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Title:

Association Between Antithyroid Antibodies and Thyroid Stimulating Hormone in Recurrent Spontaneous Abortion inWad Medani, Maternity Hospital, Gezira State, Sudan.

A Thesis Submitted in Partial Fulfillment for the Requirements of Master Degree in Medical Laboratory Sciences (Clinical Chemistry)

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DECLARATION

I authorized that my dissertation "**Detection of Antithyroid Antibodies and TSH in Recurrent Spontaneous Miscarriage in Maternity Hospital, Wad Medani, Gezira State, Sudan. (2018)**" submitted by me, under the supervision of **Dr. Mosab omer khalid mohammed zeen**for the partial fulfillment for the award of Master degree in Medical Laboratory Sciences in clinical chemistry. University of shendi higher graduate studies Scientific Research. Department of clinical chemistry; shendi, Sudan. Ideclare and affirm that this Thesis is my own original work. I had followed all ethical and technical principles in the preparation, data collection, data analysis and compilation of this Thesis and it was not submitted in part or in full, in any printed or electronic means, and is not being considered elsewhere for publication".

Name and Signature of Candidate:

Name: **Ahmed Abdullah Mubarak Almidasi**

Signature ……

Date: / / 2018

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قال تعالى :

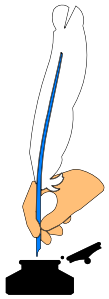
﴿ قَالُواْ سُبْحَانَكَ لاَ عِلْمَ لَنَا إِلاَّ مَا عَلَّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ ﴾

صدق الله العظيم

سورة البقرة(الآية٣٢)

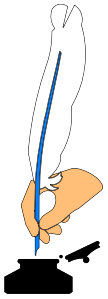
Dedication

We dedicate this work to my family.



To my teachers

To my lovely friends.



To anyone who encouraged

and supported me.

Ahmed

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Abstract

Thyroid autoimmunity in pregnant women can cause undesirable complications that can result into abortion. Abortion has been defined as the termination of pregnancy, either spontaneously or by intervention before the fetus reaches viability. Recurrent spontaneous abortion (RSA) refers to the consecutive occurrence of fatal loss (body weight < 1000 g) happening more than 2 times before (28) gestational weeks with the same sex partner. This case control study aimed to determine the association between anti-thyroid antibodies (ATA) and recurrent spontaneous abortion (RSA) in Sudanese women. In this study, a total of (90)blood samples were collected, (45)(case samples) from women with recurrent abortion while (45)(control samples) from non- abortion pregnant women. Both case and control samples were from women at reproductive age (17-44). Control samples were collected from Obstetrics and Gynecology Hospital, Wad-Madani. (5 ml) of blood specimens were collected from each participated women and dispensed into container and the serum was obtained by centrifugation at 3000 R/min for (5 mins). Detection for the levels of *ATA* using ELISA technique *and TSH* was done using electroimiluminescence technique (COBAS E 411). Case and control samples were collected from Obstetrics and Gynecology Hospital, Wad-Madani.

The result showed that out of (90) blood specimens were investigated, 13/90 (14.4%) were positive for TPO-Ab antibodies. Out of the cases samples exhibited 11/45(24.4%) were positive and 34/45(75.6%) were negative, while only 2/45 (4.4%) of the control samples tested positive and the rest of 43/45(95.6%) tested negative.

For TG-Ab, 3/45 (6.7 %) of test samples tested positive, 42/45 (93.3%) tested negative, only 1/45 (2.2%) of the control samples tested positive and 44/45 (97.8%) tested negative. The presence of both TPO-Ab and Tg-Ab in the target group was 14/90 (15.5%).

For TSH, 11/45(24.4%) of test samples had high levels, 34/45(75.6%) tested normal.2/45(4.4%) of the control samples had high levels, 41/45(91.1%) tested normal, and only 2/45(4.4%) tested low. The highest Sero frequency of TPO-Ab and TSH levels were noticed among those with first trimester abortions, (P.value = 0.012). The study observed that the TSH concentration was increase in abortion women with positive antibodies compared with the concentration of TSH in the control group with positive

antibodies. Considering occupation, education level, number of abortions, biomass index and family history of the participants there was no statistically significant correlation with ATA and TSH levels ($P > 0.05$).

This current study concluded that the seroprevalence of TPO-Ab among recurrent abortion pregnant women in AL- Gezira, State was higher compared to non-abortion pregnant women. The risk of abortion was high in positive thyroid antibodies pregnant women when the TSH level is relatively high in the first trimester. Further TPO-Ab screening in pregnancy in first trimester, may aid in early identification of the women at risk.

الخلاصة

المناعة الذاتية للغدة الدرقية لدى النساء الحوامل قد تتسبب في حدوث مضاعفات غيرمرغوب فيها يمكن أن تؤدي إلى الإجهاض . يُعرّف الإجهاض بأنه إنهاء الحمل،إما بشكل تلقائي أو بالتدخل الخارجي قبل وصول الجنين الأسبوع الثامن والعشرين . ويُعّرف الإجهاض التلقائي المتكرر بأنه حدوث ثلاثة إجهاضات متتالية او أكثر بعمرمن ثمانية وعشرين أسبوع في نفس الزوجين.

هذه الدراسة أجريت لتحديد مدى الانتشارالمصلي للأجسام المضادة للغدة الدرقية وهرمون تحفيز الغدة الدرقية بين النساء المجهضات والغير مجهضات المترددات على مستشفى النساء والولادة ود مدني الجزيرة. شملت الدراسة عينة تكونت من)٩٠(امرأة حاملا كان من ضمنهم (٤٥) امرأة تعرضت للإجهاض المتكرر (عينة تجريبيه) و (٤٥) امرأه حامل لم يحصل لهن إجهاض (عينة ضابطة). كل النساء التي شملتهن الدراسة تتراوح أعمارهن ما بين (٤٤-١٧).تم جمع (٥ مل) من عينة دم وريدي من كل النساء الحوامل وتم الحصول على البلازما بواسطة جهاز الطرد المركزي عند (٣٠٠٠) دورة لكل دقيقة لمدة (٥) دقائق، وتم فحص مصل الدم لوجود الاجسام المضادة للغدة الدرقية بإستخدام تقنية الأنزيم المناعي المرتبط (الاليزا) والهرمون المحفز للغدة الدرقية بإستخدام تقنية التوهج الكهربائي بجهاز (كوباس). أظهرت الدراسة أن (٩٠/١٣) بنسبة مئوية (١٤,٤٪) كانت إيجابية للأجسام المضادة للبيروكسيديز TPO-Ab في كل العينات، حيث كانت في المجموعة التجريبية (٤٥\١١) بنسبة مئوية (٢٤,٤) إيجابيه للأجسام المضادة للبيروكس ييز TPO-Abو (٤٥\٣٤) بنسبة مئوية (٧٥,٦٪) كانت سلبية، وفي العينة الضابطة فقط (٤٥\٢) بنسبة مئوية (٤,٤٪) كانت إيجابية للأجسام المضادة للبيروكسيديز TPO-Ab و(٤٥\٤٣) بنسبة مئوية (٩٥,٦٪) كانت سلبية للأجسام المضادة للبيروكسيديز TPO-Ab .

وقد اظهرت الدراسة ان (٤٥\١٣) بنسبة مئوية (٦,٧٪) من العينات التجريبه كانت إيجابية للأجسام المضادة للثيروقلوبيولين TG–Ab مقارنة ب (٤٥\١٤٢) بنسبة مئوية (٩٣,٣٪) كانت سلبية في العينات التجريبية، بينما في العينات الضابطة أظهرت أن (٤٥\١١) بنسبة مئوية (٢,٢٪) كانت إيجابية للأجسام المضادة للثيروقلوبيولين TG–Ab و(٤٥\١٤٤)بنسبة مئوية (٩٧,٨٪) كانت سلبية للأجسام المضادة للثيروقلوبيولين TG–Ab، وأظهرت الدراسه وجود كل منالأجسام المضادة للبيروكسيديز TPO– Ab و الأجسام المضادة للثيروقلوبيولين Tg–Ab معافي كل العينات (٩٠/١٤) بنسبة مئوية (١٥,٥٪).

وقد اظهرت الدراسة ان (٤٥\١١١) بنسبة مئوية (٢٤/٤) من العينات التجريبية كانت فوق المعدل الطبيعي للهرمون المحفز للغدة الدرقية TSH، و (٤٥\١٣٤) بنسبة مئوية (٧٥,٦٪) ضمن المعدل الطبيعي و (٤٥/٢) بنسبة مئوية (٤,٤٪) فقط بمعدل منخفض،بينما في العينات الضابطة كانت (٤٥\١٤١) بنسبةمئوية (٩١,١٪) ضمن المعدل الطبيعي،وفقط (٤٥\١٢) بنسبةمئوية(٤,٤٪) بمعدلات عالية.

لوحظ أن أعلى مستويات أنتشار مصلي للـ TPO–Abوأعلى معدلات TSHكانت بين الساء اللاتي أجهضن خلال الثلث الاول من الحمل وكانت الدالة الأحصائية لكلاً منهما (P.value=0.012) ، وأظهرتالدراسة أنتركيز TSH كان يزداد في حالات الإجهاض لدى النساء ذات الأجسام المضادة الإيجابية مقارنة بتركيز الهرمون TSH في المجموعة الضابطة ذات الأجسام المضادة الإيجابية.

عند مقارنة الأنتشار المصلي للأجسام المضادة للغدة الدرقية وتركيزالهرمون المحفز للغدة الدرقية مع ك لاً من الوظيفة ومستوى التعليم وعدد حالات الإجهاض ومؤشر الكتلة الحيوية و التاريخ العائلي للمشاركين، لم يكن هناك إرتباط ذو دلالة إحصائية (P.value > 0.05)

خلصت الدراسة إلىأن إلىأن أنالانتشارالمصلي للأجسام المضادة للبيروكسييز TPO–Ab بين النساء الحوامل ذوات الإجهاض المتكرر في ولاية الجزيرة لا كانأعلى مقارنةً

بالنساءالحوام لا للواتي لم يسبق لهن الأجهاض ، وكان خطر الإجهاض عالياً عندما يكون مستوى هرمون TSH مرتفع نسبياً في الثلث الأول من الحمل في النساء اللاتي كان لديهن إيجابية للأجسام المضادة للغدة الدرقية .وخلصت الدراسة الى أن الكشف المبكر للأجسام المضادة للغدة الدرقية TPO–Abفي الأشهر الثلاثة الأولى من الحمل قد يساعد في التعرف المبكرعلى النساء المعرضات لخطر الإجهاض.

