cardiolipin

Results showed that 17.0%, 9.0% and 15.0% of cases had antibodies to toxoplasma, Cytomegallo Virus and cardiolipin respectively (Figure 4.5). No data about these antibodies were collected for the control group.

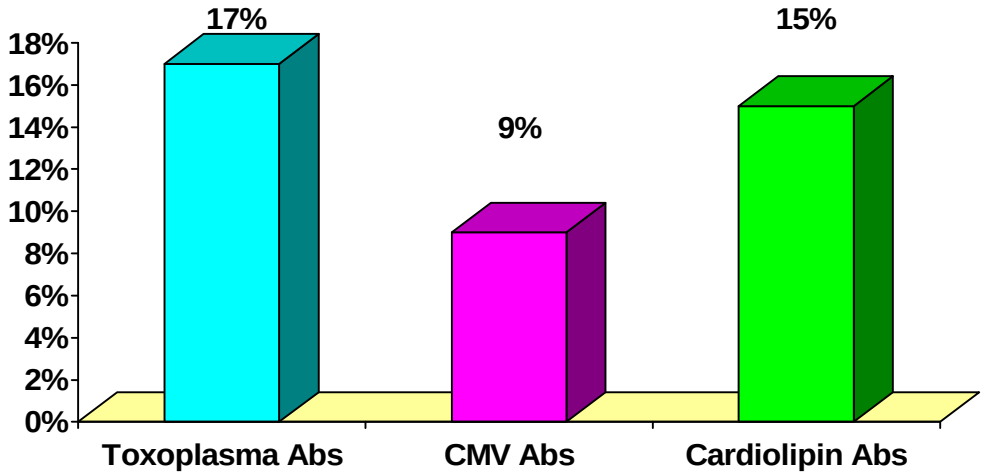

Figure 4. 5: Distribution of cases by antibodies. to Toxoplasma, CMV and Cardiolipin.

4.2 Hormones and antibodies data analysis

4.2.1 Serum TSH and FT4 hormones levels

Serum samples from the study population (cases and controls) were successfully analyzed for TSH and FT4 by MEIA. Results indicated that 10 (5.0%) of study population had elevated TSH levels (>4.6 mIU/ml), while 190 (95.0%) had normal TSH levels. The high TSH subjects were distributed between cases and controls as follow: 7 (3.5%) were in cases and 3 (1.5%) were in control group. On the other hand, the normal TSH subjects were distributed as follow: 93 (46.5%) were in cases and 97 (48.5%) were in control group, as shown in (Table 4.1). In addition, results indicated that cases and controls had normal FT4 levels.

Table 4.1 : Distribution of TSH level among cases (n=100) and controls (n=100).

TSH level	**High	*Normal
	No. (%)	No. (%)
Case	7 (3.5)	93 (46.5)
Control	3 (1.5)	97 (48.5)
Total	10 (5.0)	190 (95.0)

****High TSH level= > 4.6 mIU/ml.**
***Normal TSH level = 0.5-4.66 mIU/ml.**

4.2.2 Serum anti-TPO Abs and anti -TG Abs levels

On one hand, the analysis of the results indicated that 30 (15.0%) of the study population were positive for anti TPO antibodies while 170 (85.0%) were negative for anti TPO antibodies. Women with positive anti TPO antibodies were distributed as follows: 23 (11.5%) were cases and 7 (3.5%) were controls. In contrast, women with negative anti TPO antibodies were 77 (38.5%) cases and 93 (46.5%) controls (Table 4.2).

On the other hand, Table 4.3 showed that 26 (13.0%) of the study population were positive for anti TG antibodies while 174 (87.0%) were negative for anti TG antibodies. Women with positive anti TG antibodies were distributed as follows: 21 (10.5%) were in cases and 5 (2.5%) were in control group. In contrast, women with negative anti TG antibodies were 79 (39.5%) cases and 95 (47.5%) controls.

Table 4.2: Frequency of anti TPO antibodies among the study population

Anti TPO Abs.	**positive	*negative
	No. (%)	No. (%)
Case	23 (11.5)	77 (38.5)
Control	7 (3.5)	93 (46.5)
Total	30 (15.0)	170 (85.0)

****positive : TPO Abs level >100 IU/ml.**

***negative : TPO Abs level ≤100 IU/ml.**

Table 4.3: Frequency of anti TG antibodies among the study population

Anti TG Abs.	**positive	*negative
	No. (%)	No. (%)
Case	21 (10.5)	79 (39.5)
Control	5 (2.5)	95 (47.5)
Total	26 (13.0)	174 (87.0)

****positive : TG Abs level >150 IU/ml.**

***negative : TG Abs level ≤150 IU/ml.**

4.3 Relations of Anti TPO Autoantibodies

4.3.1 Relation between Anti TPO Abs and recurrent pregnancy loss

Results revealed a statistically significant relation between recurrent pregnancy loss and anti TPO autoantibodies ($\mathbf{X^2}$= 10.039, ***P***= 0.002), where 23.0% of the cases were positive for TPO antibodies compared to 7.0% of controls and 77.0% of cases were negative for TPO antibodies compared to 93.0% of controls (Table 4.4).

