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Anti-Phospholipid Syndrome among Aborted Women in Their Second-Trimester in Gaza: Case-Control Study

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Dedication

To my mother who teach me how to give. . .

To my wife who support me. . .

To my kids Walid, Sara, Hamza, And Mohammed. . .

To my family and friends. . .

Declaration:

I certify that this thesis submitted for the Degree of Master, is the result of my own research, except where otherwise acknowledged, and that this study (or any part of the same) hasn't been submitted for a higher degree to any other University or institution.

Signed:

Mahmoud S. El-Haj Ahmed

Date: / /2012

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Abstract

Background: Antiphospholipid syndrome (APS) is an acquired thrombophilia cause recurrent venous or arterial thrombosis and/or fetal loss, which occurs in approximately (1%) of women worldwide. **Aim:** to assess the relationship between APS and second-trimester abortion in Southern Gaza Governorates. **Methods**: The design of the study is a case-control study. Convenient sample obtained from Southern Gaza Governorates, one hundred participants of equal number of cases and control. Data collected by two methods: The first, a questionnaire interview which included socioeconomic data and group of risk factors to exclude them from the study. The second, which included withdrawal blood samples to investigate group of tests related to APS and abortion. For this study we used techniques such as ELISA, Ag-Ab reaction and spectrophotometer to measure the level of various variables such as lupus anticoagulant (LA) test, dilute Russell viper venom time (dRVVT), anticardiolipin (ACL) (IgM and IgG), toxoplasma (IgM), rubella (IgM), fasting blood sugar (FBS) and thyroid stimulating hormone (TSH). **Results**: The results of our study showed that there is a statistically significant relationship between APS and second-trimester abortion ($P < 0.05$). Also we found significant relationship between dRVVT and second-trimester abortion. As well LA test showed a significant relationship with the second-trimester abortion ($P < 0.05$). Moreover, the dRVVT was the most specific test for APS diagnosis. According to our results morbidity and mortality associated with APS second-trimester abortion could be decreased by early diagnosis of APS using dRVVT as a diagnostic test beside clinical findings. There is no relationship between toxoplasma (IgM) and second-trimester abortion. Moreover, there is no relationship between rubella (IgM) and second-trimester abortion. **Conclusion:** Our findings added an impute in the early diagnosis of APS and dRVVT is the most specific test for APS diagnosis more than other

tests. Also the tests that were positive for APLs should be repeated after 6 weeks because actual cases usually have persistent APLs in their blood. In future research there is a need to study inherited thrombophilia and its relationship to abortion.

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List of Abbreviations

Abbreviations	Full Word
Ab	Antibody
ACL	Anticardiolipin
Ag	Antigen
APC	Activated Protein Resistant
APLs	Antiphospholipid Antibodies
APS	Antiphospholipid Syndrome
APTT	Activated Partial Thromboplastin Time
ASLA	Activated Seven Lupus Anticoagulant
β_2GPI	Beta 2 Glycoprotein I
Conc.	Concentration
CMV	Cytomegalovirus
DAPTT	Dilute Activated Partial Thromboplastin Time
DM	Diabetes Mellitus
dRVVT	dilute Russell Viper Venom Time
dRVV Confirm	dilute Russell Viper Venom Confirm
dRVV Test	dilute Russell Viper Venom Test
DVT	Deep Vein Thrombosis
EBV	Ebstain Bar Virus
ELISA	Enzyme Linked Immuno Sorbent Assay
FBS	Fasting Blood Sugar
GOD	Glucose Oxidase
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HRP	Horseradish peroxidase
HMW	High Molecular Weight
Ig	Immunoglobulin
IUFD	Intra Uterine Fetal Death
IUGR	Intra Uterine Growth Restriction
KCT	Kaolin Clotting Time
Lab	Laboratory
LAs	Lupus Anticoagulants
LA test	Lupus Anticoagulant Test
LMWH	Low Molecular Weight Heparin
MCH	Maternal and Child Health Care
PAPS	Primary Antiphospholipid Syndrome
PHC	Primary Health Care
RIA	Radio Immuno Assay
PL	Phospholipid
POD	Peroxidase
PT	Prothrombin Time
SCT	Silica Clotting Time
SLE	Systemic Lupus Erythematosus
SN	Seronegative
SP	Seropositive

SVT	Superficial Vein Thrombosis
TIA	Transient Ischemic Attack
TMB	Tetra-Methyl-Benzedine
TTIT	Tissue Thromboplastin Inhibition Test
TT	Textarin Time
TSH	Thyroid Stimulating Hormone
TSVT	Taipan Snake Venom Time

Chapter 1:

Introduction

1.1 Overview

Antiphospholipid syndrome (APS) or (Hughes' syndrome) is a disorder that manifests clinically as recurrent venous or arterial thrombosis and/or fetal loss, which globally occurs in approximately (1%) of women (Zinger *et al*, 2009). APS is clinically characterized by the occurrence of repeated unexplained abortions before the 10th week, unexplained fetal death at or beyond the 10th week or premature birth before the 34th week because of pre-eclampsia (Gris *et al*, 2011). The body produces antiphospholipid antibodies (APLs) against itself in an autoimmune response to phospholipids (PLs) detected by immuno and/or clotting assays that is either as anticardiolipin (ACL), anti beta 2 glycoprotein I (β_2GPI) Abs, or lupus anticoagulants (LAs) (Amitrano *et al*, 2009).

APS is acquired thrombophilia associated with venous or arterial thrombosis or both (Gezer, 2003). APS is a systemic autoimmune disorder accompanied by mild to moderate thrombocytopenia and elevated titers of APLs, where APS was defined originally in 1983 in systemic lupus erythematosus (SLE) patients (Marai *et al*, 2004). Normal pregnancy is associated with changes in haemostasis create a state of hypercoagulability, the risk of thromboembolism during pregnancy and the postpartum period is probably increased (Hvas *et al*, 2009). The hypercoagulable state induced by the pregnancy might be aggravated by pre-existing inherited or acquired thrombophilia (Hvas *et al*, 2009). APS primarily (PAPS) occurs in the absence of associated diseases and secondary (SAPS) to

autoimmune diseases such as SLE. Among women with recurrent abortion, APLs are present in (11–22%). However, the Abs are found in more than (33%) of women with SAPS (Ghosh *et al*, 2006). The median age of new patients of APS is approximately 30 years, with a male/female ratio of about 1:3 (Riley, 2005). It is still unclear why some patients develop recurrent thromboses, mainly of large vessels (simple or classic APS), whereas others develop rapidly recurrent vascular occlusions, predominantly affecting small vessels (catastrophic APS) (Zinger *et al*, 2009). Catastrophic APS, defined as simultaneous multiorgan thromboembolic events usually following an infection, is a serious and often fatal state. Catastrophic APS is initiated in (35%) of the cases by a variety of infections. Although present in 1% of APS patients, a reported (48%) mortality was found in a study of 80 patients (Marai *et al*, 2004).

Clinically, APS has been associated with infections by diverse viruses (parvovirus B19, human immunodeficiency virus (HIV) and hepatitis C virus (HCV), bacteria and (Mycobacterium Lepra and Helicobacter pylori) parasites (leishmaniasis) (Marai *et al*, 2004). Exposure to one or more infectious agent can cause a molecular mimicry between human β_2GPI and molecules similar to β_2GPI in invading agent and result in the production of pathogenic APLs that can induce thrombosis and abortion (Sherer *et al*, 2007). Reports of association of ACL Abs and/or LAs with viral infections, including HCV, HIV, cytomegalovirus (CMV), Varicella zoster, Epstein-Barr virus (EBV), adenovirus and parvovirus B (some of which were associated with thrombosis) have been reviewed (Rand *et al*, 2003). Among the various infectious agents, EBV early (IgG) antigen, toxoplasma (IgM) and rubella (IgM) were significantly elevated in APS compared with control (Shoenfeld *et al*, 2006). A positive family history for autoimmune disease and/or APS is common in patients with PAPS or SAPS. This finding supports a genetic contribution to APS. The percentage of a positive family history for autoimmune disease

tends to be higher in patients with SAPS than in those with PAPS (Weber *et al*, 2000). Some authors have demonstrated that a history of thromboembolism predicts new thromboembolic events and new unsuccessful pregnancies. It is advisable to categorize APS patients according to the presence or not of classic thrombophilia risk factors such as hypertension, diabetes mellitus, hypercholesterolemia, or tobacco use because they may contribute to modifications in the eventual risk factor profile. In addition, it is also important to take into account whether the patient has an inherited thrombophilia (Espinosa *et al*, 2008). Without treatment, the live birth rate of an APS pregnancy is approximately (20%). The risk of pregnancy loss in women with APLs and with a previous pregnancy loss has been estimated at over (60%) (Stone *et al*, 2001).