Table of Contents:

List of figures:

List of Tables:

List of abbreviations

Abbreviation	Meaning
ATA	Antithyroid antibodies
anti-Tg	Antithyroglobulin
anti-TPO	Antithyroperoxidase
APCA	antipaternal cytotoxic antibodies
ART	Assisted reproductive technology
CNS	central nervous system
CTLA4	cytotoxic T lymphocyte–associated antigen-4 gene
DNA	Deoxyribonucleic Acid
FSH	Follicle-stimulating hormone
FT3	Free triiodothyronine
FT4	free thyroxine
GD	Graves' disease
HCG	Human chorionic gonadotrophin
HT	Hashimoto' thyroiditis
H-P-T	hypothalamic-pituitary-thyroid
LH	Luteinizing hormone
PM	Primary myxedema
RA	Recurrent abortion
RSA	Recurrent spontaneous abortion
AMP	Adenosine monophosphate
TAI	Thyroid autoimmunity
TBII	TSH-binding inhibitory immunoglobulins
TG	Thyroglobulin
TgAb	Thyroglobulin antibody
TPO	Thyroid peroxidase
TPOAb	Antithyroperoxidase antibody
TRH	Thyrotropin releasing hormone
TSBAb	thyroid stimulation blocking antibodies
TSH	Thyroid stimulating hormone
TSHR-Ab	TSH receptor antibodies
T3	Triiodothyronine
T4	Thyroxine
mins	Minutes
WHO	World Health Organization
SD	Standard deviation
HRP	Horseradish peroxidase

Abbreviation	Meaning
µL	Microliter
Nm	Nanometer
G	Gram
IU/ml	International unit per milliliter
mIU/L	Mili international unit per liter

1.1 Introduction:

Abortion is one of the most problems that most pregnant women are worried about (Singh et al., 2005).Thyroid autoimmunity (TA) is defined as the presence of antithyroid antibodies (ATA), specifically antithyroglobulin (anti-TG) and antithyroid peroxidase (anti-TPO) (He et al., 2016). characterized by abnormal lymphocytic activation directed against self-tissues. Hashimoto's thyroiditis (HT) and Graves' disease (GD) are two of the most common clinical expressions of organ-specific autoimmunity (Cogni and Chiovato, 2013)**.** Thyroid autoimmunity (TA) is the most prevalent autoimmune disease in women of reproductive age, with a prevalence of (5 -15%), and in women with infertility, the prevalence is (10 - 31%). (TA) was shown to be associated with many kinds of adverse obstetric outcomes, such as preterm delivery, placental abruption, and low birth weight. The association with abortion was first reported in 1990 by (Stagnaro-Green) Subsequently, the number of studies on the association between (TA) and abortion increased substantially, however, the results were conflicting (He et al., 2016). In the developed world, thyroid autoimmunity is the main cause of hypothyroidism, which itself results in poor obstetric outcomes. Even in women with biochemically, normal thyroid function, studies have reported an association between the presence of thyroid autoantibodies, particularly thyroid peroxidase antibodies and adverse pregnancy outcomes, including abortion, preterm birth, and adverse neurodevelopmental sequelae in children. The exact mechanisms of these associations are unknown, though two have been proposed. Firstly, the presence of thyroid autoantibodies in women with normal thyroid function could be associated with a subtle deficiency in the availability of thyroid

hormones (a fall in circulating free thyroid hormones within the reference range) or a lower capacity of the thyroid gland to adequately rise to the demand for augmented synthesis of thyroid hormones required in pregnancy (Thangaratinam et al., 2011). Secondly, the increased risk of abortion should be attributed to the direct action of the thyroid autoantibodies, as suggested by the action of anti-Tg on the placenta in mice models (Toulis et al., 2009). Many studies have suggested that the presence of antithyroid antibodies carry an increased risk for spontaneous abortion and: or obstetric complications. A significant association between thyroid autoimmunity (TAI) and risk of abortion has been shown by various, but not all studies (Sieiro Netto et al., 2004). A considerable increase in the risk of abortion has been shown among women aged (35) years or more. So, these studies addressed a population of women who already presented a high risk of spontaneous abortion (Sieiro Netto et al., 2004).

Thus, in this study aims to determine presence of anti-TPO and anti-Tg antibodies and TSH among spontaneous recurrent abortion and non-abortion women and study the correlation with different parameters like age, tribe, job, biomass index, education levels, abortion, and number of abortion and in which stage occurred.

1.2 Justification:

Beyond the study by (Zahran, 2010) no data have been recorded for the actual situation of abortion s in association with antithyroid antibodies diseases in Sudan. Increased abortion rate and recurrent spontaneous abortion was noticed through registries of the major hospital in Sudan (Zahran, 2010). increased rapidly and there was no solid data for actual percentage. The thyroid disorder contributes somehow to morbidity and mortality; there is a little data on thyroid dysfunction among women who exposed to RSA. The present study conducting on thyroid field to determine the Sero-detection of thyroid antibodies and TSH among recurrent spontaneous abortion, that may add more information in this field of study.

1.3 Objectives:

To Evaluate Association Between Antithyroid Antibodies, Thyroid Stimulating Hormone and Recurrent Spontaneous Abortion in Wad Medani, Maternity Hospital, Gezira State, Sudan.

1.3.2 Specific objectives:

- To detect antithyroperoxidase and antithyroglobulin antibodies in women with recurrent spontaneous pregnancy loss.

- To determine correlation between Thyroid Stimulating Hormone level and recurrent spontaneous pregnancy loss.

-To determine the associated factors related with antithyroid (antithyroperoxidase and antithyroglobulin) antibodies including age, trimester, occupation, etc….

2. literature review

2.1 Abortion

2.1.1 Definition of abortion

Abortion , also known as spontaneous abortion and pregnancy loss, is the natural death of an embryo or fetus before it is able to survive independently. Some use the cut off of (20) weeks of gestation after which fetal death is known as a stillbirth. The most common symptoms of abortion is vaginal bleeding with or without pain (Cannon et al., 2010). The World Health Organization (WHO) definition is the expulsion or extraction from its mother of an embryo or fetus weighing (500 g) or less (Stirrat, 1990). There are two types of abortion sporadic and recurrent. Recurrent abortion affects about (1%) of couples. By contrast, at least (25%) and probably as many as (50%) of all women experience one or more sporadic abortion s, usually due to random fetal chromosomal abnormalities, the risk of which rises with increasing maternal age (Rai and Regan, 2006). A preclinical abortion is defined as a demise, which occurred before (6) weeks of gestation. Clinical abortion can be divided into embryonic or fetal. Embryonic abortion is defined as an embryo with a crown rump length of (25 mm), without cardiac activity. A fetal abortion is defined as a fetus of (10 to 20) weeks' size, without cardiac activity (Stephenson and Kutteh, 2007).

2.1.2 Recurrent spontaneous abortion (RSA):

Recurrent abortion (RA) is an important complication of pregnancy .It affects approximately (3–5%) of couples and represents a significant clinical problem (Ticconi et al., 2011). Moreover, after three consecutive pregnancy losses, the possibility of a 4th abortion ranges from (32 to 47%) (Dendrinos et al., 2000). Potential amount of possible abortion before pregnancy is

recognized to be about (30%). In clinically recognized pregnancy, it is (10–15%) before 8th week and (3%) between 8th and 28th weeks (Lata et al., 2013).

2.1.3 Etiology of recurrent spontaneous abortion

Several factors have been implicated in the etiology of RSA (genetic, anatomic, infectious, endocrine, immunologic, etc.). Immunologic factors are considered important in (30-80%) of cases according to different studies. The immune system plays an important role in the implantation and viability of the fetus, through a mechanism of immunologic recognition by the maternal immune system. The current understanding of the immunology of pregnancy points at two separate etiologies for RSA. Alloimniune causes refer to the failure of normal immune recognition of the fetus by the maternal body, and can be detected by the failure of the maternal immune system to mount an appropriate response to paternal antigens contained in fetal tissues (negative antipaternal cytotoxic antibodies; APCA). Autoimmune causes refer to the presence of a spectrum of autoantibodies that has been linked with increased spontaneous abortion. They are broadly divided into two categories: non-organ-specific, such as antiphospholipid (lupus anticoagulant and anticardiolipin), anti DNA and antinuclear antibodies; and organ-specific, like antithyroid antibodies (ATA; thyroid peroxidase (TPO) and thyroglobulin Tg) and anti-ovarian antibodies (Dendrinos et al., 2000).

2.2 Thyroid gland:

The thyroid gland, located immediately below the larynx on each side of and anterior to the trachea, is one of the largest of the endocrine glands, normally weighing (15 to 20) grams in adults (Hall, 2011). and it consists of two lobes

connected by an isthmus. The thyroid gland develops from the floor of the primitive pharynx during the 3rd week of gestation. The mature thyroid gland contains numerous spherical follicles composed of thyroid follicular cells that surround secreted colloid fluid contains large amounts of thyroglobulin, the protein precursor of thyroid hormones. The thyroid hormone synthesis normally begins at about (11) weeks of gestation (Sadlar, 2004).Thyroid gland follicles play a critical role in compartmentalizing the necessary components for thyroid hormone synthesis (Choksi et al., 2003).

2.2.1 Thyroid hormones:

The thyroid secretes two major hormones, thyroxine and triiodothyronine, commonly called T4 and T3, respectively. Both of these hormones profoundly increase the metabolic rate of the body (Hall, 2011). With the T3 being the most active form. and they are released into systemic circulation in response to thyroid stimulating hormone (Smith et al., 2002). About (93%) of the metabolically active hormones secreted by the thyroid gland is thyroxine, and (7%) triiodothyronine. However, almost all the thyroxine is eventually converted to triiodothyronine in the tissues, so both are functionally important. The functions of these two hormones are qualitatively the same, but they differ in rapidity and intensity of action. Triiodothyronine is about four times as potent as thyroxine, but it is present in the blood in much smaller quantities and persists for a much shorter time than does thyroxine. The thyroid gland also secretes calcitonin, an important hormone for calcium metabolism (Hall, 2011).

2.2.2 Synthesis of Thyroid hormones:

One of the starting molecules for thyroid hormone synthesis is thyroglobulin (Tg), a glycoprotein that comprises (115 – 123) tyrosine residues. Epithelial

cells of the thyroid gland have a sodium-iodide symporter on the basement membranes that concentrates circulating iodide ion from the blood. Once inside the cell, iodide ion is transported to the apical membrane of thyroid follicular cells where it is oxidized by thyroid peroxidase (TPO) and hydrogen peroxide. The reactive iodine atom is added to selected tyrosyl residues within Tg. The iodotyrosines in Tg are then coupled via a reaction catalyzed by TPO and produced either T3 or T4, depending on the number of iodine atoms present in the iodotyrosines (Choksi et al., 2003, Kasper et al., 2005). After coupling, Tg is taken back into the thyroid cell where it is processed in lysosomes to release T4 and T3. Uncoupled mono- and diiodotyrosines are deiodinated by enzyme, thereby recycling any iodide that is not converted into thyroid hormones (Murray et al., 2000, Kasper et al., 2005). Free thyroid hormones diffuse into blood where they reversibly complex with liver-derived binding proteins for transport to other tissues. Thyroxine binds to several proteins in the blood and the free form (0.03%) is traditionally held to be the physiologically important form of the hormone, with the binding proteins serving three functions: firstly, is maintenance of a large pool with a constant free hormone concentration. Secondly is ensuring an even distribution of hormone among peripheral cells. The third function is minimizing hormone loss by renal filtration (Ganong, 2003, Poppe et al., 2007). Thyroid hormones have the profound effect of increasing the metabolic rate of the body. Complete lack of thyroid secretion usually causes the basal metabolic rate to fall (40 to 50%) below normal, and extreme excesses of thyroid secretion can increase the basal metabolic rate to (60-100%) above normal (Guyton and Hall, 2006). Thyroid stimulating hormone or thyrotropin (TSH), which is secreted by the anterior pituitary gland, regulates thyroid hormone synthesis and secretion. TSH is a large

glycoprotein composed of two subunits (α and β). The α–subunit is the same in LH and FSH, while the β – subunit is specific for TSH. TSH acts by binding to specific receptors on the surface of the thyroid cells and stimulates cyclic AMP synthesis, which activates protein kinases which, in turn, promotes maintenance of the thyroid gland and stimulates the synthesis and release of thyroid hormones (Gosling and Basso, 1994, Murray et al., 2000). Hypothalamic thyrotropin-releasing hormone (TRH) stimulates TSH secretion from the anterior pituitary. TSH then initiates thyroid hormone (TH) synthesis and release from the thyroid gland. Although opposing (TRH) and (TH) inputs regulate the hypothalamic-pituitary-thyroid axis, TH negative feedback is thought to be the primary regulator. Control of circulating concentrations of thyroid hormone is regulated by negative feedback loops within the hypothalamic-pituitary-thyroid (H-P-T) axis. (TH) negative feedback at the pituitary is believed to be the most important physiological regulator of serum TSH levels (Cohen et al., 2000, Nikrodhanond et al., 2006).