Table 4.4: Relation between Anti TPO Autoantibodies and RPL

Anti TPO Abs.	Case	Control	*p*-value
	NO. (%)	N0. (%)	
positive	23 (11.5)	7 (3.5)	0.02
negative	77 (38.5)	93 (46.5)	
Total	100 (50.0)	100 (50.0)	

4.3.2 Relation between anti-TPO Abs and hormones related to thyroid function (TSH and FT4)

4.3.2.1 Relation between anti-TPO Abs and TSH

Table 4.5 showed statistically significant relationship between anti-TPO Abs and TSH hormone in cases ($\mathbf{X^2}$= 9.968, **P**= 0.002) but this relation does not reach to statistical significant level in control group ($\mathbf{X^2}$= 3.294, **P**= 0.07). The percentage of cases with positive anti-TPO Abs and high TSH level was 5.0%, while the percentage of those with negative anti-TPO Abs and normal TSH level was 75.0% . The percentage of cases with positive anti-TPO Abs and normal TSH level was 18.0%, while the percentage of those with negative anti-TPO Abs and high TSH level was 2.0%. For control women, the percentage of those with positive anti-TPO Abs and high TSH level was 1.0%, while the percentage of those with negative anti-TPO Abs and normal TSH level was 91.0%. The percentage of controls with positive anti-TPO Abs and normal TSH level was 6.0%, while the percentage of those with negative anti-TPO Abs and high TSH level was 2.0%.

Table 4.5: Relation between anti-TPO Abs and TSH

		TSH		p-value
		Normal	High	
Anti-TPO Abs		No. (%)	No. (%)	
Case	positive	18 (18.0)	5 (5.0)	
	negative	75 (75.0)	2 (2.o)	**0.002**
	Total	**93 (93.0)**	**7 (7.0)**	
control	**Positive**	6 (6.0)	1 (1.0)	
	negative	91 (91.0)	2 (2.0)	**0.07**
	Total	**97 (97.0)**	**3 (3.0)**	

4.3.2.2 Relation between anti-TPO Abs and FT4

Results showed that all cases and controls were within the normal FT4 level (Table 4.6).Twenty three percent of cases had positive anti-TPO Abs and normal FT4 level.

compared to 7.0% of controls with positive anti-TPO Abs and normal FT4 level. No relation was detected between anti-TPO Abs and FT4.

Table 4.6: Relation between anti-TPO Abs FT4

		FT4		p-value
		Normal	High	
Anti-TPO Abs		No. (%)	No. (%)	
Case	positive	23 (23.0)	0 (0.0)	>0.05
	negative	77 (77.0)	0 (0.0)	
	Total	100 (100.0)	0 (0.0)	
control	Positive	7 (7.0)	0 (0.0)	>0.05
	negative	93 (93.0)	0 (0.0)	
	Total	100 (100.0)	0 (0.0)	

4.3.3 Relation between anti-TPO Abs and anti-TG Abs

Results showed statistically significant relation between anti-TPO Abs and anti-TG Abs for both cases and control group, where $\mathbf{X^2}$=17.497, **P**= 0.000 in cases and X^2=22.710, P= 0.000 in control group. The percentage of cases with positive anti-TPO Abs and anti-TG Abs was 12.0%, while the percentage of those of negative anti-TPO Abs and anti-TG Abs was 68.0% (Table 4.7). The percentage of cases with positive anti-TPO Abs and negative anti-TG Abs was 11.0%, while the percentage of those with negative anti-TPO Abs and positive anti-TG Abs was 9.0%. This means that 32.0% of cases were positive with one or both of anti-TPO Abs and/or anti-TG Abs, while 68.0% were negative for these Abs.

On the other hand the percentage of controls with positive anti-TPO Abs and anti-TG Abs was 3.0%, while the percentage of those of negative anti-TPO Abs and anti-TG Abs was 91.0%. The percentage of controls with positive anti-TPO Abs and negative anti-TG Abs was 4.0%, while the percentage of those with negative anti-TPO Abs and positive anti-TG Abs was 2.0%. This means that 9.0% of controls were positive with one or both of anti-TPO Abs and/or anti-TG Abs.

Table 4.7: Relationship between anti-TPO Abs and anti-TG Abs

Anti -TG Abs		Anti -TPO Abs			p-value
		positive	Negative	Total	
		No. (%)	N0.(%)	%	
Case	positive	12 (12.0)	9 (9.0)	**21.0%**	**0.000**
	negative	11 (11.0)	68 (68.0)	**79.0%**	
	Total	**23 (23.0)**	**77 (77.0)**	**100%**	
control	Positive	3 (3.0)	2 (2.0)	**5.0%**	**0.000**
	negative	4 (4.0)	91 (91.0)	**95.0%**	
	Total	**7 (7.0)**	**93 (93.0)**	**100%**	

4.3.4 Relation between anti-TPO Abs and age

Table 4.8 showed no statistically significance relation between anti-TPO Abs and the age of study population ($\mathbf{X^2}$= 1.214, ***P***=0. 271). Ten (5.0%) and 20 (10.0%) of the study population whom age were 18-23 and 24-30 years, respectively had positive anti-TPO Abs.

Table 4.8: Relationship between anti-TPO Abs and age

Variable	Anti -TPO Abs		*p*-value
Age by year	Positive	Negative	
	No. (%)	No. (%)	
18 - 23	10 (5.0)	75 (37.5)	**0.271**
24 -30	20 (10.0)	95 (47.5)	
Total	**30 (15.0)**	**170 (85)**	

4.3.5 Relation between Anti-TPO Abs and Governorate

Table 4.9 illustrates the distribution of the seropositivity for anti TPO autoantibodies in the five governorates of the Gaza strip. There was no statistically significance relation between anti TPO autoantibodies and the governorate of the subjects (F= 0.222, *P*=0.926).