The presence of persistent LAs and/or Abs against either ACL or β_2GPI is currently the cornerstone for a laboratory diagnosis of APS (Tebo *et al*, 2008). Each test may identify different autoantibodies; a single test makes the diagnosis possible when positive on two or more occasions at least 6 weeks apart, single test positivity may be unrelated to pathogenic Abs (Pengo *et al*, 2010). Yet others have shown that triple APLs positivity is a marker of poor pregnancy outcome (Ruffatti *et al*, 2011).

1.2 Problem Statement

APS was first described in 1983 (Gezer, 2003). There is a little database about the prevalence of APS and abortion worldwide (Ricard *et al*, 2010). The highest rates of APS and abortion present in United Kingdom (31.3%) (Cervera *et al*, 2010) and the magnitude of the problem caused by APLs is largely unknown, especially in the developing countries (Mathai, 2010). Generally speaking, there are many studies on the causes of abortion, but

there is a lack of studies on the relationship between APS and abortion in Gaza Governorates. Therefore there is a need to have more studies about this topic.

1.3 Justification

LAs have been reported and found to be associated with the increase of abortion (Gezer, 2003). The abortion rate in Gaza Governorates represents (10.6%) of governmental and nongovernmental sectors (MOH, 2010). There are 194 patients in El-Helal El-Emirati hospital take heparin, 140 patients in Naser hospital, 130 patients in El-Aqsa Martyrs and 407 patients in Al-Sheffa hospital (MOH, 2010). However, this clinical indication needs to be investigated in more details especially in Gaza Governorates. This study was the first one, investigating the APS among aborted women in their second-trimester in Gaza Governorates.

1.4 Study Objectives

1.4.1 General Objective

The general objective of the study is to assess the relationship between APS and second-trimester abortion in Gaza Governorates.

1.4.2 Specific Objectives

1. To determine the level of the following tests among cases and control for APS diagnosis:

A. LA test.

B. dRVVT.

C. ACL (IgM).

D. ACL (IgG).

2. To determine the level of the following tests among aborted and non aborted women for APS diagnosis:

A. LA test.

B. dRVVT.

3. To determine the best sensitive and specific test for APS diagnosis.

4. To investigate the relationship between APS and some sociodemographic factors such as employment status, a place and age group.

5. To determine the level of the following tests among cases and control to exclude confounder variables:

A. Toxoplasma (IgM).

B. Rubella (IgM).

6. To determine the following tests among cases and control to confirm negative results of participants:

A. FBS.

B. TSH.

C. Hypertension.

1.5 Questions

1. Is there a relationship between APS and second-trimester abortion?
2. Is there a difference between APS among employee and non employee women?
3. Is APS percent differ from Rafah Governorate than KhanYounis Governorate?
4. Is there a relationship between APS and age group?
5. Is there a relationship between LA test and second-trimester abortion?
6. Is there a relationship between dRVVT and second-trimester abortion?
7. Is there a relationship between ACL (IgM) and second-trimester abortion?
8. Is there a relationship between ACL (IgG) and second-trimester abortion?
9. Which test has the highest sensitivity and specificity in APS diagnosis?
10. Is there a relationship between toxoplasma (IgM) and second-trimester abortion?
11. Is there a relationship between rubella (IgM) and second-trimester abortion?

1.6 Context of the Study

1.6.1 Gaza Governorates Demographic Characteristics

Gaza strip is a small piece of land located in the Southern area of Palestine with about 1.56 million inhabitants (MOH, 2010). It is divided into five Governorates: North Gaza, Gaza, Mid Zone, KhanYounis Governorate and Rafah Governorate (Palestinian Central Bureau of Statistics, 2008). Gaza Governorates are characterized with high population density with more than 4,700 individuals per square kilometer. The Israeli Occupation imposed buffer zone is excluded from the total area of 387 km^2. This high population density and narrow

place of land creates high demands for health care services and possible work overload for health care providers (PCBS, 2008).

1.6.2 Health Care System

Palestinian health care system is complex as there are the four main providers for healthcare services; MOH, United Nations Relief and Works Agency (UNRWA), Non-Governmental Organizations (NGOs) and the private for-profit service providers. MOH is the main health care provider in the Governorates; it provides primary health care (PHC), secondary and tertiary services for the whole population. The MOH purchases advanced medical services through referring patients to the neighboring countries and other private and NGOs health care facilities. UNRWA PHC services to the refugee population, and purchases secondary and tertiary care services when needed. The NGOs sector ranges from missionary hospitals, to facilities supported by international organizations, to community health centers. The private for-profit health sector also provides the three levels of care through a wide range of practices (Mathia, 2005).

1.6.3 Maternal and Child Health Care (MCH)

It is a PHC component where these services should be available affordable and accessible to all the target population in their communities. Maternal and child care services provided by the MOH, UNRWA and NGOs together. Services are free of charge for children under 3 years and pregnant. MCH services are provided for who covered by governmental health insurance. Maternal health include antenatal care, natal care, postnatal care, family planning and family health counseling aim to insure complete health care for all women

during their reproductive life and children in the community. MCH activities for women health include: Provision of antenatal care including regular examination (CBC and urine analysis…etc), immunization, proper nutrition and self care provision of safe delivary site postnatal follow up, family planning services and health education.

There are 45 maternal and child health centers (25 MOH and 20 UNRWA). The number of pregnant women registered in PHC of MOH is 13793 and 43395 in UNRWA centers. The rate of new pregnant women of high risk group is (23.4%) from the total of new pregnant women. The rate of delivary in clinical centers is (99.9%) and (0.04%) in houses. MOH total (normal and cesarean) delivary represents 39238 (71.3%), NGOs delivary represents 15844 (28.7%). MOH abortion number represents 5824 (12.9%) and NGOs abortion number represents 755 (4.5%) (MOH, 2010).

1.6.4 MOH Hospitals

MOH owns and operates 13 hospitals with 1993 beds. While in 2007 1587 beds, the increasing of beds related to change in the definition of hospital beds "all the available beds and ready to services whether they are inpatient or outpatient". Outpatient beds include emergency and daily care (MOH, 2010).

Table 1.1: MOH-Hospitals Distribution by Specialty and Number of Utilized Bed in the Year 2009

Specialty	No. of Hospital	No. of Beds
General	7	1554
Pediatrics	3	303
Maternity	1	64
Psychiatrics	1	26
Ophthalmic	1	46
Total	13	1993

During the year 2009, about 35418 deliveries were recorded at MOH hospitals. The caesarian deliveries constituted (18.0%) of the total deliveries in MOH hospitals. The highest rate of delivery was in Al-Shifa hospital (44.4%) and the lowest rate (13.5%) was in Al Aqsa hospital (MOH, 2010).