2.2.3 Thyroid hormones during pregnancy:

The main change in thyroid function associated with the pregnant state is the requirement of an increased production of thyroid hormone (Glinoer, 2004). Those changes require an increased availability of thyroid hormones by (40 to 100%) in order to meet the needs of mother and fetus during pregnancy (Smallridge et al., 2005). the requirement of an increased production of thyroid hormone depends directly upon the adequate availability of dietary iodine and integrity of the glandular machinery. Physiologic adaptation takes place when the iodine intake is adequate, while this is replaced by pathologic alterations when there is a deficient iodine intake (Glinoer, 2004). These

physiological changes during pregnancy can affect the reference intervals of TSH and thyroid hormones in the serum. Serum thyrotropin (TSH) level decreases during the first trimester, and then increases gradually. The serum free thyroxine (FT4) level usually increases in early pregnancy and then decreases (Shan et al., 2009). The hypermetabolic state of normal pregnancy can mimic the features of thyroid disease. Human chorionic gonadotrophin (hCG) can stimulate the thyroid gland during the first trimester because of its structural similarity to thyrotropin (TSH). Increased sialylation, mediated by oestrogens, reduces the clearance of thyroxine-binding globulin, resulting in increased levels of total T4 and T3. Changes in albumin and free fatty acid concentrations affect the binding of T4 and T3 to carrier proteins, lowering the blood levels of free hormones (FT4 and FT3) as pregnancy progresses (Panesar et al., 2001).

2.2.4 Maternal thyroid hormones and fetal development:

Optimal functioning of thyroid gland is essential at all stages of life, including pregnancy and fetal development. Sufficient evidence is available showing that thyroid dysfunction during pregnancy can affect not only the maternal outcome but also the neuropsychological development of fetus (Marwaha et al., 2008). Thyroid hormones play a crucial role in the development and physiological functioning of the central nervous system (CNS). Thyroid hormones regulate the neuronal growth and receptors, so any deficiency or increase of them (hypo- or hyperthyroidism) during these periods may result in an irreversible impairment, morphological abnormalities, disorganization, maldevelopment and physical retardation (Kilby et al., 2005). A large body of evidence strongly suggests that thyroid hormone is an important factor contributing to normal fetal brain

development. In the first trimester of gestation the fetus is completely depend on thyroxine from the mother for normal neurologic development (Smallridge et al., 2005). Mild maternal thyroid dysfunction has been reported to be associated with spontaneous abortion, fetal death preterm delivery, small head circumference and low birth weight, and impaired neuropsychological development. In addition, a low maternal T4 level is a risk factor for breech presentation, whereas maternal hyperthyroidism increases the incidence of hip dysplasia (Su et al., 2011).

2.2.5 Thyroid disorders:

2.2.5.1 Hyperthyroidism (Thyrotoxicosis):

Thyrotoxicosis describes a constellation of clinical features arising from elevated circulating levels of thyroid hormone. The most common causes are Graves' disease, multinodular goitre and autonomously functioning thyroid nodules (toxic adenoma) (Walker and Colledge, 2014).

2.2.5.1.1 Symptoms and Signs of Hyperthyroidism:

Hyperactivity, irritability, altered mood, insomnia, heat intolerance, increased sweating, palpitations, fatigue, weakness, dyspnea, weight loss with increased appetite (weight gain in 10% of patients), pruritus, increased stool frequency, thirst and polyuria, oligomenorrhea or amenorrhea and loss of libido (Weetman, 2000). sinus tachycardia, atrial fibrillation, fine tremor, hyperkinesis, hyperreflexia, warm, moist skin, palmar erythema, onycholysis, hair loss, muscle weakness and wasting, congestive (high-output) heart failure, chorea, periodic, paralysis (primarily in Asian men) and psychosis (Weetman, 2000).

2.2.5.1.2 Treatment of Hyperthyroidism:

Definitive treatment of thyrotoxicosis depends on the underlying cause and may include antithyroid drugs, radioactive iodine or surgery (Walker and Colledge, 2014).

2.2.5.2 Hypothyroidism:

is a common condition with various causes, but autoimmune disease (Hashimoto's thyroiditis) and thyroid failure following ^{131}I or surgical treatment of thyrotoxicosis account for over (90%) of cases, except in areas where iodine deficiency is endemic. Women are affected approximately six times more frequently than men (Walker and Colledge, 2014).

2.2.5.2.1 Clinical features of Hypothyroidism:

Hypothyroidism produces many symptoms. The alternative term 'myxoedema' refers to the accumulation of mucopolysaccharide in subcutaneous tissues. The classic picture of the slow, dry-haired, thick-skinned, deep-voiced patient with weight gain, cold intolerance, bradycardia and constipation makes the diagnosis easy. Milder symptoms are, however, more common and hard to distinguish from other causes of non-specific tiredness. Many cases are detected on biochemical screening (Ballinger, 2009).

2.2.5.2.2 Treatment of Hypothyroidism:

is with levothyroxine replacement (Walker and Colledge, 2014).

2.3 Thyroid autoimmunity:

Autoimmune diseases are a group of heterogeneous disorders characterized by abnormal lymphocytic activation directed against self-tissues. Hashimoto's thyroiditis (HT) and Graves' disease (GD) are two of the most common clinical expressions of organ-specific autoimmunity (Stathatos and

Daniels, 2012).They fulfill all the required criteria for autoimmune diseases including:

1) infiltration of the thyroid by lymphocytes, which are auto-reactive to thyroid antigens;

2) presence of circulating thyroid autoantibodies;

3) immunological overlap with other autoimmune diseases;

4) a story of familiar occurrence, mainly in females;

5) the possibility to produce both experimental autoimmune thyroiditis and, to a lesser extent, Graves' disease in laboratory animals (Rose and Bona, 1993).Thyrotropin receptors are primarily expressed on the surface of thyrocytes. Thyrotropin (TSH). produced by the anterior pituitary binds to the receptor and stimulates thyroid hormone production (T3, triiodothyronine and T4, tetra iodothyronine). Increasing levels of thyroid hormones send negative signals to the hypothalamus and downregulate thyrotropin-releasing hormone (TRH) production, which, in turn, downregulates TSH production. As the circulating levels of thyroid hormones decline, there is upregulation of TRH and TSH production, leading to the activation of the thyroid gland (Segerson et al., 1987, Wondisford et al., 1989). The biological effects of TSH include the differentiation, proliferation, and regulation of the function of thyroid cells. In patients with thyrotropin receptor-mediated autoimmune diseases, this normal homeostasis is perturbed, leading to thyroid dysfunction. Autoimmunity to the thyrotropin receptor is unique in that the clinical outcome is dependent upon the nature of the antibodies generated against the receptor. If the autoantibodies bind to the receptor and stimulate the thyroid gland (thyroid stimulatory antibodies, TSAb), it can lead to Graves' disease

(GD) characterized by hyperthyroidism. On the other hand, if the antibodies block either TSH binding (TSH-binding inhibitory immunoglobulins, TBII) or TSH-mediated of the thyroid (thyroid stimulation blocking antibodies, TSBAb), then it can cause Hashimoto thyroiditis primary myxedema (PM) characterized by hypothyroidism (Patibandla and Prabhakar, 1996).

2.3.1 Graves' disease:

Graves' disease is an autoimmune syndrome in which thyroid-stimulating antibodies bind to and activate the thyrotropin receptor on thyroid cells, resulting in hyperthyroidism (Weetman, 2000). This is the most common cause of hyperthyroidism and is due to an autoimmune process. Serum IgG antibodies bind to TSH receptors in the thyroid, stimulating thyroid hormone production, i.e. they behave like TSH. These TSH receptor antibodies (TSHR-Ab) are specific for Graves' disease, can be measured in serum, are present in (85–90%) of cases and decline with treatment (Ballinger, 2011). Graves' disease shares many immunologic features with autoimmune hypothyroidism, including high serum concentrations of antibodies against thyroglobulin, thyroid peroxidase, and possibly the sodium– iodide cotransporter in thyroid tissue. The serum concentrations of these antibodies vary among patients, and the antibodies themselves may modify the stimulatory effects of thyroid-stimulating antibodies. In some patients, the simultaneous production of antibodies that block the thyrotropin receptor reduces the stimulatory action of thyroid-stimulating antibodies. For these reasons there is no direct correlation between serum concentrations of thyroid-stimulating antibodies and serum thyroid hormone concentrations in patients with Graves 'hyperthyroidism (Weetman, 2000).

2.3.1.1 Pathogeneses of Grave's disease:

Is characterized by a breakdown in self-tolerance to thyroid autoantigens, of which the most important is the TSH receptor. The result is the production of multiple autoantibodies, including: -

• Thyroid-stimulating immunoglobulin: An IgG antibody that binds to the TSH receptor and mimics the action of TSH, stimulating adenyl cyclase, with resultant increased release of thyroid hormones. Almost all persons with Graves' disease have detectable amounts of this autoantibody, which is relatively specific for Graves' disease.

• Thyroid growth-stimulating immunoglobulins: Also directed against the TSH receptor, these antibodies have been implicated in the proliferation of thyroid follicular epithelium.

• TSH-binding inhibitor immunoglobulins: These anti-TSH receptor antibodies prevent TSH from binding to its receptor on thyroid epithelial cells and in so doing may actually inhibit thyroid cell function. The coexistence of stimulating *and* inhibiting immunoglobulins in the serum of the same patient is not unusual—a finding that may explain why some patients with Graves' disease spontaneously develop episodes of hypothyroidism. A T cell–mediated autoimmune phenomenon also is involved in the development of the infiltrative ophthalmopathy characteristic of Graves' disease (Kumar et al., 2012).