Table 4.9: Relationship between Anti-TPO Abs and Governorate

Variable	Anti -TPO Abs		F	*p*-value
Governorate	positive	negative		
	N0. (%)	N0. (%)		
North Gaza	5 (2.5)	35 (17.5)	0.222	0.926
Gaza	12 (6.0)	58 (29.0)		
Mid zone	6 (3.0)	34 (17.0)		
Khan Younis	5 (2.5)	25 (12.5)		
Rafah	2 (1.0)	18 (9.0)		
Total	30 (15.0)	170 (85.0)		

4.3.6 Relationship between anti-TPO Abs and Abs to Toxoplasma among cases (n=100)

Table 4.10 showed that there was no statistically significant relation between anti-TPO Abs and Abs to Toxoplasma (X^2=0.475, *P*= 0.490), where 5 (5.0%) were positive for both anti-TPO Abs and Abs to Toxoplasma, and 18 (18.0%) were positive for anti-TPO Abs and negative for Abs to Toxoplasma.

Table 4.10: Relationship between anti-TPO Abs and Abs to Toxoplasma among cases (n=100)

Variable	Anti -TPO Abs		*p*-value
Toxoplasma	Positive	Negative	
Abs.	N0. (%)	N0. (%)	
Positive	5 (5.0)	12 (12.0)	0.490
negative	18 (18.0)	65 (65.0)	
Total	23 (23.0)	77 (77.0)	

4.3.7 Relationship between anti-TPO Abs and Abs to CMV among cases (n=100)

Table 4.11 showed that there was no statistically significant relation between anti-TPO Abs and Abs to CMV ($\mathbf{X^2}$= =0.003, ***P***= 0.954), where 2 (2.0%) were positive for both anti-TPO Abs and Abs to CMV, and 21 (21.0%) were positive for anti-TPO Abs and negative for Abs to CMV.

Table 4.11: Relationship between anti-TPO Abs and Abs to CMV among cases (n=100)

Variable	Anti -TPO Abs		*p*-value
CMV Abs.	Positive	Negative	
	N0. (%)	N0. (%)	
Positive	2 (2.0)	7 (7.0)	0.954
negative	21 (21.0)	70 (70.0)	
Total	23 (23.0)	77 (77.0)	

4.3.8 Relationship between anti-TPO Abs and Abs to Cardiolipin among cases (n=100)

Results also showed that there was no statistically significant relation between anti-TPO Abs and Abs to Cardiolipin ($\mathbf{X^2}$= =1.064, ***P***= 0.302) as shown in (Table 4.12), where 5 (5.0%) were positive for both anti-TPO Abs and Abs to cardiolipin, and 18 (18.0%) were positive for anti-TPO Abs and negative for Abs to Cardiolipin.

Table 4.12: Relationship between anti-TPO Abs and Abs to Cardiolipin among cases (n=100)

Variable	Anti -TPO Abs		*p*-value
ACL Abs.	Positive	Negative	
	N0. (%)	N0. (%)	
Positive	5 (5.0)	10 (10.0)	0.302
negative	18 (18.0)	67 (67.0)	
Total	23 (23.0)	77 (77.0)	

4.4 Relations of Anti TG Autoantibodies

4.4.1 Relation between Anti TG Abs and recurrent pregnancy loss

Analysis of the results showed a statistically significant relation between recurrent pregnancy loss and anti TG autoantibodies ($\mathbf{X^2}$= 11.317, ***P***= 0.001), where the majority of positive results were in cases as follow: 21.0% of cases were positive for TG antibodies compared to 5.0% of control group, and 79.0% of cases were negative for TG antibodies compared to 95.0% of control group (Table 4.13).

Table 4.13: Relation between Anti TG Autoantibodies and RPL among the study population

Anti TG Abs.	Case	Control	
	NO. (%)	N0. (%)	*p*-value
positive	21 (10.5)	5 (2.5)	0.001
negative	79 (39.5)	95 (47.5)	
Total	100 (50.0)	100 (50.0)	

4.4.2 Relation between anti-TG Abs and hormones related to thyroid function (TSH and FT4)

4.4.2.1 Relation between anti-TG Abs and TSH

Data pointed out statistically significant relationship between anti-TG Abs and TSH in cases ($\mathbf{X^2}$= 5.977, **P**= 0.034) as demonstrated in (Table 4.14). However, this relationship does not reach to statistical significant level in control group ($\mathbf{X^2}$= 5.227, **P**= 0.144). The percentage of cases with positive anti-TG Abs and high TSH level was 4.0%, while the percentage of those with negative anti-TG Abs and normal TSH level was 76.0% . The percentage of cases with positive anti-TG Abs and normal TSH level was 17.0%, while the percentage of those with negative anti-TG Abs and high TSH level was 3.0%. On the other hand the percentage of controls with positive anti-TG Abs and high TSH level was 1.0%, while the percentage of those with negative anti-TG Abs and normal TSH level was 93.0%.

The percentage of controls with positive anti-TG Abs and normal TSH level was 4.0%, while the percentage of those with negative anti-TG Abs and high TSH level was 2.0%.