Table 1.2: Distribution of Deliveries by Mode in the MOH Hospitals, in the Years 2007, 2008 and 2009

Years	Caesarian	Normal	Total deliveries	% of caesarian
2007	4445	29831	35276	15.4
2008	5743	28582	34325	15.4
2009	6384	29034	35418	18.0

1.6.5 MOH Laboratory Facilities

MOH offers laboratory services through three levels: Central, intermediate and peripheral. The peripheral is the highest (37 labs) with total number of tests 3619519 in the 2009. There were 419 employees offering laboratory services in MOH, 250 laboratory technician were in hospitals, 30 in central lab and 139 in PHC centers (MOH, 2010). The laboratories of MOH does not investigate tests of APS diagnosis but tests such as TSH, blood sugar, toxoplasma (IgM) and rubella (IgM), sometimes investigated. The book permission of MOH for researchers gives only the right to obtain part of patient's blood (MOH, 2010).

1.7 Operational Definitions

1.7.1 Antiphospholipid Syndrome

APS: is an acquired thrombophilia, characterized by the occurrence of venous and arterial events **(**Giannakopoulos *et al*, 2009). APS is characterized by thrombosis and pregnancy loss in the presence of APLs, mainly ACL, anti β_2GPI and LAs (Marai *et al*, 2001).

1.7.2 Antiphospholipid Antibodies

APLs produced the body against itself in an autoimmune response to PLs detected by immuno and/or clotting assays1 that is either as ACL, β_2GPI, or LAs (Amitrano *et al*, 2009). APLs are immunoglobulin's, used as an important marker for recurrent thrombosis (Kaul et *al*, 2007). APLs (synonym: Inhibitors) are endogenously produced substances which may interfere with coagulation either *in vivo* or *in vitro* (Triplett, 2000).

1.7.3 Abortion

Abortion: is the loss of a pregnancy during the first 20 weeks of gestation, at a time that the fetus cannot survive (Cunningham *et al*, 2008).

1.7.4 Anticardiolipin Antibodies

ACL: is a molecule found in blood platelets and various cell membranes, cardiolipin is diphosphatidylglycerol, has a negatively charged PL, present mainly in the inner surface of plasma membranes and regulate blood clotting throughout body. (IgG) and IgA anticardiolipin more related than (IgM) to thrombotic phenomena (Marai *et al*, 2001).

1.7.5 Lupus Anticoagulants

LAs: is an immunoglobulin's, usually of the (IgG) class, also referred as APLs because they interfere with PL-dependent coagulation tests by reacting with the PLs in the test system. They are not associated with a bleeding disorder unless thrombocytopenia or antiprothrombin Abs are already present (Rand *et al*, 2003).

1.7.6 dilute Russell Viper Venom Time

dRVVT: is the most widely used clinical laboratory assay for confirmation of the presence of a PL-dependent ab (Riley, 2005). This *in vitro* diagnostic test is based on the ability of the venom of the Russell's viper to induce thrombosis. The coagulant protein of (RVV) is an enzyme (serine protease) in the venom directly activates factor X, in the presence of calcium ion, factor Xa cleaves prothrombin to thrombin, which converts fibrinogen to fibrin leading to clot formation in the presence of factor V and PL (Kaul *et al*, 2007).

1.7.7 Sensitivity

Sensitivity: is the ability of a test to identify correctly those who have the disease.

1.7.8 Specificity

Specificity: is the ability of a test to identify correctly those who do not have the disease.

1.7.9 Stillbirth or Fetal Death

The absence of signs of life at or after birth (Cunningham *et al*, 2008).

1.7.10 Elective or Voluntary Abortion

Elective or voluntary abortion: is the interruption of pregnancy before viability at the request of the woman but not for reasons of impaired maternal health or fetal disease. Most abortions performed today fall into this category. Induced abortion is the medical or surgical termination of pregnancy before the time of fetal viability (Cuningham *et al*, 2008).

Chapter 2:

Literature Review

2.1 Prevalence of APS and Abortion

The prevalence of APS and abortion was reported in UK (31.3%) (Cervera *et al*, 2010), Spain (30%) (Zinger *et al*, 2009), Bahrain (27%) (Naqdy *et al*, 2005) and Oman (27%) (Naqdy *et al*, 2005), followed by Jordan (19.2%) (Daboubi, 2001). In a multicenter study, (53.1%) of patients had primary APS, while (36.2%) had SLE with secondary APS. The most frequently presenting manifestations of APS are deep venous thrombosis (DVT) (31.7% of patients), thrombo-cytopenia (21.9%), livedo reticularis (20.4%) and stroke (13.1%). Less frequent manifestations include superficial thrombophlebitis (9.1%), pulmonary embolism (9%), TIA (7%) and hemolytic anemia (6.6%). Fetal loss is the presenting manifestation in (14%) of female patients (Rai *et al*, 2010).

2.2 Abortion Definition

Abortion is premature birth before a live birth is possible, in this sense it is synonymous with miscarriage, it also means an induced pregnancy termination to destroy the fetus, both terms are used interchangeably in a medical context, popular use of the word abortion by laypersons implies a deliberate pregnancy termination, such a loss may be involuntary (spontaneous), or it may be voluntary (induced or elective) (Cunningham *et al*, 2008).

Second-trimester abortion complicates (1%) of pregnancies and the previous abortions in the first trimester represents (85%) of cases (Gaufberg *et al*, 2010).

2.3 Abortion Classifications

An abortion can be classified clinically by a number of ways:

2.3.1 Clinical Classification of Abortion

1. Complete Abortion: Uterine contractions are felt, the cervix dilates and blood loss continues, the fetus and placenta are expelled complete, the uterus contracts and bleeding stops, no further treatment is needed (Hanretty, 2003).
2. Incomplete Abortion: in spite of uterine contractions and cervical dilatation, only the fetus and some membranes are expelled, the placenta remains partly attached and bleeding continues, this miscarriage must be completed by surgical methods (Hanretty, 2003).

2.3.2 Classification of Abortion According to Number of Loss

1. Recurrent Abortion: Recurrent abortion or habitual abortion refers to three or more consecutive spontaneous abortions (Cunningham *et al*, 2008).
2. Sporadic Abortion: Sporadic abortion refers to one spontaneous abortions (Cunningham *et al*, 2008).

2.3.3 Classification of Abortion According to Time of Loss

1. First-trimester abortion.
2. Second-trimester abortion: A late or second trimester-abortion occurs between 12 and 24 weeks gestation. After 24 weeks gestation, the baby is potentially viable and birth is referred to as a preterm delivery (Baker, 2006).
3. Third-trimester abortion.

2.3.4 Classification of Abortion According to Number of Weeks

1. Biochemical loss: < 6weeks.
2. Early pregnancy loss: 6–8 weeks.
3. Late pregnancy loss: >10 weeks (Edmonds, 2007).

2.3.5 Second-Trimester Abortion According to Clinical Classifications

1. Threatened: Bleeding contractions, abnormal vaginal discharge, minimal cervical dilatation and intact membranes.
2. Inevitable: Any of the above or may be minimal, cervical dilatation > 3cm and membranes ruptured.
3. Missed: Usually none of the above and fetal death on ultrasound.
4. Septic: Above and malaise, fever, tachycardia, hypotension and uterine tenderness (Baker, 2006).

2.3.5.1 Causes of Second-Trimester Abortion

1. Chronic maternal health factors account for the majority of second-trimester abortions include:
   A. Severe hypertension.
   B. Renal disease.
   C. Hypothyroidism and hyperthyroidism: The published data on the association between overt hyperthyroidism and venous thrombosis are limited to few case reports (Franchini *et al*, 2011).
2. Other maternal health factors such as:
   A. Infections such as rubella, CMV, Mycoplasmal, Ureaplasmal, Listeria and Toxoplasma.
   B. Trauma.
   C. Severe emotional shock (Gaufberg *et al*, 2010).
3. Uterine defects, are frequently associated with second-trimester abortion.
4. Environmental factors, are related to exposures to prescription drugs, chemicals, or radiation (Gaufberg *et al*, 2010).
5. Immunologic factors such as APS.