2.3.1.2 Clinical manifestations of Grave's disease:

The clinical manifestations of Graves' disease can be divided into those common to any form of hyperthyroidism and those specific to Graves' disease, the severity and duration of Graves' disease and the age of the patient determine the manifestations of hyperthyroidism (Weetman,

2000).The most common symptoms are weight loss, heat intolerance, difficulty sleeping, tremor, increased frequency of defecation, proximal-muscle weakness, irritability, and menstrual irregularity. Signs include tachycardia, stare, eyelid lag, proptosis, goiter, resting tremor, hyperreflexia, and warm, moist, and smooth skin. Rare findings (in <1% of patients) include localized dermopathy (i.e., pretibial myxedema) and thyroid acropachy (Brent, 2008). Graves' disease is associated with a decreased quality of life because of both the metabolic effects of elevated levels of thyroid hormone and thyrotropin-receptor antibodies (e.g., disturbed sleep and emotional lability) and the cosmetic effects (e.g., goiter and ophthalmopathy). Men with Graves' disease may have gynecomastia, reduced libido, and erectile dysfunction. Women often have irregular menses (Brent, 2008).

2.3.2 Hashimoto thyroiditis:

Hashimoto's disease is a chronic autoimmune thyroiditis characterized by diffuse lymphocytic infiltration, thyroid follicles of reduced size containing sparse colloid, and fibrosis replacing the thyroid parenchyma (Dayan and Daniels, 1996). And is the most common thyroid disorder in iodine-sufficient areas. It is characterized by the presence of complement-fixing autoantibodies to thyroid peroxidase autoantibodies (anti-TPO), which are closely associated with overt thyroid dysfunction and tend to correlate with progressive thyroidal damage and lymphocytic inflammation. This form of autoimmune thyroiditis, again more common in women and most common in late middle age, produces atrophic changes with regeneration, leading to goiter formation. The gland is usually firm and rubbery but may range from soft to hard. TPO antibodies are present, often in very high titers (> 1000

IU/L). Patients may be hypothyroid or euthyroid, though they may go through an initial toxic phase, 'Hashi-toxicity'. Levothyroxine therapy may shrink the goitre even when the patient is no hypothyroid (Ballinger, 2011).

2.3.2.1 Pathogenesis of Hashimoto thyroiditis:

is caused by a breakdown in self-tolerance to thyroid autoantigens. Thus, circulating autoantibodies against thyroid antigens are present in the vast majority of patients, who demonstrate progressive depletion of thyroid epithelial cells (thyrocytes) and their replacement by mononuclear cell infiltration and fibrosis. The inciting events leading to breakdown in self-tolerance have not been fully elucidated, but multiple immunologic mechanisms that may contribute to thyrocyte damage have been identified, including: -

• CD8+ cytotoxic T cell–mediated cell death: CD8+ cytotoxic T cells may cause thyrocyte destruction.

• Cytokine-mediated cell death: Excessive T cell activation leads to the production of inflammatory cytokines such as interferon-γ in the thyroid gland, with resultant recruitment and activation of macrophages and damage to follicles.

• Binding of antithyroid antibodies (antithyroglobulin, and antithyroid peroxidase antibodies), followed by antibody- dependent cell–mediated cytotoxicity. A significant genetic component to the disease pathogenesis is supported by the concordance of disease in as many as (40%) of monozygotic twins, as well as the presence of circulating antithyroid antibodies in approximately (50%) of asymptomatic siblings of affected patients. Increased susceptibility to Hashimoto thyroiditis is associated with polymorphisms in multiple immune regulation–associated genes, the most

significant of which is the linkage to cytotoxic T lymphocyte–associated antigen-4 gene (CTLA4), which codes for a negative regulator of T cell function (Kumar et al., 2012).

2.3.2.2 Clinical feature of Hashimoto thyroiditis:

The clinical manifestations and signs of Hashimoto thyroiditis are usually similar to those common to any form of hypothyroidism.

2.4 Thyroid autoimmunity and abortion

The relation of thyroid autoimmunity to abortion is an important issue that has attracted the interest of many investigators. Most studies have shown a significant positive association between the presence of thyroid autoantibodies and abortion rate. It is of interest that women with high titers do not show a higher abortion rate when compared with women having low titers, although, there is no general agreement on this issue (Kaprara and Krassas, 2008). There are three possible explanations for the assumed association of thyroid autoimmunity with abortion 1) pregnancy loss is an epiphenomenon and not a direct effect of the thyroid autoantibodies, the presence of thyroid autoantibodies reflecting a generalized activation of the immune system; 2) delayed conception from the presence of thyroid autoantibodies; hence, when women with thyroid autoimmunity become pregnant, face a higher risk of abortion because of older age; and 3) the pregnancy loss is secondary to a subtle deficiency in thyroid hormone concentrations or a lower capacity of the thyroid to adequately adapt to the demands of pregnancy (Kaprara and Krassas, 2008**).** The association between spontaneous abortion and thyroid autoimmunity (TAI) defined as the presence of autoantibodies against thyroid peroxidase (TPO-Ab) and/or thyroglobulin (Tg-Ab) was initially reported by (Stagnaro-Green et al.,

1990). subsequently investigated in numerous studies and finally confirmed by two meta-analyses. Causality remains unclear; co-presence of (TAI) with other autoimmune syndromes, direct action of TPO-Ab and Tg-Ab on placenta, hampered adaptability of the thyroid gland to the increased demands of pregnancy in the presence of (TAI) and higher age of women with (TAI) have all been implicated (Toulis et al., 2010). Over the past decade, many reports have linked thyroid autoimmunity (TA) with recurrent abortions and it has been suggested that thyroid autoantibodies may serve as a marker for at-risk pregnancies. Furthermore, it was recently shown that thyroxine administration to pregnant women with positive thyroid autoantibodies and a history of recurrent abortions may improve the final outcome (Kaprara and Krassas, 2008). Many studies have suggested that the presence of antithyroid antibodies carry an increased risk for spontaneous abortion and/or obstetric complications. In fact, different researchers have found a highly significant correlation between thyroid autoimmunity and increased rate of abortion. The absence of other non-organ-specific antibodies among the patients included in these studies suggests that thyroid antibodies should be considered an independent indication of the risk of pregnancy loss (Vaquero et al., 2000). Risk of abortion was significantly higher among women (35) years or older, TPO-Ab positive and presenting high levels of TSH (Sieiro Netto et al., 2004). Furthermore, such association is usually reversible after appropriate treatment of the underlying thyroid disease and normalization of thyroid function (Abramson and Stagnaro-Green, 2001). In meta-analysis done by (Thangaratinam et al., 2011) involved (12126) women, of the (31) studies evaluating abortion , (28) showed a positive association between thyroid autoantibodies and abortion . In population of women in Iran, TG-Ab and TPO-Ab were identified more

frequently in women with recurrent abortions compared with controls, and thyroid autoimmunity was independently associated with a higher risk of recurrent abortion (Iravani et al., 2008).

2.5 Previous studies:

2.5.1 Detection of at-risk pregnancy by means of highly sensitive assays for thyroid autoantibodies (Stagnaro-Green et al., 1990)

In this study they screened (552) women who presented to their obstetrician in the first trimester of pregnancy using highly sensitive enzyme-linked immunosorbent assays for the presence of thyroglobulin and thyroid peroxidase autoantibodies and found an incidence of positivity of (19.6%). The tendency to secrete detectable levels of thyroid autoantibodies was significantly correlated with an increased rate of abortion. Thyroid autoantibody-positive women miscarried at a rate of (17%), compared with (8.4%) for the autoantibody-negative women. They concluded that thyroid autoantibodies are an independent marker of "at-risk" pregnancy.

2.5.2 The association of antithyroid antibodies in euthyroid non-pregnant women with recurrent first trimester abortions in the next pregnancy (Pratt et al., 1993)

Among (42) such women, (30) successfully completed a new pregnancy and among them only five (17%) had thyroid antibodies. This was in sharp contrast with (12 of 42) women who aborted again during a subsequent pregnancy and among whom eight (67%) had thyroid antibodies.

2.5.3 Thyroid autoantibodies in euthyroid non-pregnant women with recurrent spontaneous abortions (Bussen and Steck, 1995)

A total of (22) euthyroid non-pregnant habitual aborters were analyzed, using enzyme-linked immunosorbent assay, for thyroglobulin and thyroid

peroxidase antibodies; (22) nulligravidae and (22) multigravidae without endocrine dysfunction served as controls. Eight of the (22) women with recurrent spontaneous abortions (36%) but only two of the (22) nulligravidae controls (9%; chi 2 test, P = 0.03) and one of (22) multigravidae subjects (5%; chi 2 test, P < 0.01) demonstrated positive titers (> 100 IU/ml) of thyroglobulin, thyroid peroxidase or both antibodies. In conclusion, the incidence of thyroid antibodies in euthyroid women with habitual abortions appears to be significantly increased compared with the controls of reproductive age without previous abortions.

2.5.4 Presence of thyroid antibodies in early reproductive failure: biochemical versus clinical pregnancies (Singh et al., 1995)

In this study found that of the (487) patients studied, there were (106) women who were antibody positive for anti-TG, antithyroid peroxidase, or both, and (381) who were negative. The overall incidence of positivity was (22%). In the antibody-positive group there was a (32%) clinical abortion rate in comparison to (16%) in the antibody-negative group.

2.5.5 Effects of autoantibodies on the course of pregnancy and fetal growth (Iijima et al., 1997)

(1179) healthy women with singleton gestations were screened in early pregnancy for seven kinds of autoantibodies: antithyroid microsomal antibody, antithyroglobulin antibody, two kinds of rheumatoid factor, antinuclear antibody, anti-DNA antibody, and antimitochondrial antibody. In (228) cases , rate of spontaneous abortion was observed in those with antithyroid microsomal (10.4%), compared with negative subjects (5.5%).

2.5.6 Increased prevalence of antithyroid antibodies identified in women with recurrent pregnancy loss but not in women undergoing assisted reproduction (Kutteh et al., 1999)

Included were (700) women with a history of two or more consecutive pregnancy losses, (688) women with a history of infertility who were undergoing ART, and (200) healthy, reproductive-aged female controls. Result(s): Antithyroid antibodies were identified in (29/200) (14.5%) of controls and (158/700) (22.5%) of women with recurrent pregnancy loss and (132/688) (19.2%) of women undergoing ART. Conclusion(s): Antithyroid antibodies are identified more frequently in women with recurrent pregnancy loss than in controls but not in women undergoing ART. These autoantibodies may be markers of autoimmune activation and have been associated with an increased risk of pregnancy loss and postpartum thyroid disease.