Table 4.14: Relation between Anti TG Autoantibodies and TSH

		TSH		p-value
		Normal	High	
Anti-TG Abs		No. (%)	No. (%)	
Case	positive	17 (17.0)	4 (4.0)	0.034
	negative	76 (76.0)	3 (3.0)	
	Total	**93 (93.0)**	**7 (7.0)**	
control	Positive	4 (4.0)	1 (1.0)	0.144
	negative	93 (93.0)	2 (2.0)	
	Total	**97 (97.0)**	**3 (3.0)**	

4.4.2.2 Relation between anti-TG Abs and FT4

As indicated in (Table 4.15), all cases and controls were within the normal FT4 level. Twenty one (21.0%) of cases and 5 (5.0%) of controls were with positive Anti-TG Abs and normal FT4 level. No significant relation was detected between Anti-TG Abs and FT4.

Table 4.15: Relation between Anti-TG Abs and FT4

		FT4		p-value
		Normal	High	
Anti-TG Abs		No. (%)	No. (%)	
Case	positive	21 (21.0)	0 (0.0)	>0.05
	negative	79 (79.0)	0 (0.0)	
	Total	**100 (100.0)**	**0 (0.0)**	
control	Positive	5 (5.0)	0 (0.0)	>0.05
	negative	95 (95.0)	0 (0.0)	
	Total	**100 (100.0)**	**0 (0.0)**	

4.4.3 Relation between Anti-TG Abs and age

Results showed that there was no statistically significant relation between anti-TG Abs and the age of the study population (X^2= 0.199, *P*=0.655) as shown in (Table 4.16). Ten (5.0%) of the study population, whom age was 18-23 years and 16 (8.0%) whom age was between 24-30 years had positive anti-TG Abs.

Table 4.16: Relation between Anti-TG Abs and age

Variable	Anti -TG Abs		*p*-value
Age by year	Positive	negative	
	No. (%)	No. (%)	
18 - 23	10 (5.0)	75 (37.5)	
24 -30	16 (8.0)	99 (49.5)	**0.655**
Total	**26 (13.0)**	**174 (87.0)**	

4.4.4 Relation between Anti-TG Abs and Governorate

Table 4.17 illustrates the distribution of the seropositivity for anti TG autoantibodies in the five governorates of the Gaza strip. There was no statistically significant relation between anti TG autoantibodies and the Governorate of the subjects (F= 0.364, *P*=0.834).

Table 4.17: Relationship between Anti-TG Abs and Governorate

Governorate	Anti -TG Abs		F	*p*-value
	positive	negative		
	NO. (%)	NO. (%)		
North Gaza	6 (3.0)	34 (17.0)		
Gaza	8 (4.0)	62 (31.0)		
Mid zone	4 (2.0)	36 (18.0)		
Khan Younis	4 (2.0)	26 (13.0)	0.364	0.834
Rafah	4 (2.0)	16 (8.0)		
Total	26 (13.0)	174 (87.0)		

4.4 5 Relationship between anti-TG Abs and Abs to Toxoplasma among cases (n=100)

Data showed that there was no statistically significant relation between anti-TG Abs and Abs to Toxoplasma (X^2=0.139, *P*= 0.709) as shown in Table 4.18. Three (3.0%) of cases were positive for both anti-TG Abs and Abs to Toxoplasma, and 18 (18.0%) of them were positive for anti-TG Abs and negative for Abs to Toxoplasma.

Table 4.18: Relationship between anti-TG Abs and Abs to Toxoplasma among cases (n=100)

Variable	Anti –TG Abs		*p*-value
Toxoplasma Abs.	Positive	negative	
	N0. (%)	N0. (%)	
Positive	3 (3.0)	14 (14.0)	0.709
negative	18 (18.0)	65 (65.0)	
Total	21 (21.0)	79 (79.0)	

4.4.6 Relationship between anti-TG Abs and Abs to CMV among cases (n=100)

Also, there was no significance relation between anti-TG Abs and Abs to CMV ($\mathbf{X^2}$= =0.538, ***P***= 0.445) as shown in (Table 4.19), where 1 (1.0%) of cases were positive for both anti-TG Abs and Abs to CMV, and 20(20.0%) of them were positive for anti-TG Abs and negative for Abs to CMV.

Table 4.19: Relationship between anti-TG Abs and Abs to CMV among cases (n=100)

Variable	Anti –TG Abs		*p*-value
CMV Abs.	Positive	negative	
	N0. (%)	N0. (%)	
Positive	1 (1.0%)	8 (8.0)	0.445
negative	20 (20.0)	71 (71.0)	
Total	21 (21.0)	79 (79.0)	

4.4.7 Relationship between anti-TG Abs and Abs to Cardiolipin among cases (n=100)

Results also showed no significant relation between anti-TG Abs and Abs to Cardiolipin ($\mathbf{X^2}$= =1.618 ***P***= 0.203) as shown in (Table 4.20), where 5 (5.0%) of cases were positive for both anti-TG Abs and Abs to Cardiolipin, and 16 (16.0%) of them were positive for anti-TG Abs and negative for Abs to Cardiolipin.

Table 4.20: Relationship between anti-TG Abs and Abs to Cardiolipin among cases (n=100)

Variable	Anti –TG Abs		*p*-value
Abs. to Cardiolipin	Positive	negative	
	N0. (%)	N0. (%)	
Positive	5 (5.0)	10 (10.0)	
negative	16 (16.0)	69 (69.0)	0.203
Total	21 (21.0)	79 (79.0)	

4.5 Comparison between Anti TPO Abs levels and Anti TG Abs levels among cases and controls

4.5.1:Comparison between Anti TPO Abs levels among cases and controls

Table 4.21 shows the levels of serum Anti TPO Abs in both cases and controls. Anti TPO Abs levels of cases were higher than that of controls (86.8±128.5 vs. 37.1± 64.1, % difference= 133.9, **t**=3.46, **P**=0.001). These changes were statistically significant.