2.4 APS

The APS defines the occurrence of thrombosis, and pregnancy morbidity in the presence of APLs detected by immuno and/or clotting assays that is either as ACL, β_2GPI, or LAs, the targets of APL are PL-binding plasma proteins or complexes of these proteins with PLs (Amitrano *et al*, 2009). APS is an autoimmune disease characterized by the presence of

APLs of IgG or (IgM) class, which are thought to be involved in the development of venous and/or arterial thrombosis and pregnancy morbidity (Devreese, 2006).

2.4.1 APS Classifications

Some authors divide APS into one of six subgroups:

Type 1: Consists of DVT and pulmonary embolism.

Type 2: Basically involves arterial thrombosis, such as coronary artery thrombosis, peripheral artery thrombosis, aortic thrombosis and carotid artery thrombosis.

Type 3: Includes artery thromboses that are basically intracranial, such as retinal artery or retinal vein thrombosis, cerebrovascular thrombosis and transient ischemic attack (TIA).

Type 4: Is rare, composed of a mixture of types 1, 2 and 3.

Type 5: Comprises fetal wastage syndrome secondary to placental vascular thrombosis and rarely maternal thrombocytopenia.

Type 6: Found in patients who harbor APLs without any clinical manifestations of thrombosis as yet.

There is usually correlation with type or titer of ACL and type of syndrome; however subclassifications of APS might be important from the standpoint of therapy (Gezer, 2003).

2.4.2 Clinical Symptoms and Etiology of APS

A number of retrospective studies have established a strong relationship between the presence of circulating LAs and ACL and pregnancy loss. The rate of fetal loss in untreated women with LAs and previous pregnancy failure may be as high as (80%). This

relationship has been confirmed by cohort studies in unselected populations and in women with APS. Remarkably, APLs related pregnancy losses often occur in the second or third trimester (Gronowski, 2004). APS is a disorder that manifests clinically as recurrent venous or arterial thrombosis and/or fetal loss, clinical features can vary widely and can involve any organ system but the features for both primary and secondary APS are identical (Thomas, 1998).

2.4.3 Physical Findings and Clinical Presentation of APS

Thrombosis: Patients with APS are at risk for both venous and arterial thromboses. Venous thromboses are more common, occurring as the initial manifestation of APS in approximately (30%) of APS patients. Of all patients with venous thrombosis, (5%) to (20%) have APL. The most common site for deep vein thrombosis is the calf, but thromboses may also occur in the renal, hepatic, axillary, subclavian, vena cava and retinal veins. The most common site of arterial thrombosis is the cerebral vessels. Other common sites are the coronary, renal, mesenteric and bypass arteries. Recurrent thrombosis is common with APS.

1. Central nervous system: Stroke, transient ischemic attack, migraine, multiinfarct dementia, epilepsy, movement disorders, transverse myelopathy, depression and Guillain-Barre syndrome.
2. Pulmonary: Pulmonary embolism and infarction, pulmonary hypertension, acute respiratory distress syndrome, intraalveolar pulmonary hemorrhage, a postpartum syndrome characterized by fever, pleuritic chest pain, dyspnea and patchy infiltrates with pleural effusion on chest radiograph.

3. Cardiology: Libman-sacks endocarditis, intracardiac thrombosis, coronary artery disease and myocardial infarction.
4. Gastrointestinal: Abdominal pain, gastrointestinal bleed secondary to ischemia, splenic or pancreatic infarction, hepatic vein thrombosis and budd-chiari syndrome (second most common cause of syndrome).
5. Renal: Proteinuria, acute renal failure, hypertension, renal infarct, renal artery or vein thrombosis and postpartum hemolytic-uremic syndrome.
6. Hematology: Thrombocytopenia and hemolytic anemia.
7. Endocrine: Addison's disease secondary to adrenal hemorrhage and less frequently thrombosis.
8. Cutaneous: Such as livedo reticularis, cutaneous necrosis, skin ulcerations and gangrene of digits.
9. Obstetrics: Recurrent spontaneous abortion (secondary to placental vessel thrombosis and ischemia).
10. Catastrophic APS: Widespread thrombotic disease with visceral damage (Ferri, 2010).

2.4.4 Overview of APLs

Are a heterogeneous group of Abs about 40 types which were originally thought to be directed against anionic PLs (Demarco *et al*, 2006). APLs are used to detect several specific PL-binding proteins that the body produces against itself in an autoimmune response to PLs found in cell membranes and platelets, PLs are a normal part of the body (Demarco *et al*, 2006). APLs are present in (1-5%) of the general population, most APLs in apparently healthy individuals are low titer and transient (Danowski *et al*, 2009).

2.4.4.1 Pathogenesis of APLs

APLs present in the blood appear to react with cell membranes, causing the cells to behave as though they have been irritated or stimulated; this disturbs the normally well-controlled coagulation system. The exact mechanism by which APLs induce a thrombophilia state is not known.

There was several mechanisms have been proposed by which APLs may result in these complications of pregnancy including the following:

1. Interference with the components of intrinsic pathway.
2. Inhibition of factor XI activation.
3. Patients with APS were often found to have factor XII deficiency or inactivation.
4. Inhibition of anti-thrombin activity.
5. Interference with components of the protein C pathway.
6. Inhibition of activated protein resistant (APC) anticoagulant pathway.
7. Association of APS with protein S deficiency.
8. Interference with the activation of protein C by the thrombomodulin-thrombin Complex.
9. Inhibition of thrombin formation.
10. Binding to coagulation activated cofactors Va and VIIIa (Vlachoyiannopoulos *et al*, 2007).
11. Activation of endothelial cells with up-regulation of adhesion molecules and subsequent increased endothelial adherence by platelets and monocytes.
12. Suppression of the functions of other coagulation inhibitors (β_2GPI and annex in V) (Gronowski, 2004).

13. An Ab may interact with platelet, causing them to release their cellular constituents and form a clot.
14. An Ab may also attach to the surface of endothelial cells that line blood vessels. This will cause the normally nonreactive endothelial surface to appear reactive to circulating blood, and a clot will form (Gezer, 2003).

2.4.4.2 ACL Abs Detection

The detection of ACL by solid phase immunoassays was historically the first specific APL assay. Initially, ACL were detected by radio immuno assay (RIA), which was soon replaced by enzyme linked immuno sorbent assay (ELISA). ACL are usually detected in serum, although plasma samples have also been used. However, plasma samples may give lower readings due to the presence of anticoagulant; therefore, when ACL is tested in plasma, it should be mentioned in the report of the results. Typically, both the (IgG) and the (IgM) isotypes are measured, as the diagnostic criteria for APS do not attribute more diagnostic value to the IgG isotype. The (IgA) isotype is also measured in several laboratories, but the clinical significance of elevated ACL (IgA) is not clear. ACL (IgA) may help define a subgroup of patients at higher risk of skin disease and thrombocytopenia. The advantages of ACL ELISAs are the speed and the simplicity of the assay as well as the availability of many commercial kits. Additionally, ACL ELISA results are not influenced by anticoagulant drug use or the existence of other coagulation abnormalities, in contrast to LAs determinations. Despite its widespread use, ACL ELISAs have some major drawbacks. They have low specificity (Vlachoyiannopoulos *et al*, 2007).

ACL ELISA are found in a variety of infectious and post infectious disorders in addition to the APS, rendering a positive result as non-specific. It has been shown the difference between ACL Abs in patients with APS and other diseases (Passam and Krilis 2004).