2.5.7 Thyroid autoimmunity and abortion: a prospective study in women undergoing in vitro fertilization (Muller et al., 1999)

In Netherlands, in women undergoing in vitro fertilization**,** (173) women were observed, of whom (54/173) (31%) became pregnant. Pregnancy occurred in (12/25) (48%) of the antibody-positive women and in (42/148) (28%) of the antibody negative women. Among those who became pregnant, abortion occurred in (4/12) (33%) of TPO antibody– positive women and in (8/42) (19%) of TPO antibody–negative women

2.5.8 Thyroid autoimmunity in patients with recurrent spontaneous abortion s (Dendrinos et al., 2000)

Thirty euthyroid women with RSA (three or more consecutive abortion s) aged (25-37) years were compared with (15) matched controls. Thyroid

peroxidase (TPO) and thyroglobulin antibodies were tested with a chemiluminescence immunoassay and APCA were tested with a cross-match reaction. Results were compared using the chi-squared test. There was a higher frequency of ATA in women with RSA compared to controls (37% versus 13%, $p < 0.05$). Twenty of the women (67%) with RSA were tested negative for APCA, indicating an alloimmune contribution to their infertility. In this subgroup of women, the frequency of ATA continued to be higher than controls (40% versus 13%, $p < 0.05$). In conclusion, women with RSA, independent of APCA status, have a higher frequency of ATA.

2.2.9 Autoimmune thyroid disease in pregnancy and the postpartum period: relationship to spontaneous abortion (Bagis et al., 2001)

from (876) subjects completed the study, (12.3%) were thyroid antibody-positive (4.5%) tested positive for both thyroid peroxidase antibody[TPO-Ab] and thyroglobulin autoantibody [Tg-Ab], (4.79%) were TPO-Ab–positive only, and (3.1%) were Tg-Ab–positive only). (50%) of the ATA-positive women and (14.1%) of the ATA-negative group had a history of spontaneous abortion.

2.5.10 Influence of thyroid autoimmunity and maternal age on the risk of abortion (Sieiro Netto et al., 2004)

study in Brazil found that from (534) pregnant women only (29) (5.4%) were positive to TPO-Ab, (3) (10.3%) of them had abortion compared to only (10) in the (495) TPO-Ab negative pregnant women (P =0.029).

2.5.11Thyroid autoimmunity and recurrent spontaneous abortion in Iran: a case-control study (Iravani et al., 2008)

A total of (641) patients and (269) controls were included. Mean age (± SD) was (30.6 ± 6.4) years (range, 16-51 years) in the patient group and (30.05 ±

6.6) years (range, 18-48 years) in the control group. Thyroid antibodies were present in (157/641) patients (24.5%) and in (34/269) controls (12.6%) (*P*<.001). The presence of thyroid antibodies was significantly associated with recurrent abortion independent of the impact of age with an odds ratio of (2.24) (95% confidence interval, 1.5-3.35).

2.5.12 Thyroid Function and Thyroid Antibodies in Recurrent Abortion Women (Zahran, 2010)

found that the presence of positive TPO-Ab in the target and control groups was (26.66%) and (42.5%) respectively. The presence of positive Tg-Ab in the target group was (3.33%). The presence of both TPO-Ab and Tg-Ab in the target group was (1.66%). The study observed that the TSH concentration was increase in abortion women with positive antibodies compared with the concentration of TSH in the control group with positive antibodies, none of the control group had got positive Tg-Ab. The presence of both TPO-Ab and Tg-Ab in the target group was (1.66%).

2.5.13 Thyroid autoantibodies in euthyroid women with recurrent abortions and infertility (Soltanghoraee et al., 2010)

In this study the prevalence of (TPO- & Tg) autoantibodies in euthyroid controls was about (25%) and the percentage of people with an anti-Tg > 500 was two times bigger in the abortion group compared to the control group and the proportion of people with an anti-Tg > 500 in younger cases in the abortion group was significantly higher than the rest of the cases ($p<0.05$). Anti-TPO distribution had no significant differences.

2.5.14 Thyroid autoimmunity and recurrent abortion (Ticconi et al., 2011)

In this case-control study, a total of (160) women with RM and (100) healthy women were investigated for the presence of serum ATA directed against thyreoglobulin (TG-Ab), thyroid peroxidase (TPO-Ab) and TSH receptor (TSHr-Ab), which were determined by either chemiluminescence or radioimmunoassay. Antithyroid autoantibodies were detected in (46) (28.75%) women with RM and in (13) (13%) women of the control group (P <0.05). The frequencies for TG-Ab and TPO-Ab were higher in RM than in control women. Among the women of RM group, (91.3%) of ATA+ women were positive also for other autoantibodies. The majority of study women were euthyroid. Antithyroid autoantibodies, particularly TG-Ab, are associated with RM and could be an expression of a more general maternal immune system abnormality leading to RM. ATA could have a role in RM irrespective of thyroid hormone status.

2.5.15 Thyroid autoimmunity and obstetric outcomes in women with recurrent abortion a case–control study (Lata et al., 2013)

a study showed that of (100) pregnant patients with previous recurrent abortion, thyroid autoimmunity (thyroid peroxidase antibody (TPOAbC) > 34 U/ml) was found in (31%) of the cases. The incidence of subclinical hypothyroidism was higher in TPOAb positive group than in TPOAb negative group (52 vs 16%; P= 0.0002)

2.5.16 Thyroid autoantibodies are not associated with recurrent pregnancy loss (Esplin et al., 1998)

The results were showed that (22) of the women with a history of recurrent pregnancy loss (29.3%) and twenty-eight of the control subjects (37%) had

positive results for either one or both of the thyroid autoantibodies (P >. 05), the mean antibody titers of both antibodies (TPO and Tg antibodies) were similar in both case and control groups, also found that there was no difference in thyroid function between the study and control subject according to the assessment of TSH level and concluded that thyroid autoantibody was not associated with recurrent pregnancy loss.

2.5.17 Prospective pregnancy outcome in untreated recurrent miscarries with thyroid autoantibodies (Rushworth et al., 2000)

A total of (870) consecutive, non-pregnant women with a history of three or more pregnancy losses and normal parental karyotypes were investigated for the presence of thyroglobulin antibodies (TgAb) and for thyroid microsomal antibodies (TmAb). Thyroid antibodies were found in (162) (19%) women. TgAb only were found in eight women (5%); TmAb only in (98) (60%) and both TgAb and TmAb were found in 56 (35%). Thirteen women had a history of thyroid disease and a further (15) women were found to have abnormal thyroid function. All (28) were excluded from the pregnancy outcome study. Among the remaining (134) thyroid antibody positive women, (36) women were not tested and normal thyroid stimulating hormone results were obtained for (98). In the group proven euthyroid, (14/24) untreated pregnancies resulted in live births (58%). Among the (710) thyroid antibody negative women, (47) of (81) untreated pregnancies resulted in live births (58%). The future risk of pregnancy loss in women with unexplained recurrent abortion is not affected by their thyroid antibody status.

3. Materials and Methods

3.1 Study area and duration:

This study was conducted in Gazeira State (Wad Madani Teaching Hospital Department of Obstetrics &gynecological) from March 2018 to August 2018.

3.2 Study population:

Pregnant women with and without history of abortion were included.

3.3 Study design:

Prospective - Case control study, analytical, laboratory hospital based.

3.4 Sample size:

The study included (100) individuals, (50) females with at least three or more consecutive recurrent abortion (case) and (50) with no history of abortion (control). Then were collected blood samples for measurement the serum cytomegalovirus antibody level at both groups.

For limitation fund and period of research (90) women were involved in this research.

3.5 Study Criteria:

3.5.1 Inclusion criteria:

For case samples pregnant women with three or more consecutive recurrent abortion s.

For control samples pregnant women with no history of abortion

3.5.2 Exclusion criteria:

All pregnant women with history of abortion less than three times, pregnant women with any disorders that can effect TPO-Ab. Tg-Ab and TSH or

disorders can cause pregnancy loss such as (metabolic or endocrinologic disorders, preeclampsia, etc.…), pregnant women with negative Rhesus factor and pregnant women who refused to participate.

3.6 Sample and data collection:

About five ml of venous blood samples were collected from selected patients attending Obstetrics and Gynecology departments at Wad Madani teaching hospital. An equivalent volume of venous blood samples were collected from the control subjects. Each specimen of blood was allowed to clot. The serum was obtained by centrifugation at 3000 g for five minutes, and then serum was separated, aliquoted and stored at -20 ºC till analyzed. All samples were kept frozen at Central Laboratory Research.

Personal and clinical data were collected from patients and controls by a structured questionnaire and filled by the investigator during each time when blood samples collected.

3.7 Data analysis:

Data were analyzed and tabulated using statistical package for social sciences (SPSS) program version 21, unpaired sample test, a crosstabs and correlation were performed by using Chi2 test estimated difference by P value and evaluated the Odd ratio with confidence interval range Lower and upper limitation.

3.8. Ethical consideration:

This study was approved by college of Medical Laboratory Science ethical committee, University of shendi. Permission from hospital was applied and verbal consent was taken from participants involved in the study.

3.9 Methodology:

3.9.1Enzyme linked immunosorbent assay (ELISA) processing:

The specimens were analyzed for quantitative determination of TPO and Tg antibodies by commercially available enzyme–linked immunosorbent assay 'TPO ELISA" kit (AESKU.DIAGOSTIC GmbH & Co. KG, Mikroforum Ring2, 55234 Wendelsheim, Germany) that has 80.0% specificity and functional sensitivity was determined to be: (10 IU/ml) according to the information included in the kit's insert. The assays were performed following the instructions of the manufacturer. And commercially available enzyme–linked immunosorbent assay 'Tg ELISA" kit (Demeditec Diagnostics GmbH • Lise-Meitner-Straße 2 • D-24145 Kiel (Germany)) that has functional sensitivity was determined to be: (10 IU/ml) according to the information included in the kit's insert. The assays were performed following the instructions of the manufacturer.

3.9.2 Enzyme linked immunosorbent assay (ELISA) for detection of TPO antibodies:

3.9.2.1 Principle of TPO antibodies test:

Serum samples diluted (1:101) are incubated in the microplates coated with the specific antigen. Patient´s antibodies, if present in the specimen, bind to the antigen. The unbound fraction is washed off in the following step. Afterwards anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) are incubated and react with the antigen-antibody complex of the samples in the microplates. Unbound conjugate is washed off in the following step. Addition of TMB-substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color changes

to yellow). The intensity of color formation from the chromogen is a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of the respective antibodies in the patient sample.

3.9.2.2 Procedure of TPO antibodies test:

The reagents and specimens were allowed to reach room temperature and prepared enough microplate modules for all calibrators, controls and patient samples. (100 µl) of calibrators and controls were added to their respective wells, then (100 µl) of each diluted specimen was added to each well except the previous wells. The plate was covered with the cover sealer and incubated for (30 mins) at room temperature (37° C). Then discarded the contents of the microwells and washed (3) times with 300 µl of working wash buffer, (100 µl) of conjugate was added to each well. The plate was covered and incubated again, for (30 mins) at (37°C). Then discarded the contents of the microwells and washed (3) times with working wash buffer, (100 µl) of TMB substrate were added to each well, and then the plate was incubated at (37°C) for (30 mins). At the end of incubation period, (100 µl) of stop solution was added, and then the plate was incubated at (37°C) for (5 mins). Finally, the optical density was read at (450 nm) within 30 min.