Table 4.21: Comparison between the levels of serum Anti TPO Abs. among cases (n=100) and controls (n=100)

Ant TPO Abs. level	Mean ±SD (IU/ml)	Range		% difference	t	P – value
		Min.	Max.			
Cases	86.8± 128.5	5	654	133.9	3.46	**0.001**
Controls	37.1± 64.1	4	426			

4.5.2 Comparison between Anti TG Abs levels among cases and controls

As depicted from (Table 4.22), Anti TG Abs levels of cases were higher than that of controls (74.1±113.9 vs. 31.3± 44.8, % difference=136.7, **t**=3.5, **P**=0.001).

Table 4.22: The levels of serum Anti TG Abs among cases (n=100) and controls (n=100)

Ant TG Abs. level	Mean ±SD (IU/ml)	Range		% difference	t	P - value
		Min.	Max.			
Cases	74.1± 113.9	7.0	544.0	136.7	3.5	**0.001**
Controls	31.3± 44.8	4.0	242.0			

4.6 Comparison between TSH levels and FT4 levels among cases and controls

4.6.1 Comparison between TSH levels among cases and controls

Table 4.23 shows the levels of serum TSH in both cases and controls. Serum TSH levels of cases were higher than that of controls (3.1± 1.7 vs. 2.6± 1.5, % difference= 19.2, **t**=2.7, **P**=0.040).

Table 4.23: Comparison between the levels of serum TSH among cases (n=100) and controls (n=100)

TSH level	Mean ±SD (IU/ml)	Range		% difference	t	P - value
		Min.	Max.			
Cases	3.1±1.7	0.6	9.1	19.2	2.07	**0.040**
Controls	2.6±1.5	0.8	7.8			

4.6.2 Comparison between FT4 levels among cases and controls

As presented in (Table 4.24), the levels of serum FT4 levels in cases were lower than in controls (1.2± 0.338 vs.1.3± 0.348, % difference=-17.6). This change was not statistically significant (**P**=0.259).

Table 4.24: Comparison between the levels of serum FT4 among cases (n=100) and controls (n=100)

FT4 level	Mean ±SD (IU/ml)	Range		% difference	t	P - value
		Min.	Max.			
Cases	1.2± 0.3	0.8	2.0	-17.6	-1.13	**0.259**
Controls	1.3± 0.3	0.8	1.9			

4.7 Relationships between Anti-thyroid Abs and type of miscarriage

4.7.1:Relationship between Anti-TPO Abs and type of miscarriage

Table 4.25, illustrates the distribution of the seropositivity for anti TPO autoantibodies by the type of miscarriage. There was no statistically significant relation between anti TPO autoantibodies and the type of miscarriage, ($\mathbf{X^2}$= 0.076 ***P***=0.782),where 24.1% of the primary aborters (n=13) were positive for anti TPO autoantibodies, while 21.7% of the secondary aborter (n=10) were positive for anti TPO autoantibodies.

Table 4.25: Relationship between Anti-TPO Abs and type of miscarriage

Type of miscarriage	Anti TPO Abs		P - value
	Positive	Negative	
	N0. (%)	N0. (%)	
Primary	13 (94.1)	41 (75.9)	**0.782**
Secondary	10 (21.7)	36 (78.3)	

4.7.2:Relationship between Anti-TG Abs and type of miscarriage

Table 4.26 illustrates the distribution of the seropositivity for anti TG autoantibodies by the type of miscarriage. There was no statistically significant relation between anti TG autoantibodies and the type of miscarriage ($\mathbf{X^2}$=1.717, ***P***=0.190), where 25.9% of the primary aborters (n=10), were positive for anti TG autoantibodies, while 15.2% of the secondary aborters (n=7) were positive for anti TG autoantibodies.

Table 4.26: Relationship between Anti-TG Abs and type of miscarriage

Type of miscarriage	**Anti TG Abs**		**P - value**
	Positive	**negative**	
	N0. (%)	**N0. (%)**	
Primary	14 (25.9**)**	40 (74.1)	**0.190**
Secondary	7 (15.2)	39 (84.8)	

5.1 Relation between anti thyroid autoantibodies Anti TPO and Anti TG Abs and recurrent pregnancy loss

Women with a history of RPL (cases) were compared to multigravida women without any abortion (control) in the Gaza strip. The levels of anti thyroid antibodies (anti TPO antibodies and anti TG antibodies) before pregnancy were determined. The study showed that the majority of positive results for both anti TPO antibodies and anti TG antibodies were in cases. We found that 32.0% of cases (n=100) were positive for one or both anti TPO antibodies and/or anti TG antibodies. On the other hand, 9.0% of control group (n=100) were positive for one or both anti TPO antibodies and/or anti TG antibodies. There was significant relationship between the presence of anti thyroid antibodies anti TPO antibodies and anti TG antibodies and RPL, where ($\mathbf{X^2}$= 10.039, ***P***= 0.002) for anti TPO autoantibodies. There was a statistically significant relation between recurrent pregnancy loss and anti TPO autoantibodies, as well as there was a statistically significant relation between recurrent pregnancy loss and anti TG autoantibodies ($\mathbf{X^2}$= 11.317, ***P***= 0.001).