Table 2.1: Prevalence of Anticardiolipin in Various Disease States

Condition	**Prevalence (%)**
Stroke, all ages	10–15
Stroke < 50 yrs	50
Deep venous thrombosis	24–30
Myocardial infarction < 50 y	21
Recurrent miscarriages	25–30
Systemic lupus erythromatosus	23–47

2.4.4.3 Lupus Anticoagulants (LAs) Detection

LAs: are Abs that interfere with the PL-dependent steps in the coagulation cascade, causing *in vitro* prolongation of the respective coagulation times. The paradox of LAs is that, although they are acquired coagulation inhibitors, they are associated with thrombosis rather than bleeding. Several clinical studies have shown that among the APL, LAs is the strongest risk factor for thrombosis. LAs are a heterogeneous group of Abs directed against PL-binding Proteins. LAs are detected on the basis of their functional activity, i.e., by their ability to prolong the clotting time in phopspholpid- dependent coagulation assays, such as APTT. However, detection of LAs is complicated, and the Scientific and Standardization Committee of the International Society of Thrombosis and Haemostasis (SSC/ISTH) has established four criteria for their detection. Because no single test is able to confirm the presence of the detection is based not on one but on a combination of coagulation assays.

2.4.4.4 LAs Coagulation Assays

Official guidelines advise using at least two different assays to test for LAs. Practically any general physician is familiar with the Prothombin Time (PT) and the APTT, which are widely available. The APTT is especially helpful when there is a suspicion of APS and a prolongation of the APTT may lead to further, specific testing. It must be noted that many cases of asymptomatic APS are suspected on the basis of a prolonged APTT. The tests included in the LAs assays are the APTT and variations of the PT-dilute prothrombin time (dPT), dRVVT and kaolin clotting time (KCT). In all tests the sensitivity may be increased by diluting the PL content and preincubating prior to recalcification. APTT is a screening test for the intrinsic coagulation pathway (sensitive to acquired or inherited deficiencies of factor VIII, IX, XI, XII, high molecular weight (HMW) kininogen and prekallikrein) and common pathway (factors II, V, X and fibrinogen). In APS, the APLs causes APTT prolongation by preventing the assembly of the prothrombinase complex. The PT assay is known to assess the extrinsic pathway (tissue factor and F VII) and common pathway.

Citrated plasma is recalcified and tissue factor is added. When the thromboplastin is diluted and the test plasma is preincubated the test is called tissue thromboplastin inhibition assay (LAs sensitive). Variations of this method include dilution of the PL and addition of a snake venom, which directly activates F X (in the dRVVT), or KCT, or other venoms (in the Textarin-Ecarin). The KCT tests a broader range of the clotting cascade (including contact activation), whereas the dRVVT focuses on prothrombinase activation (Passam and Krilis 2004). As shown below figure (2.1) summarized the tests of LAs.

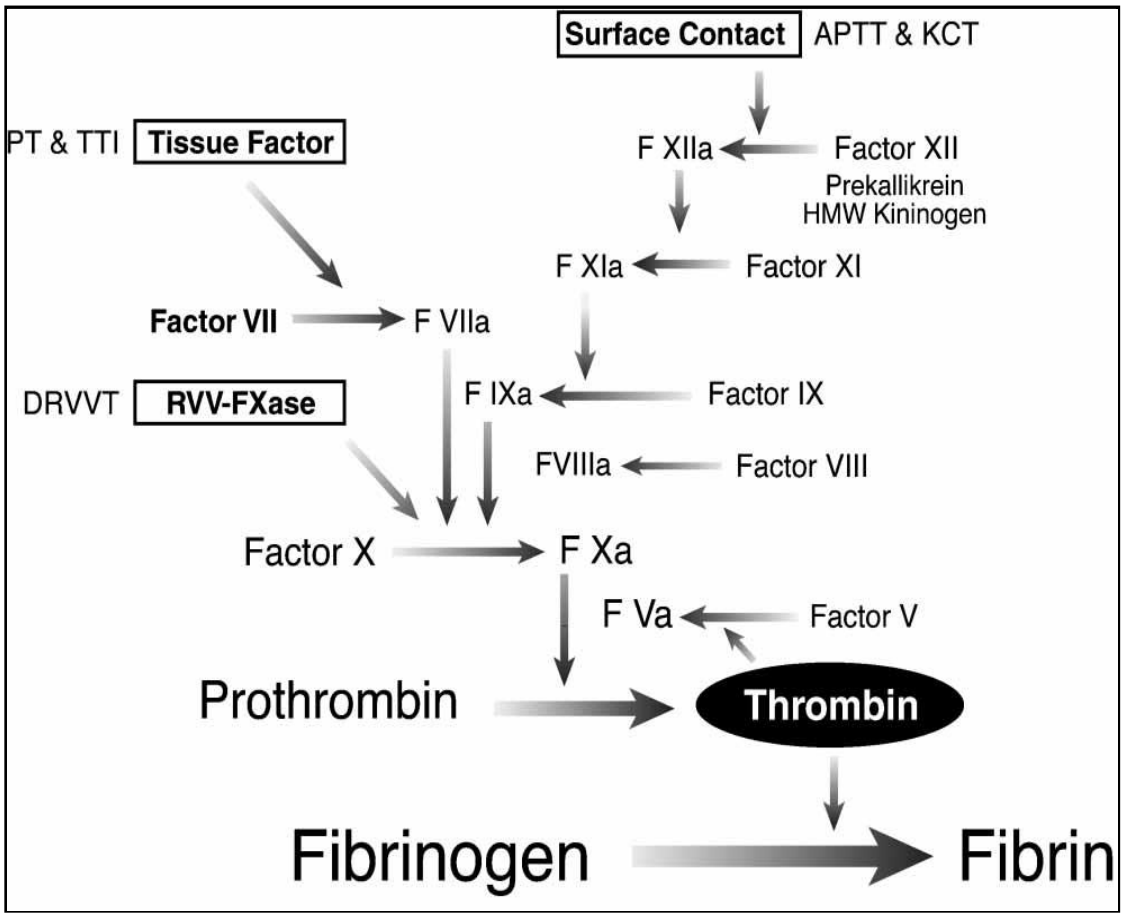

Figure 2.1: LAs Tests within Fibrin Cascade (Passam and Krilis 2004)

2.4.4.5 Advantages of LAs Assays

1. Increased specificity for APS diagnosis.

2. The only established method for detecting ACL negative cases.

3. Higher predictive value. In a systematic review of the literature, the relative risk for thrombosis comparing LAs and ACL assays has shown a higher predictive value for LAs (Passam and Krilis, 2004).

2.4.4.6 Disadvantages of LAs Assays

1. Tedious methodology.

2. Low reproducibility of results.

3. Dependence on platelet contamination or anticoagulant treatment.

Regarding the second limitation, the dRVVT at low and high PL concentrations has been shown to be reliable for LAs determination in patients receiving oral anticoagulant (Passam and Krilis 2004).

2.4.4.7 dRVVT Detection

Russell's viper venom (RVV) is the most widely used clinical laboratory assay for confirmation of the presence of a PL-dependent ab. It causes massive thrombosis when injected *in vivo* (Riley, 2005). This *in vitro* diagnostic test is based on the ability of the venom of the Russell's viper to induce thrombosis. The coagulant protein of (RVV) is an enzyme (serine protease) in the venom directly activates factor X, in the presence of Calcium ion, factor Xa cleaves prothrombin to thrombin, which converts fibrinogen to fibrin leading to clot formation in the presence of factor V and PL. In the dRVVT assay, low, rate-limiting concentrations of both Russell's viper venom and PL are used. This makes the test sensitive to the presence of LAs, because these Abs interfere with the clot-promoting role of PL *in vitro*, and their presence results in a prolonged clotting time. A mixing study is then performed, which consists of adding an equal volume of the patient's plasma to normal plasma. In this study, one would expect the clotting time to return to the normal range if there was only a deficiency of coagulation factors alone. A prolonged clotting time of 30 seconds or greater that does not correct despite the mixing study

suggests the presence of a LA (Thiagarajan *et a,l* 1986). An abnormal result for the initial dRVVT assay should be followed by a dRVVT confirmatory test. In this test, the inhibitory effect of LA on PLs in the dRVVT can be overcome by adding an excess of PL to the assay. The clotting times of both the initial dRVVT assay and confirmatory test are normalized and then used to determine a ratio of time without PL excess to time with PL excess. In general, a ratio of greater than 1.2 is considered a positive result and implies that the patient may have APLs. The dRVVT test is more sensitive than the APTT test for the detection of LAs, because it is not influenced by deficiencies or inhibitors of clotting factors VIII, IX or XI. APS is an important marker for recurrent thrombosis, and often warrants indefinite anticoagulant (blood thinner) therapy. The criteria were defined in 1999, and revised in 2006 (Kaul *et al*, 2007).