3.9.2.3 Calculation of TPO antibodies results:

By using the standard curve, the result was calculated by plotting the optical density (OD) of each calibrator (y-axis) with respect to the corresponding concentration values in IU/ml (x-axis). From the OD of each sample, the corresponding antibody concentrations were read and expressed in IU/ml.

3.9.2.4 Interpretation of TPO antibodies results:

Normal Range ≤ 40 IU/ml.

Equivocal Range 40 - 60 IU/ml.

Positive Results > 60 IU/ml.

3.9.2.5 Quality control of TPO antibodies test:

Reagents, Standard and control were checked for storage, stability and preparation before starting work. Each microwell plate was considered separately when the results were calculated and interrelated.

3.9.3 Enzyme linked immunosorbent assay (ELISA) for detection of Tg antibodies:

3.9.3.1 Principle of Tg antibodies test:

Highly purified human thyroglobulin (TG) is bound to microwells. Antibodies against the coated antigen, if present in diluted patient sample, bind to the respective antigen. Washing of the microwells removes unbound unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human antibodies immunologically detect the bound patient antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue colour. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow colour is measured photometrically at (450 nm). The amount of colour is directly proportional to the concentration of antibodies present in the original sample.

3.9.3.2 Procedure of Tg antibodies test:

The reagents and specimens were allowed to reach room temperature and prepared enough microplate modules for all calibrators, controls and patient

samples. (100 µl) of calibrators and controls were added to their respective wells, then (100 µl) of each diluted specimen was added to each well except the previous wells. The plate was covered with the cover sealer and incubated for (30 mins) at room temperature (37° C). Then discarded the contents of the microwells and washed (3) times with (300 µl) of working wash buffer, (100 µl) of conjugate was added to each well. The plate was covered and incubated again, for (15 mins) at (37°C). Then discarded the contents of the microwells and washed (3) times with working wash buffer, (100 µl) of TMB substrate were added to each well, and then the plate was incubated at (37°C) for (15) minutes. At the end of incubation period, 100 µl of stop solution was added, and then the plate was incubated at (37°C) for (5 mins). Finally, the optical density was read at (450 nm) within (30 mins).

3.9.3.3 Calculation of Tg antibodies results:

By using the standard curve, the result was calculated by plotting the optical density (OD) of each calibrator (y-axis) with respect to the corresponding concentration values in IU/ml (x-axis). From the OD of each sample, the corresponding antibody concentrations were read and expressed in IU/ml.

3.9.3.4 Interpretation of Tg antibodies results:

Negative < 100 IU/ml

Borderline 100 - 150 IU/ml

Positive > 150 IU/ml.

3.9.3.5 Quality control of Tg antibodies test:

Reagents, Standard and control were checked for storage, stability and preparation before starting work. Each microwell plate was considered separately when the results were calculated and interrelated.

3.9.4 quantitative determination of the TSH test method by cobas e 411 Immunoassay Analyzers based on electrochemiluminescent technology:

3.9.4.1 principle of TSH test:

Sandwich principle. Total duration of assay: (18 mins).

• 1st incubation: (50 µl) of sample, a biotinylated monoclonal TSH-specific antibody and a monoclonal TSH-specific antibody labeled with a ruthenium complex react to form a sandwich complex. 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with by a photomultiplier. Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

3.9.4.2 Procedure of TSH test:

The specimens were allowed to reach room temperature and the device was opened and calibrated. Then go to the workplace in device. Then the test was selected. The rack number, position and sample ID were entered. Then the test/profile was selected and saved. loaded the samples on rack. Then pressed on start selection and wait for the assay process was completed. Finally, the results were printed.

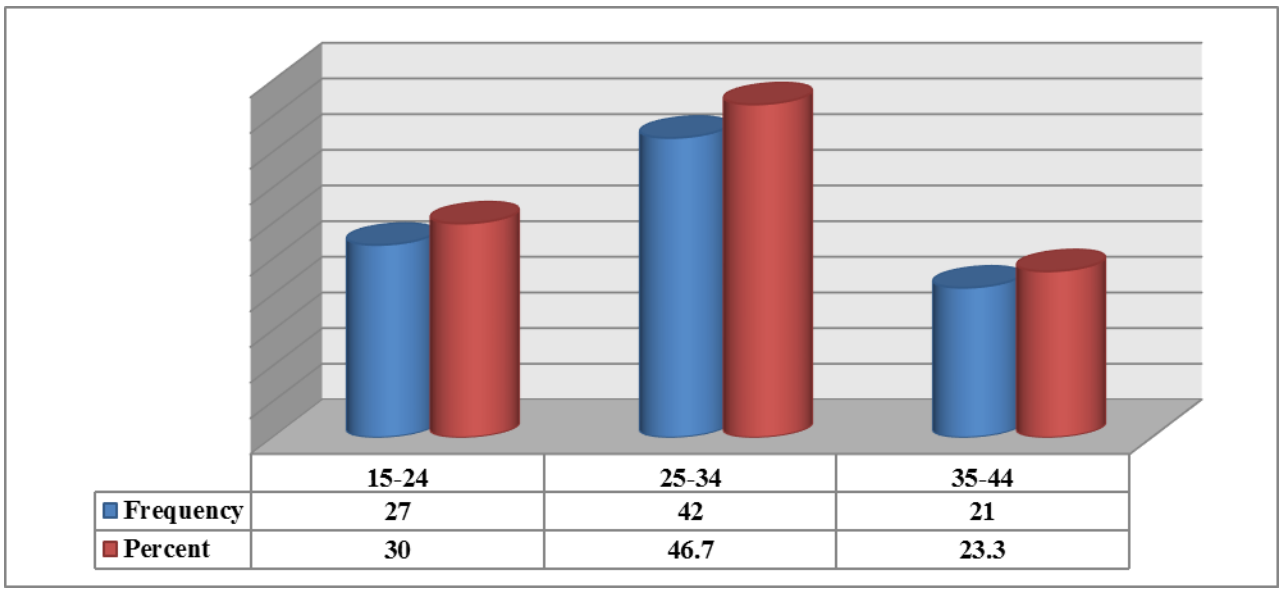
3.9.4.3 Interpretation of TSH results:

Normal Range 0.4 – 6.2 miu/l.

High > 6.2 miu/l.

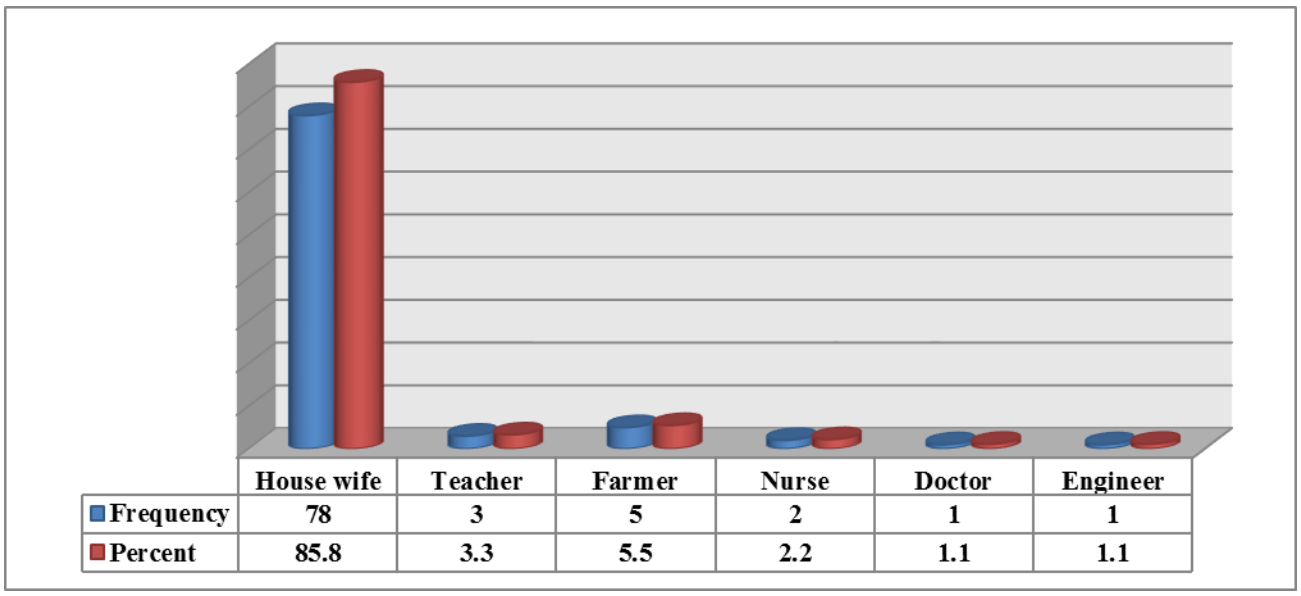
Low < 0.4 miu/l.

4. Results:



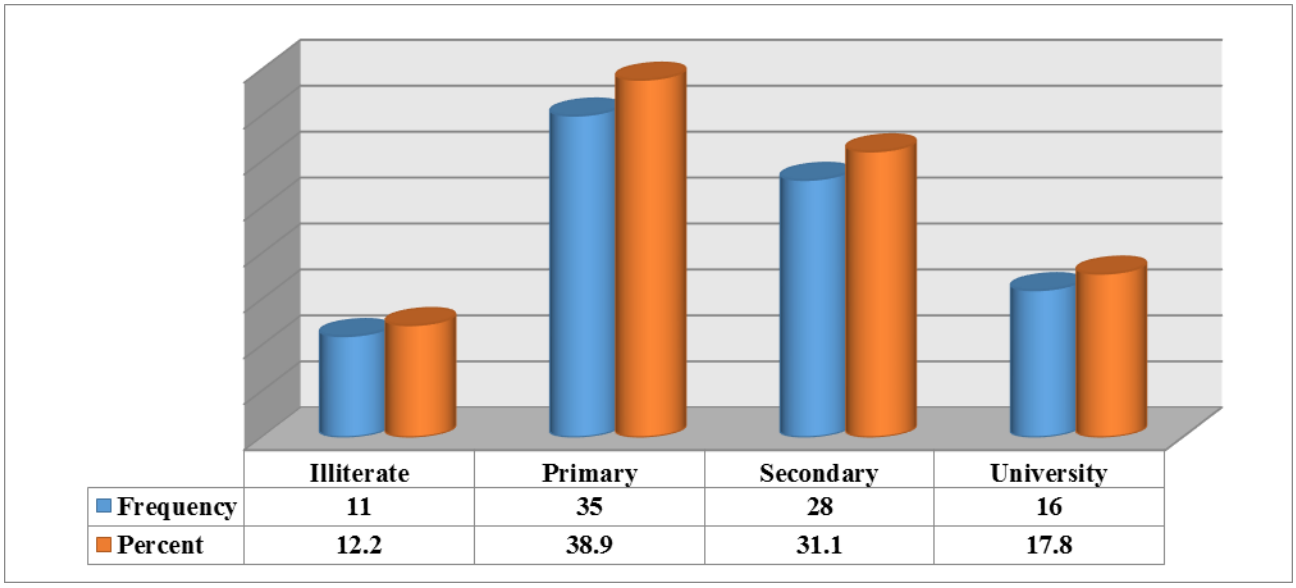
	15-24	25-34	35-44
Frequency	27	42	21
Percent	30	46.7	23.3

Figure 4.1 Frequency of age groups among study population.