Antithyroid antibodies are known to occur in normal, healthy population, and these autoantibodies are five times more common in women than in men. Because of prominent prevalence of antithyroid antibodies in normal women, interpreting the significance of these antibodies in women with reproductive problems remains difficult **(136).** It is also suggested that the presence of thyroid autoantibodies reflects a generalized activation of the immune system particularly of T cells, which are ultimately responsible for the loss of the pregnancy **(137).** In the most studies for determining the relationship between autoantibodies and miscarriage, both of anti-TPO and anti-TG antibodies have been measured, in addition to TSH level and FT4 level. Our results were in agreement with other researchers who found a relationship between thyroid autoantibodies and abortion. Stagnaro-Green and his colleague Glinoer were reported a doubling of the miscarriage rate in women who were positive with Antithyroid Abs compared to those with negative Abs (***p*** = 0.011, ***p***<0.001), rspictively **(138, 139).** Also, Lejeune and his colleague were reported

that the miscarriages are associated with Antithyroid Abs and took place early, within the first trimester of the pregnancy **(140).** Several researchers reported that women with TPO Abs and/or TG Abs had a higher miscarriage rate than women who were negative for these Abs (**P**< 0.05) respectively **(141, 142, 143, 133).**

On the other hand, the present results were not concordant with some other studies in this field. Pratt and his colleagues were failed to reach statistical significance (**p**=0.2) when they were tried to evaluate women with recurrent abortions for the presence of thyroid Abs compared with the controls **(128).** Also, Esplin and his colleagues compared the rate of thyroid auto Abs positivity in women with recurrent abortion with controls (p> 0.05 not statistically significant) **(137).** In a study conducted in the same year, no difference was found in miscarriage rates between Ab-negative and Ab-positive women (**p**=0.29) **(144).** In addition, it was concluded that the risk of pregnancy loss in women with unexplained recurrent miscarriages is not affected by their thyroid Abs status **(145).**

5.2 Relation between anti thyroid autoantibodies Anti TPO and Anti TG Abs and thyroid related hormones (TSH and FT4)

It has long been recognized that 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 of thyroid function in pregnancy and has been associated with fetal loss, pregnancy-induced hypertension, preterm delivery, placental abruption, and reduced intellectual function in the offspring. These adverse outcomes have been associated with both overt hypothyroidism (elevated serum TSH concentration and reduced free T_4 concentration), which found in about 0.2% of pregnancies, as well as subclinical hypothyroidism (elevated serum TSH concentration and normal serum free T_4 concentration), found in about 2.3% of pregnancies. Maternal overt hyperthyroidism (suppressed serum TSH and elevated serum free T_4 and T_3 concentrations) is less common, affecting approximately two of 1000 pregnancies. The adverse events for mother and fetus include fetal loss, fetal growth restriction, preeclampsia, and preterm delivery **(146).** Mild or subclinical hyperthyroidism (suppressed serum TSH alone) is found in about 1.7% of

pregnancies and has not been specifically associated with adverse pregnancy outcomes **(147).** Our results data showed statistically significant relationship between anti-TPO Abs and TSH hormone in cases ($\mathbf{X^2}$=9.968, **P**=0.002), but this relation does not reach to statistical significant level in control group ($\mathbf{X^2}$=3.294, **P**=0.07). In our study we found that , there were 5 cases with positive anti-TPO Abs and high TSH compared to 2 cases with negative anti-TPO Abs and high TSH level. These 7 cases were within normal FT4 level, clinically these cases described as subclinical hypothyroidism.

Our results also, showed statistically significant relationship between anti-TG Abs and TSH in cases ($\mathbf{X^2}$= 5.977, **P**= 0.034), but such relation does not reach to statistical significant level in control group ($\mathbf{X^2}$= 5.227, **P**= 0.144). There were 7 cases with high TSH level, distributed as 4 with positive anti-TG Abs and high TSH level compared to 3 cases with negative anti-TG Abs and high TSH level. These cases were also described as subclinical hypothyroidism.

The vast majority of pregnant women with thyroid disease have subclinical hypothyroidism, and any consideration of thyroid function test screening in pregnancy depends on the clinical significance of this condition**(148).** Several studies have shown that adverse events are found in pregnant women with overt and subclinical hypothyroidism but not in women with hypothyroidism adequately replaced with thyroxin replacement therapy (e.g. Leuvotroxine) **(149).** These findings provide evidence for the value of identification and treatment of subclinical hypothyroidism in pregnancy **(133).**

Bagis and his colleagues found that the women who were thyroid Abs positive had a significantly higher TSH and lower free thyroxin compared with the control group **(150).** Sieiro Netto and his colleagues found that risk of miscarriage was significantly higher in TPO Abs positive women and in women with high TSH levels **(151).**

5.3 Relation between anti thyroid autoantibodies (Anti TPO and Anti TG Abs) and age

In the present study, we did not find any correlation between age and presence of anti-TPO, where the age of our study population was 18-30. Similar study have revealed an increase in the prevalence of autoantibodies with age **(152).** In one study, most of the patients with RPL demonstrated an elevated autoantibody titer as age increased up to the age range of 31-35 **.** In addition, an increase in the amount of anti-TPO with age was reported **(153).** However, this correlation was not statistically significant.