2.4.5 Diagnosis of APS

The diagnosis of APS is based on the modified clinical and laboratory criteria as proposed by the International Antiphospholipid Symposium in Sapporo. Definite APS is defined by the presence of at least one clinical criterion and one laboratory criterion (figure 2.2). The Sapporo criteria for definite APS did not define other categories of APS (Marai *et al* 2004). There are now internationally agreed criteria for classification of APS, updated in 2006 (Edwards, 2008), depend on Sapporo criteria were used for the definition of APS as more recent guidelines were only published in 2006 (Bramham *et al*, 2010). Diagnosis of APS requires at least one clinical and another laboratory criterion (Gaufberg *et al*, 2010). Clinical studies do not distinguish between patients with different laboratory profiles. Triple positivity appears the only pattern that identifies high-risk patients with APS (Pengo *et al*, 2010).

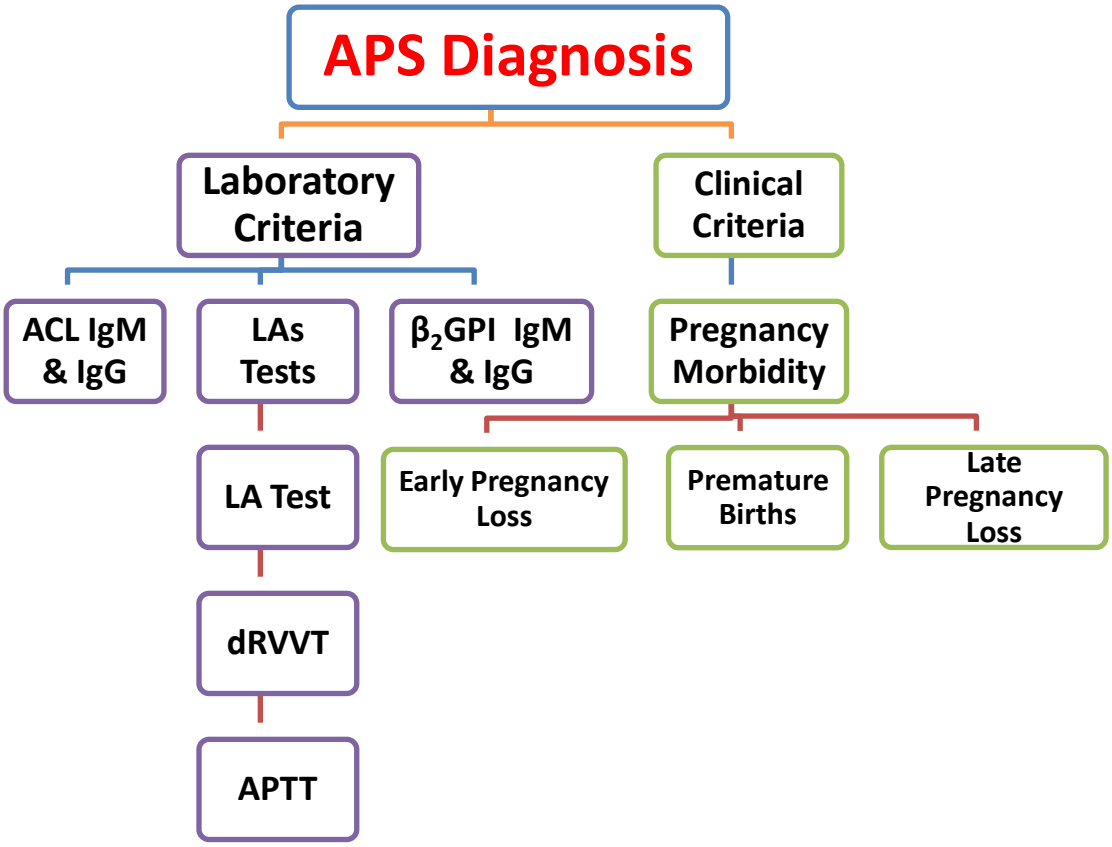

Figure 2.2: APS Diagnosis (Belilos *et al* 2004)

2.4.5.1 Clinical Criteria

Vascular thrombosis: One or more clinical episodes of arterial, venous, or small-vessel thrombosis, occurring within any tissue or organ.

Pregnancy Morbidity

1- One or more late-pregnancy loss (>10 weeks gestation).

2- One or more premature births of a morphologically healthy neonate at or before 34 weeks' gestation because of severe preeclampsia or eclampsia or severe placental insufficiency.
3- Three or more unexplained, consecutive, early pregnancy loss (<10 weeks gestation) **(**Belilos *et al* 2004)**.**

2.4.5.2 Laboratory Criteria

Laboratory criteria include the presence of medium or high titers of β_2GPI and/or ACL, LAs in plasma on two or more occasions at least 6 weeks apart. Patients with APS may have one or more abnormal results.

The following laboratory tests should be considered in a patient suspected of having APS:

1- LA test detected in the blood on two or more occasions at least 6 weeks apart.
2- dRVVT detected in the blood on two or more occasions at least 6 weeks apart.
3- ACL (IgG) present at moderate or high levels in the blood on two or more occasions at least 6 weeks apart.
4- ACL (IgM) present at moderate or high levels in the blood on two or more occasions at least 6 weeks apart.
5- β_2GPI (IgG) present at moderate or high levels in the blood on two or more occasions at least 6 weeks apart.
6- β_2GPI (IgM) present at moderate or high levels in the blood on two or more occasions at least 6 weeks apart.
7- APTT.

8- Syphilis Abs.

9- Platelet count **(**Belilos *et al* 2004**).**

2.5 Theoretical Framework

Loss of pregnancy is a physically and emotionally challenging ordeal. When pregnancy loss is repetitive, these feelings are magnified and the result is a distressing and frustrating problem for both the patients and the physicians. Early pregnancy losses are losses of embryos during the first trimester of pregnancy and have been termed abortions or miscarriages. Although most miscarriages are sporadic and not repetitive, there is a subset of couples that suffer recurrent miscarriage. the risk of miscarriage increases with maternal age.