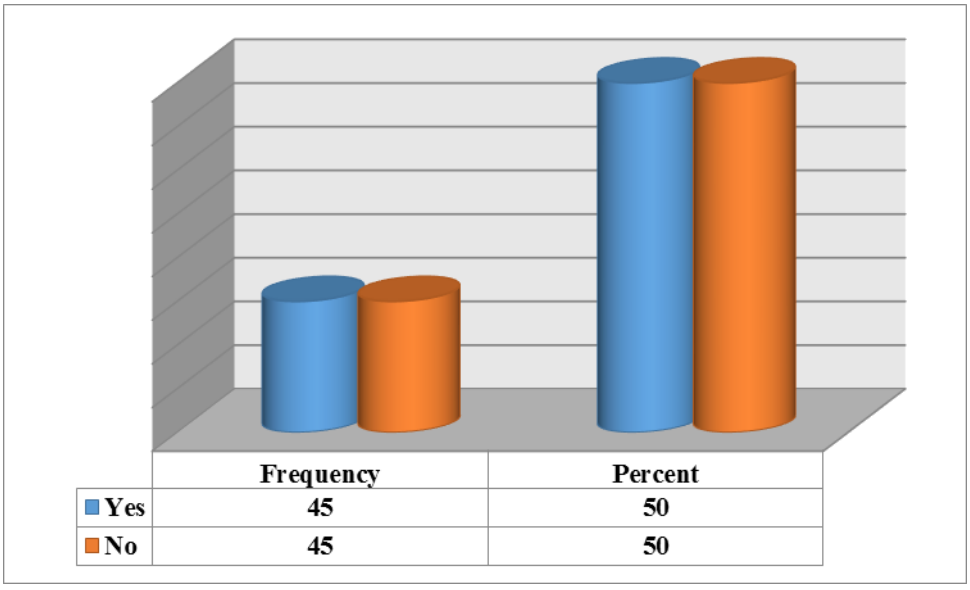
	House wife	Teacher	Farmer	Nurse	Doctor	Engineer
Frequency	78	3	5	2	1	1
Percent	85.8	3.3	5.5	2.2	1.1	1.1

Figure 4.2 Frequency of job among study population.



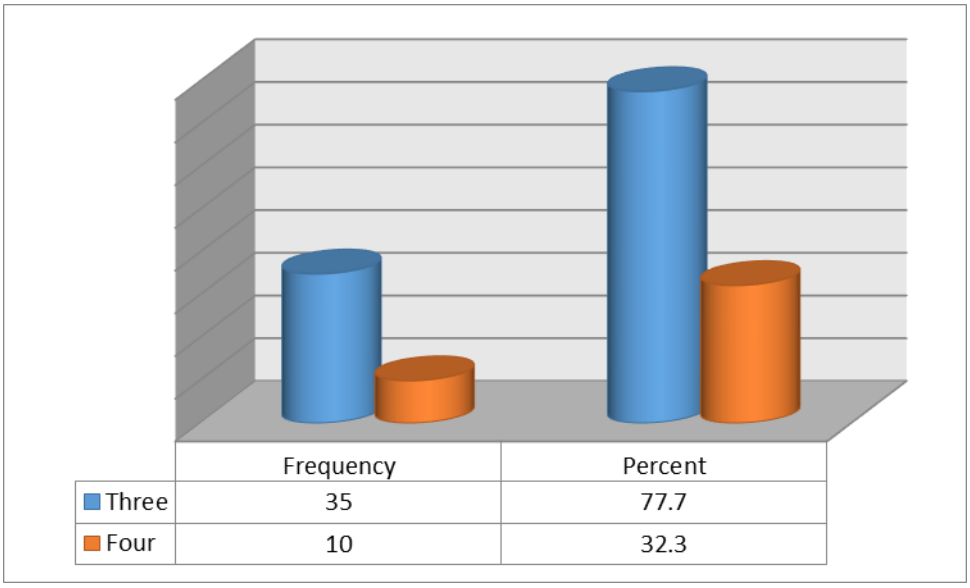
	Illiterate	Primary	Secondary	University
Frequency	11	35	28	16
Percent	12.2	38.9	31.1	17.8

Figure 4.3 Frequency of education level among study population.



	Frequency	Percent
Yes	45	50
No	45	50

Figure 4.4 Frequency of abortion among study population.



	Frequency	Percent
Three	35	77.7
Four	10	32.3

Figure 4.5 Frequency of number of abortion among case group.

Table 4.1: Mean and standard deviation of recurrent abortion and TPO-Ab concentration.

Variables	No	Mean IU/ml	SD	P.value
Control	45	19.85	7.11	0.007
Case	45	384.41	154.54	
Total	90			

*The mean difference is significant.

* P.value < (0.05).

Table 4.2: Mean and standard deviation of age groups and TPO-Ab concentration.

Age groups	No	Mean IU/ml	SD	P.value
15-24	27	115.29	384.57	0.874
25-34	42	167.37	547.15	
35-44	21	306.12	1.29	
Total	90			

* The mean difference is insignificant.

* P.value > (0.05).

Table 4.3: Mean and standard deviation of trimesters stage and TPO-Ab concentration among case group.

Trimester's stages	No	Mean IU/ml	SD	P.value
Stage I	33	396.96	1.165	0.012
Stage II	11	54.19	114.85	
Stage III	1	1.98	0.00	
Total	45			

* The mean difference is significant.

* P.value < (0.05).

Table 4.4: Mean and standard deviation of recurrent abortion and Tg-Ab concentration.

Variables	No	Mean IU/ml	SD	P.value
Control	45	87.88	6.79	0.245
Case	45	65.04	20.76	
Total	90			

* The mean difference is insignificant.

* P.value > (0.05).

Table 4.5: Mean and standard deviation of age groups and Tg-Ab concentration.

Age groups	No	Mean IU/ml	SD	P.value
15-24	27	55.09	34.46	0.272
25-34	42	81.76	101.52	
35-44	21	93.345	155.48	
Total	90			

* The mean difference is insignificant.

* P.value > (0.05).

Table 4.6 Means and standard deviation of trimesters stage and Tg-Ab concentration among case group.

Trimester's stages	No	Mean IU/ml	SD	P.value
Stage I	33	70.55	93.69	0.491
Stage II	11	141.50	230.33	
Stage III	2	70.18	0.00	
Total	45			

* The mean difference is insignificant.

* P.value > (0.05).

Table 4.7: Means and standard deviation of recurrent abortion and TSH concentration.

Variables	No	Mean mIU/L	SD	P.value
Control	45	3.55	0.29	0.028
Case	45	4.64	0.30	
Total	90			

* The mean difference is significant.

* P.value < (0.05).

Table 4.8: Means and standard deviation of age groups and TSH concentration.

Age groups	No	Mean mIU/L	SD	P.value
15-24	27	3.42	2.04	0.834
25-34	42	4.67	1.82	
35-44	21	3.83	2.17	
Total	90			

* The mean difference is insignificant.

* P.value > (0.05).

Table 4.9: Means and standard deviation of trimesters stage and TSH concentration among case group.

Trimester's stages	No	Mean mIU/L	SD	P.value
Stage I	33	4. 78	2.10	0.012
Stage II	11	4.68	1.86	
Stage III	2	4.60	0.00	
Total	45			

* The mean difference is significant.

* P.value < (0.05).

5.1 Discussion

This study was a case-control study conducted in Al Gezira state (Wad Madani teaching hospital Department of Obstetrics &gynecological). The aim of this study is to determine the thyroid antibodies and TSH among recurrent spontaneous abortion. The target group composes of (45) women with Recurrent spontaneous abortion and the control group contains (45) non-abortion women. Thyroid antibodies (TPO-Ab and Tg-Ab) were analyzed by using (ELISA) technique and TSH was analyzed by using electroimiluminescence technique (cobas e 411) for both target and control groups. Statistical analysis of each parameter was performed using SPSS program, descriptive cross tabulations, T test for means, and ANOVA test for variances were done.

In present study the means of TPO-Ab, TG-Ab and TSH in control and cases were (19.852±7.11, 348.41±154.54) (65.04 ± 6.79, 87.88 ±20.76) (3.55 ±0.29, 4.64±0.30) respectively. The results of present study showed significant increase in concentrations of TPO-Ab in cases compared to controls (P.value = 0.007). And there was insignificant of Tg-Ab concentration in cases which was not statistically significant (P.value = 0.245). Also highly significant increase in concentrations of TSH in case compared to controls which statistically significant (P.value = 0.028). The study observed that the TSH concentration was increase in abortion women with positive antibodies compared with the concentration of TSH in the control group with positive antibodies.

The high TPO-Ab values that reported in the recurrent abortion group may suggest that the interaction between hormones and elevated thyroid antibodies may directly result in pregnancy loss. Other investigators

consider thyroglobulin and TPO-Abs to be secondary markers of autoimmune disease rather than the actual cause of pregnancy loss. These ATAs may reflect an abnormal immunological response that results in pregnancy loss. The higher TSH values that reported in the recurrent abortion group means that the TH in blood circulation is not high enough to stop the stimulation of the hypothalamus to release TRH to the anterior pituitary to inhibit the stimulation of the synthesis and secretion of TSH which was competitively interacting by ATA. Consequently, the thyroid hormones are not sufficient to meet the needs of both mother and fetus resulting in termination of the pregnancy.

The results of present study were agreed the results that obtained by (Bagis et al., 2001) who found (12.3%) of 876 were positive for (ATA), (50%) of them had a history of Recurrent spontaneous abortion compared to only (14.1%) in negatives. The thyroid-stimulating hormone (TSH) levels in the ATA-positive group were significantly higher than those in the ATA-negative group. This study results also agreed the results that obtained by (Zahran, 2010) who found seventy percent (70%) of the control group had TSH levels below normal., and positive TPO-Ab in the target and control groups was (26.66%) and (42.5%) respectively. The presence of both TPO-Ab and Tg-Ab in the target group was (1.66%), and, disagreed with present study, in the TSH concentration was increased in abortion women with positive antibodies compared with the concentration of TSH in the control group with positive antibodies.

The study results were agreed the results that obtained by (Glinoer et al., 1991) who found the positive for both ATA in cases and controls were (13.3%) and (3.3%), $p < 0.001$. similar to results obtained by (Iijima et al.,

1997) showed that women positive for thyroid autoantibodies had a higher abortion rate than women who were negative (10.4 vs 5.5%, $p<0.05$). Results of present study were disagreed with study done by (Esplin et al., 1998) and study conducted by (Rushworth et al., 2000) who revealed no correlation between ATA and recurrent spontaneous abortion .

In present study the highest concentrations for TPO-Ab and TSH was in first trimester,which was statistacally significant (p.value = 0.012).This results were agreed the results that obtained by (Lejeune et al., 1993) who found that Twenty one women suffered spontaneous abortion during the first trimester (23.8%). Thyroid auto-antibodies were present in five of these cases.