5.4 Relation between anti thyroid autoantibodies (Anti TPO and Anti TG Abs) and Antibodies to Toxoplasma, CMV and Cardiolipin

5.4.1 Antibodies to Toxoplasma and CMV

Some maternal infections, especially during the early gestation, can result in fetal loss or malformations because the ability of the fetus to resist infectious organisms is limited and the fetal immune system is unable to prevent the dissemination of infectious organisms to various tissues **(154).** The fetus and/or neonate are infected predominantly by viral but also by bacterial and protozoal pathogens. Infections with various pathogens cause miscarriage or may lead to congenital anomalies in the fetus while others are associated with neonatal infectious morbidity. There are also reports saying that most of the infections of the mother will not result in fetal infection **(155).** Recognizing the prevalence infections with intrauterine and other pathogens in both mother and fetus is an important part of prenatal care **(156).** We did not collect data or analyzed serum to detect anti Cardiolipin Abs. in the control group. Analysis of our study results showed that 17 of the cases (n=100) were positive for abs to Toxoplasma. and 9 were positive for abs. to CMV, these cases represent recurrent pregnancy loss in the Gaza strip. We reported that 5 out of 17 cases with positive abs to Toxoplasma were positive with anti TPO autoantibodies, while 3 out of 17 cases with positive abs to Toxoplasma were positive with anti TG autoantibodies. As well as 2 out of 9 cases with positive abs to CMV were positive with anti TPO autoantibodies, while 1 out of 9 cases with positive abs to CMV was positive with anti TG autoantibodies. Our

results showed that there is no relationship between anti TPO autoantibodies and abs to Toxoplasma, or abs. to CMV (P= 0.490, 0.945) respectively. Also, we did not find statistical significance between anti TG autoantibodies and abs to Toxoplasma, or abs. to CMV (P= 0.709, 0.445), respectively.

Kaur and his colleagues observed antibodies to Toxoplasma in 50.7% of the miscarriage cases and this was significant, compared to it's occurrence in the normal **(157).** As per our study, 17.0% of the cases has Toxoplasma IgG, the immunity status found among this population is low (compared to the 35.6% **(158),** 47.5% **(159)** and 53.6% **(160)**)observed in other populations. The high rate of resistance against Toxoplasma may depend upon the environment and life style of the people here.

As Toxoplasma, the role of CMV is also not very significant in the miscarriage cases. In our study 9.0% of the miscarriage cases were seropositive to CMV. Only 3.2% of the general population is susceptible to this intrauterine pathogen as per their IgG estimation, since 96.8% were immune to it, as observed through their IgG estimation **(161).** Other workers have also observed more or less the same resistance rates as 92.1% **(158),** 87.8% **(159)** and 97.2% **(160)** among their people. As per this study, CMV and Toxoplasma infections have significant role in causing miscarriage. Our results showed that there was no significant difference in the distribution of (TG-abs and TPO-abs) in women who had suffered from recurrent pregnancy loss and positivty of abs. to Toxoplasma or CMV.

5.4.2 Anti Cardiolipin autoantibodies

Anti-Cardiolipin (ACA) is probably the most 'classical' anti phospholipids (APL), as it is used for the definition of APS. It is highly associated with recurrent pregnancy loss **(162).** Previous studies have subsequently confirmed the adverse effects of ACA on pregnancy with the experimental mouse model. The experimental induction of APA causes the increased resumption rate and at the same time decreases placental and embryo weight in pregnant mice **(163).** In our study we found that 15% of the cases were positive for antibodies to Cardiolipin. The percentages of anti- Cardiolipin antibody in patients with recurrent abortions and fetus death in the different studies **(164).**

We reported that 15 out of 100 cases were positive for anti- Cardiolipin but 85 were negative and 5 out of 15 cases were positive for anti TPO autoantibodies, as well as 5 out of 15 cases with positive for anti TG autoantibodies. We did not collect data or analyzed serum to detect anti Cardiolipin Abs. in the control group. Our results showed that there is no relationship between anti TPO autoantibodies and abs to Cardiolipin (P=0.302). Also, we had not found relationship between anti TG autoantibodies and abs to Cardiolipin (P= 0.203). It was reported that APA was found in 10-15 % of women with fetal death **(165).** Although APA may be a risk factor for pregnancy losses **(166)**. An association of APA with recurrent idiopathic pregnancy losses was reported **(167)** and fetal death is associated with APA **(168),** our results showed that there was no significant difference in the distribution of (TG-abs and TPO-abs) in women who had suffered from recurrent pregnancy loss and positivity of abs. to Cardiolipin. In other prospective studies, investigators did not find any relation between APA and fetal growth restriction **(169).** Kdous and his colleagues reported that the exact mechanism of APA remains controversial **(170).**

CHAPTER SIX CONCLUSIONS & RECOMMENDATIONS

6.1 Conclusions

Auto immune disease is considered as a risk factor for RPL in Gaza strip, where 32% of healthy non pregnant women with history of spontaneous recurrent abortion during the first trimester have one or both anti-thyroid autoantibodies (TG-abs and/or TPO-abs), it appears to be increased compared with controls of reproductive age women without previous abortions were 9.0% of them were positive for TG-abs and/or TPO-abs. However, we conclude that:

- There is a relationship between anti-thyroid autoantibodies TG-abs and TPO-abs and recurrent pregnancy loss in women in Gaza strip.
- The presence of one or both TG-abs and/or TPO-abs increased the risk of recurrent pregnancy loss in the women in Gaza strip.
- There is significant difference in the distribution of TG-abs and TPO-abs in women who had suffered from recurrent pregnancy loss and control ones in Gaza strip.
- The levels of TG-abs and TPO-abs were higher in women who had suffered from recurrent pregnancy loss than in controls.
- The levels of TSH were elevated or normal in both women with positive TG-abs and/or TPO-abs and control subjects.
- There is no significant difference in the distribution of free T4 in women who had suffered from recurrent pregnancy loss and control subjects in Gaza strip.