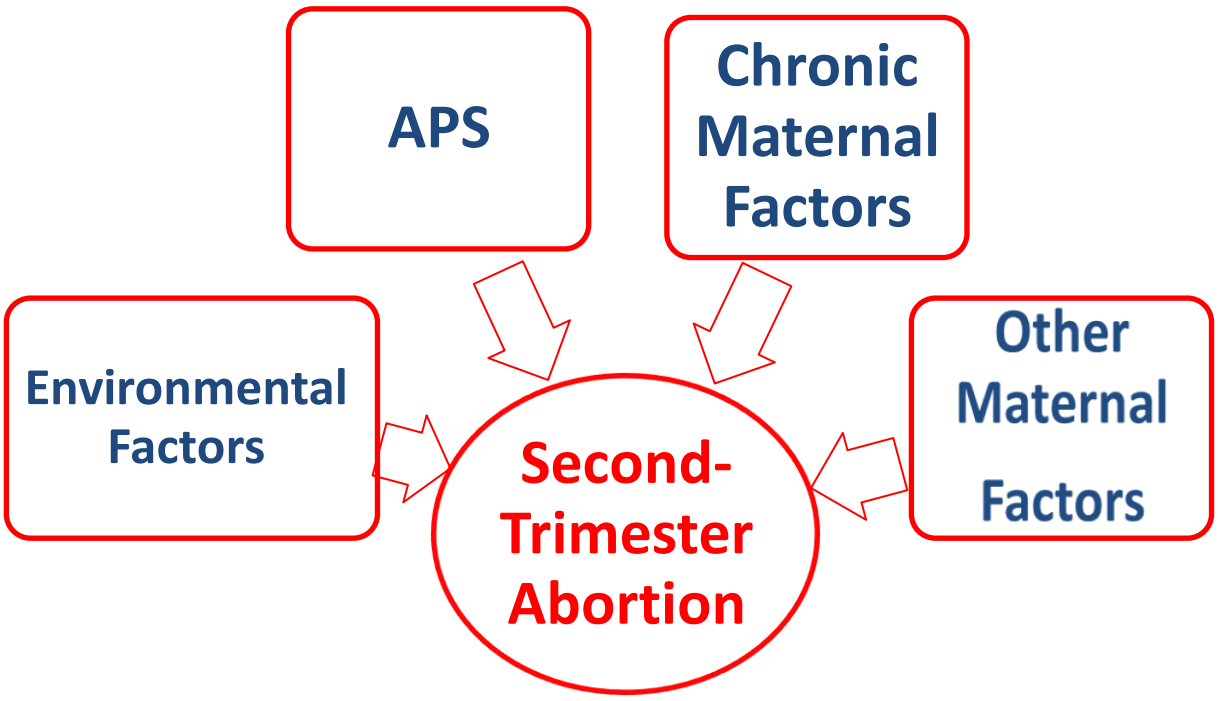

Figure 2.3: Theoretical Framework

Framework can be used as the basis for the measurement of the performance of the public health system as a whole or for a specific public health organization. The developed model allowed public health researchers, practitioners, and policymakers to more effectively examine the relationship between the practice of public health and population outcomes which could contribute to the development of a science base for the public health system. The approach adopted by the researcher has been through identifying five categories forming the dimensions of second-trimester abortion, which are: Chronic maternal factors, other maternal factors, environmental factors, immunologic factors and uterine defect (figure 2.3). This work studied the relationship between APS and second-trimester abortion.

2.6 Previous Studies

Most APS clinical studies include patients with single APLs results and/or low-titer APLs ELISA results. Furthermore, study designs are mostly retrospective and not population based, with a limited number of prospective and/or controlled population studies. Another impediment to APS clinical research is that there is a limited number of multicenter collaborative studies to recruit adequate numbers of subjects (Erkan *et al*, 2011). Due to the limited availability of national surveys, studies with local datasets, the researcher retrieved surveys concerning prevalence of APS and its relationship with abortion from different countries; literature review performed using Pub Med and Hinari. The researcher first retrieved all medical subject headings related to the concepts of APS, second-trimester abortion prevalence, as well as keywords for categorizing articles according to countries of the Middle East, then developing countries then international.

We employed these Pub Med words, limiting the search to the time period 2000–2011. The researcher obtained 20 results only.

2.6.1 Regional Coverage of Studies

The number of studies from the prevalence of APS and second-trimester abortion were as follows: 6 from the USA, three from occupied Palestine, two from Spain, two from UK, followed by Jordan one, Oman one, Kuwait one and Bahrain one. It was not possible to retrieve any studies from Syria, Emirate, Saudi Arabia, Qatar, Egypt, Iran and Yemen. Of the 20 selected articles only 7 investigated, a sample size greater than 50 individuals (Annex 1). They were designated as cohort, case control and cross sectional studies. The studies covered an age group of reproductive females diagnosed with APS with relatively small sample size as shown in (Annex 2). Different surveys and standards were employed in purpose of defining laboratories cutoff point criteria and reference value respectively, especially ACL (IgM) and ACL (IgG). After analyzing the studies shown in (Annex 2), the author have summarized the prevalence rates from surveys covering the countries employed in (Annex 3).

2.6.2 Occupied Palestine Studies

A study was carried out in the occupied Palestine, assessed APLs titer among 90 patients and its correlations with the clinical manifestations and symptoms of the disease. The purpose of the study was to review the manifestations of the APS in occupied Palestine, and to investigate the difference between patients with primary or secondary syndrome (Marai, 2001). Analysis of the correlation between the manifestations of the disease and

the ab titers LAs, ACL (IgM) and ACL (IgG) was also performed. In the study there were 90 patients with APS in the Sheba Medical Center. The clinical findings for all patients were recorded according to established protocol, arterial thrombotic events were present in (51.1%) of the patients; cerebral ischemic attacks were the most frequent arterial events, venous thrombotic events were present in (45.6%) patients, DVT was the most frequent venous event, obstetric complications were found in (37.3%) of patients; the most frequent complication was abortions. The clinical findings of patients with primary or secondary syndrome were similar venous or arterial thrombosis and obstetric complications are the most frequent clinical findings in APS. There was no different in clinical manifestations between primary and secondary syndrome.

In study on autoantibody explosion in APS occupied Palestine, which describe the autoantigen properties, prevalence and clinical importance of 30 different Abs in APS. Among the other Abs characterizing APS are autoantibodies directed to platelets, glycoprotein's, various coagulation factors, amines, mitochondrial antigens and cell surface markers. Few of these autoantibodies are correlated with the presence of other Abs and some may have an additive role in the pro-thrombotic tendency of the syndrome. This autoantibody explosion might be important in early identification of the syndrome and its manifestations (Shoenfeld *et al*, 2008).

2.6.3 Developing Countries Studies

Case-control study was carried out in Jordan to determine whether the level of ACL in women with recurrent abortion differed from that in the general population (Daboubi, 2001). A group of 26 patients defined as habitual aborters (at least three consecutive

spontaneous abortions) and in a control group of 26 patients each of whom had at least one live birth without pregnancy wastage. High level of ACL activity was detected among (19.2%) of the habitual aborters but in non of the control, indicating an association between ACL level and habitual abortion.

In a study on a favorable outcome of pregnancies in women with primary and secondary recurrent pregnancy loss associated with APS in Kuwait, 43 patients with recurrent pregnancy loss associated with APS diagnosed before pregnancy and subdivided into primary 18 and secondary 25 subgroups. They were closely monitored all through pregnancy with serial blood tests and ultrasonography until the pregnancy ended in abortion or delivery. The patients were treated with low-dose aspirin and heparin and/or steroids and IV given to some selected patients. The maternal and fetal outcomes were analyzed (85.0%) of all the previous abortions were in the first trimester. There was no significant difference in the incidence of live births in the primary (77.8%) and secondary (84.0%) groups (Diejomaoh *et al*, 2002).

Case-control study was conducted in Oman to detect ACL and β_2GPI in Omani patients with APS (Naqdy *et al*, 2005). ACL were detected in (23%) with SLE, (27%) suffering from recurrent abortion and (36%) of patients with thrombosis/thrombocytopenia while anti β_2GPI Abs were detected in (16.6%), (18%) and (22%) of same patients, respectively. Study concludes data demonstrate a high prevalence of ACL and β_2GPI of either combined or separate pattern among the Omani patient groups studied.

A cross-sectional study was carried out in Brazil to study determinants of risk for venous and arterial thrombosis in primary APS and in APS with SLE (Danowski *et al*, 2009). A total of 122 patients reviewed for the following variables: Gender, ethnicity, hypertension, triglycerides, cholesterol, smoking, DM, homocysteine, cancer, HCV, hormone replacement therapy/oral contraceptives, hereditary thrombophilia, ACL (IgM), ACL (IgG) and LAs. The frequency of thrombosis and pregnancy loss is greater in APS associated with SLE than in primary APS. Risk factors differ for venous and arterial thrombosis in APS. The study concludes treatment of hypertension may be the most important intervention to reduce arterial thrombosis. Elevated triglycerides are a major associate of venous thrombosis, but the benefit of treatment is not known. Hereditary thrombophilia is an associate of venous but not arterial thrombosis, making it cost-effective to investigate only in venous thrombosis.