In present study, the means of age in control and cases was (26.02±o.85,30.89±0.95) respectively, and insignificant correlation was found for age means with TPO-Ab and TSH. In present stud, according to age group the highest concentrations was among those of age group (25-34) years that was disagreed the results concluded by (Poppe and Glinoer, 2003) who suggested that autoimmunity could be related to older-aged pregnancies.

In this study there was no statistically significance when correlate ATA and TSH with tribe, education level, job, number of abortion s, biomass index and family history of recurrent spontaneous abortion.

5.2. Conclusion

1- The current study revealed higher concentrations of TPO-Ab and TSH among recurrent abortion pregnant women than that pregnant women without recurrent abortion and no effect of Tg- Ab.

2- The pregnant women in the first trimester of gestational stage had the highest frequency, this reflected as strong association between recurrent abortion and seropositivity of ATA as a causative factor and can be delivery outcome-related.

3- The thyroid antibodies can only affect pregnant women when their serum TSH level is relatively above the normal range.

4- Pregnant women can reach term and have babies when their TSH levels are relatively lower or within the normal range.

5- The current study revealed no statistically significant correlation between occupation, education level, number of abortion s, biomass index and family history of RSA with ATA and TSH levels.

5.3 Recommendations

1.Thyroid antibodies and TSH serum levels should be followed as early as the first trimester in pregnant women with history of RSA.

2. It is necessary to screen the pregnant women for the possibility of ATA so that it could be minimized by proper medication.

3.Further research, such as high-quality, well-designed randomized prospective clinical trials, are needed to clarify the causal relationship between them, to explore the mechanisms of pregnancy loss in women with (TAI).

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6.2 Appendixes

Appendix (1): Clinical Evaluation Form (**Questionnaire**)

بسم الله الرحمن الرحيم

Shendi University

Faculty of Graduate Studies and Scientific Research

Detection of Antithyroid Antibodies and TSH in Recurrent spontaneous abortion in Wad Medani, Maternity Hospital, Gezira State, Sudan.

General information:

Date: …… /…… /…… Time: _____ (am/pm) ☐ NO

Name:

_____

Age: ……year.

Tribe: ……..…….. Job: ……....

Occupation: …… Education level: …….

Tall …….. Weight ……

Biomass index ……

Clinical information:

- Have you ever been pregnant before?
- Yes……..……No…….
- Did you have abortion ?
- Yes……..……No…….
- Did you have abortion before that?
- Yes…….No ……
- If present how many time?
- One ◯ Two ◯ Three ◯ More ◯

- At what stage occur:

- 1st ◯ 2nd ◯ 3rd ◯

- Did you have a family history of abortion ?

- Yes……. No……

- How long is it science your last abortion ?

- …….

- Prior to pregnancy was your menstrual cycle regular?

- Yes……. No……

- Cigarette smoking?

- Yes……. No……

- (TORCH) infection?

- Yes…… No……

- Other chronic disease?

- Thyroid……. Hypertension……... Diabetes…….
  Anemia ……... Other auto-immune-diseases…….
  Preeclampsia ……

Appendix (2): consent form

بسم الله الرحمن الرحيم

إقرار بالموافقة

الأسم: .....

العمر : ..... العنوان : .....

أوفق بمحمض إرادتي بالمشاركمة في البحث العلمي المتعلق بدراسة

Detection of Antithyroid Antibodies and TSH in Recurrent spontaneous abortion in Maternity Hospital, Wad Medani, Gezira State, Sudan.

بعد أن شرحت لي بأنه لا يترتب عليه أي أذى جسدي أو نفسي وأعلم أن المشاركة في هذا البحث لن يؤثر بأي حال من الأحوال في العناية الطبية التي أتلقاها كما أنه يحق لي بدون إبداء أسباب الانسحاب من هذا البحث في مرحلة من مراحلة.

البحث بإشراف :

د. مصعب عمر خالد محمد زين

التوقيع: ..... التاريخ : .....

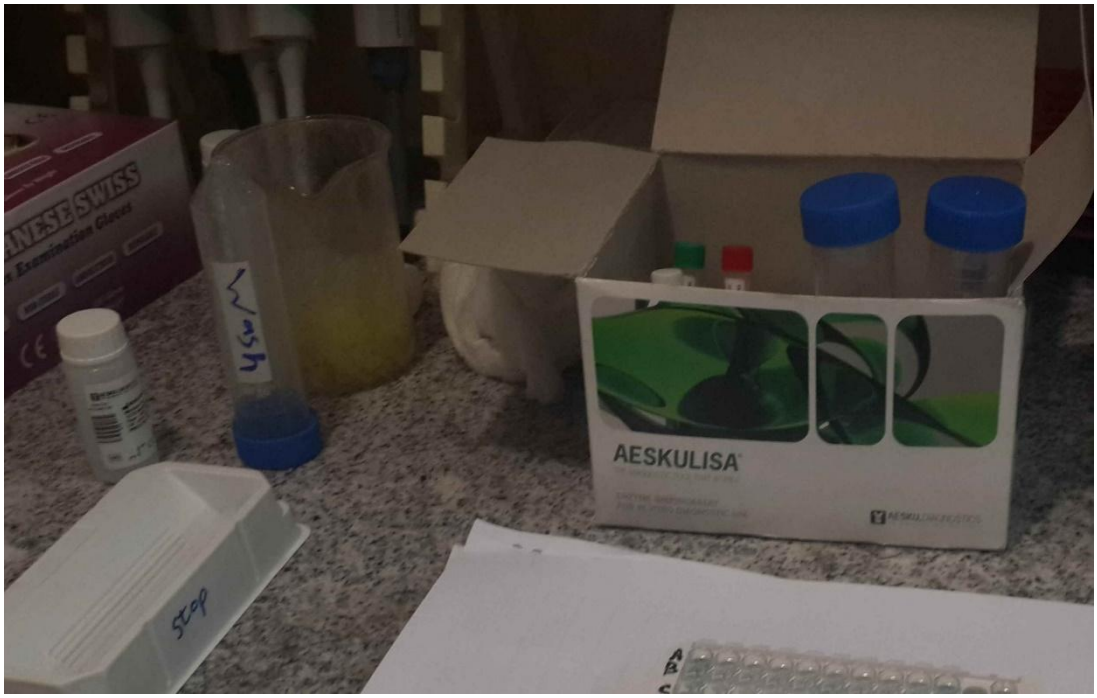
Appendix (3): Layout shows the seropositivity and seronegativity for TPO antibody.

TPO	`	2	3	4	5	6	7	8	9	10	11	12
A	PC	3	11	19	27	35	43	51	59	67	75	83
B	NC	4	12	20	28	36	44	52	60	68	76	84
C	STD1	5	13	21	29	37	45	53	61	69	77	85
D	STD2	6	14	22	30	38	46	54	62	70	78	86
E	STD3	7	15	23	31	39	47	55	63	71	79	87
F	STD4	8	16	24	32	40	48	56	64	72	80	88
G	1	9	17	25	33	41	49	57	65	73	81	89
H	2	10	18	26	34	42	50	58	66	74	82	90

CONTROL	POSITIVE
CASE	POSITIVE

Negative Result	< 40 IU/ml
positive Result	> 60 IU/ml
borderline	40 -60 IU/ml

Appendix (4): ELISA kit of TPO-Ab (Aeskulisa product)



Appendix (5): Microtitre showing antibody reactivity for TPO-Ab.

Appendix (6): Layout shows the seropositivity and seronegativity for Tg antibody.

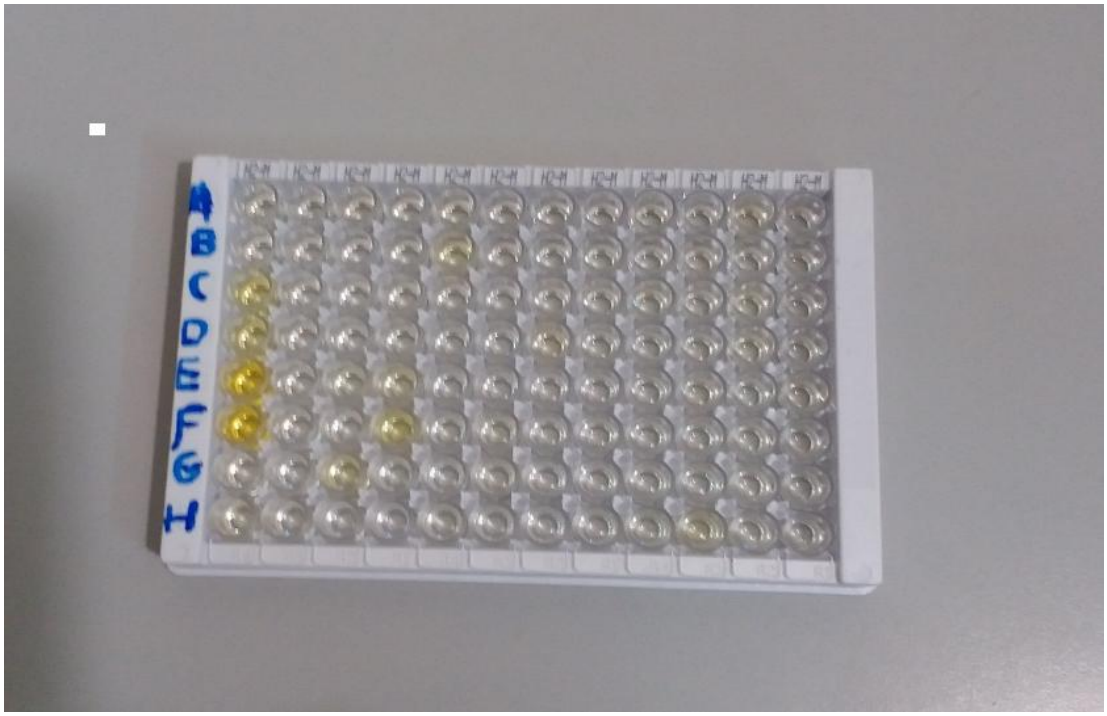
TG	`	2	3	4	5	6	7	8	9	10	11	12
A	PC	3	11	19	27	35	43	51	59	67	75	83
B	NC	4	12	20	28	36	44	52	60	68	76	84
C	STD1	5	13	21	29	37	45	53	61	69	77	85
D	STD2	6	14	22	30	38	46	54	62	70	78	86
E	STD3	7	15	23	31	39	47	55	63	71	79	87
F	STD4	8	16	24	32	40	48	56	64	72	80	88
G	1	9	17	25	33	41	49	57	65	73	81	89
H	2	10	18	26	34	42	50	58	66	74	82	90

CONTROL	POSITIVE
CASE	POSITIVE

Negative Result	< 100IU/ml
positive Result	> 150 IU/ml
borderline	100 -150 IU/ml

Appendix (7): ELISA KIT OF TG-Ab **(**Euroimmune product)

Appendix (8): Microtitre showing antibody reactivity for Tg-Ab.



Appendix (9): Easy wash for automated wash use for ELISA.

Appendix (10): Photometric measurement for detection of ELISA cooler density.

Appendix (11): Cobas e 411 instrument for measuring TSH concentration.


Roche
HITACHI