- the positivity of TG-abs and TPO-abs was not increased with age in our study population due to the low range of their age (18-30)..

- There was no significant difference in the distribution of TG-abs and TPO-abs in women who had suffered from recurrent pregnancy loss and residency.

- There was no significant difference in the distribution of (TG-abs and TPO-abs) in women who had suffered from recurrent pregnancy loss and positivity of abs. to Cardiolipin, Toxoplasma or CMV.

6.2 Recommendations

- Women with Anti-thyroid autoantibodies (TG-abs and/or TPO-abs) in early stages of pregnancy are still at risk of developing hypothyroidism later; therefore, their serum TSH levels should be monitored.
- If hypothyroidism has been diagnosed before pregnancy, it is recommended to adjust rapidly preconception thyroxine doses to reach a TSH level prior to pregnancy not higher than 5.0mU/L.
- Thyroxine replacement is recommended in women with Subclinical hypothyroidism.
- In all pregnant women with a goiter, high antithyroid antibody titer, family history of thyroid disease, any history of other autoimmune endocrine disease, or symptoms suggestive of hypothyroidism, serum TSH testing should be performed.
- In pregnant women who are found to have an increased serum TSH level, even if mild, hormonal replacement therapy e.g. levothyroxine, should be promptly initiated.
- Pregnant women who have high TPO-Abs titers but normal serum TSH levels should undergo careful postpartum and long-term follow-up because of the high probability of subsequent clinical hypothyroidism.
- Serum TSH testing should be done in all women considering pregnancy so that hypothyroidism can be diagnosed early and treated before pregnancy.

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Annex I

Palestinian National Authority
Ministry of Health
Helsinki Committee

السلطة الوطنية الفلسطينية
وزارة الصحة
لجنة هلسنكي

التاريخ9/9/2009

Name:

I would like to inform you that the committee has discussed your application about:

Anti thyroid antibodies in Palestinian women suffering from Recurrent Abortion in Gaza Strip

الاسم:سامي سعيد الديراوي

نفيدكم علماً بأن اللجنة قد ناقشت مقترح دراستكم

حول:- ***الاجسام المضادة للغدة الدرقية في المرأة الفلسطينية التي تعاني من الاجهاض المتكرر في قطاع غزة***

In its meeting in september 2009

and decided the Following:-

To approve the above mention research study.

و ذلك في جلستها المنعقدة لشهر 9 2009

و قد قررت ما يلي:-

الموافقة على البحث المذكور عاليه.

Signature
توقيع

Member عضو

Member عضو

Chairperson

Conditions:-

- Valid for 2 years from the date of approval to start.
- It is necessary to notify the committee in any change in the admitted study protocol.
- The committee appreciates receiving one copy of your final research when it is completed.

Annex II

الجـــــــامعة الإسلامية – غزة كلية العلوم

The Islamic University of Gaza

مدير برنامج ماجستير العلوم الحياتية

التاريخ/ 2007/5/21م

الأخت الدكتورة / رندة الخضري مدير دائرة المختبرات في وزارة الصحة حفظه الله ،،،

السلام عليكم ورحمة الله وبركاته ،،،

الموضوع / تسهيل مهمة باحث

نود أن نعلم سيادتكم بان الطالب / سامي الديراوي. طالب في ماجستير العلـــوم الحياتية - تخصص تحاليل طبية في الجامعة الإسلامية يقوم بإجراء.

بحث بعنوان :

Anti Thyroid Antibodies in Palestinian Women Suffering From Recurrent Abortion in Gaza Strip.

والطالب يحتاج الحصول علي العينات والمعلومات حتى يتمكن من إجراء البحث.

لذا نرجو من سيادتكم تسهيل مهمة الباحث.

ولكم جزيل الشكر والتقدير ،،،

مدير برنامج ماجستير العلوم الحياتية
د. عبود ياسر القيشاوي

Islamic University P. Box 108 AlRimal Gaza Palestine الجامعة الإسلامية-غزة - الرمال ص.ب:108 فلسطين
Tel:(970/8)2860700 Fax:(970/8)2860700 2863552 e-mail:public@mail.iugaza.edu Web Site:www.iugaza.edu

Annex III

Questionnaire for Recurrent Aborters Women

==========

Case No._____ **Date: ____ /____ /___ _____**
Name: _____ **Age: _____**
Address_____ **Tel. No._____**
==========

Number of miscarriages: ☐

Gestational age before miscarriage: ☐

Having a live birth ☐

Number of a live birth before RPL ☐

Time after last miscarriage (months) ☐

Have you ever been told in the past, that you have had any of the following?

Autoimmune Disorder ☐
If yes, Autoantibodies……..
…….

Endocrine Disease ☐

If yes,……

Thyroid Problems ☐

If yes, HYPO ☐ **HYPER** ☐

Receiving any medication? ☐

If yes,……

Infectious diseases? ☐

If yes,……

Hematological disorder ☐

If yes,……

……