2.6.4 Developed Countries Studies

The familial history for evidence of APS and autoimmune disease in rheumatology department patients with primary or secondary APS was evaluated (Weber *et al*, 2000). Thirty nine patients had PAPS and 69 SAPS. Family history data were obtained for 29 (74%) and 55 (80%) PAPS and SAPS patients, respectively (78% of the 108 patients). Twelve PAPS (41%) and 19 SAPS (35%) patients had one or more relatives with evidence of at least one clinical feature of APS such as thrombosis or recurrent fetal loss; of these patients, seven in the PAPS (24%) and 11 in the SAPS (20%) group had two or more relatives with evidence of a clinical feature of APS. Three PAPS (10%) and 14 SAPS (25%) patients had one or more family members with an autoimmune disease. The study

concludes that A positive family history for autoimmune disease and/or APS is common in patients with PAPS or SAPS. This finding supports a genetic contribution to APS. The percentage of a positive family history for autoimmune disease tends to be higher in patients with SAPS than in those with PAPS.

Gali et al. (2003) established the risk of LAs and ACL for arterial and venous thrombosis. They ran a MEDLINE search of the literature from 1988 to 2000. Studies were selected the detection of LA and ACL at medium or high titers helps to identify patients at risk for thrombosis. The study concluded that ACL were not such strong risk factors for thrombosis as LA and only (50%) of their associations with thrombosis reached statistical significance.

The clinical significance of LAs and APLs toward thrombosis and abortions was assessed (Gali *et al*, 2007). They measured them in 112 patients whose samples were available at enrollment in the warfarin in the APS study. The main demographic, clinical and laboratory features of the sample population are reported, 87 patients were diagnosed with APS because they had thrombotic and/or obstetric complications, whereas APLs were present in 25 patients either alone or in combination with clinical manifestations other than those qualifying for APS. The study confirmed the usefulness of the inclusion of (IgG) β_2GPI Abs among the laboratory diagnostic criteria of APS, it was found that levels of these Abs were significantly associated with all of the clinical end points qualifying for the syndrome. Conversely, ACL Abs did not show any significant association. These observations are well in agreement with the results of systematic review of the literature on APS which reported a higher frequency of significant associations with thrombosis for β_2GPI than for ACL Abs.

This review article explained how APLs may predispose to disease in humans. Furthermore, it also examines the evidence relating to the immunologic mechanisms that may contribute to the breakage of peripheral tolerance in this disorder. Fetal morbidity and mortality in APS may be due not only to placental thrombosis but also placental inflammation due to complement activation and to impairment of trophoblast function. The factors that determine whether APLs induce a thrombotic or no thrombotic disease phenotype in the placenta are not known. It is likely that an interplay between patient background traits and distinct APLs subgroups determines disease manifestation. Large multicenter, multipatient studies looking at the association between the various clinical manifestations of APS and the distinct APLs subgroups are a key area in assessing the contribution of the latter to the various manifestations (Giannakopoulos *et al*, 2007).

Giannakopoulos et al. (2009) examined the laboratory and key clinical aspects of APS established LAs tests and ACL assays, for diagnosing and risk stratifying patients suspected of having APS. A strong association in a number of retrospective analyses has been noted between positivity on multiple tests (LAs, ACL and β_2GPI) and thrombosis and miscarriages, compared with patients positive on one or 2 assays.

In a prospective comparative study, Saha et al. (2009) they screened 112 women with a past history either of pre-eclampsia, eclampsia, recurrent abortion, IUGR, IUFD or abruption placenta, with no apparent etiology and a demographically matched cohort of 106 women having a past history of uncomplicated pregnancy outcome for the presence of APL and their significance. In the former group, the prevalence of APL ranged from 10– (46.8%) compared with (8.4%) in the later group. In women with the presence of APL, the incidence of pre-eclampsia, early onset pre-eclampsia and abruption placenta were (25%),

(14.5%) and (18.7%), respectively. In the same group, the abortion rate was (25%) and live-birth rate was (64.6%) with IUFD rate of (10.4%). Fetal morbidity rates were also higher in the mothers with APL positivity, the incidence of IUGR was (27.1%). All these complications were statistically significant when compared with those of APL negative mothers.

Detailed analysis in Spain of APS among 280 patients included in the caps registry shows that (72%) are women, with a mean age of 37 years (11–60 yrs). PAPS represent (46%), (40%) from SLE, (5%) from lupus-like disease and (9%) from other autoimmune diseases. The study concludes catastrophic APS is an uncommon but potentially life threatening condition requiring a high degree of clinical awareness (Cervera, 2010).

In another study investigated the main causes of morbidity and mortality in patients with APS determined the clinical and immunological parameters with prognostic significance during a 5-year (Cervera *et al*, 2010). The clinical and immunological features of a cohort of 1000 patients with APS from 13 European countries who had been followed up from 1999 to 2004 were analyzed. Two hundred (20%) of patients developed APS-related manifestations during the 5-year study period, recurrent thrombotic events appeared in 166 (16.6%) patients and the most common were Strokes (2.4% of the total cohort), TIA (2.3%), DVT (2. 1%) and pulmonary embolism (2.1%). The study concludes that patients with APS still develop significant morbidity and mortality despite current treatment (oral anticoagulants or antiaggregants, or both).

Bramham *et al*, 2010 assessed pregnancy outcome in different clinical phenotypes of APS in United Kingdom. Eighty three percent pregnancies in 67 women with APS were included in the study, including (21%) with recurrent abortion (group 1), (21%) with late

fetal loss or early delivery due to placental dysfunction (group 2) and (41%) with thrombotic APS (group 3). Group 3 had higher rates of preterm delivery (26.8% versus 4.7%) than group 1 and smaller for gestational age babies than group 2 (39.5% versus 4.8%). The study concludes that women with thrombotic APS (group 3) have higher rates of pregnancy complications than those with obstetric APS (groups 1 and 2). Treatment with aspirin and LMWH is associated with improved outcomes for women with previous late fetal loss or early delivery due to placental dysfunction (group 2) (Bramham *et al*, 2010).

On observational study of 1592 no thrombotic women who had experienced three consecutive spontaneous abortions before the 10th week of gestation or one fetal death at or beyond the 10th week of gestation was carried out (Gris *et al*, 2011). They compared the frequencies of thrombotic events among women positive for APL, women carrying the F5 6025 polymorphism, and women with negative thrombophilia screening results. The annual rates of deep venous thrombosis (DVT; 1.5%), pulmonary embolism (0.4%), superficial vein thrombosis (SVT; 0.4%) and cerebrovascular events (0.3%) were significantly higher in APL women than in the other groups despite low-dose aspirin primary prophylaxis. Women carrying one of the two polymorphisms did not experience more thrombotic events than women who screened negative for thrombophilia. LAs was a risk factor for unprovoked proximal and distal DVT and SVT and women in the upper quartile of LAs activity had the highest risk. Despite data suggesting that APL may induce pregnancy loss through non-thrombotic mechanisms, women with purely obstetric APS are at risk for thrombotic complications.

Randomized or quasi-randomized, controlled trials of interventions in pregnant women with a history of pregnancy loss and APLs were examined (Empson *et al*, 2011).

In a retrospective study Garcia et al. (2012) evaluated patients with clinically well-defined APS and persistently negative APL. The authors assessed clinical manifestations of APS in 154 patients: 87 patients with seropositive (the so-called 'seropositive APS', SP-APS) APS and 67 patients with thrombosis and/or pregnancy morbidity persistently negative for APL and presenting with at least two additional non-criteria manifestations of APS (the so-called 'seronegative APS', SN-APS). Patients were interviewed at the time of recruitment. There were no significant differences in the frequency of thrombotic events or obstetric morbidity in patients with SN-APS versus patients with seropositive APS: DVT (31.4% versus 31.0%), pulmonary embolism (23.8% versus 28.7%), stroke (14.9% versus 17.2%), TIA (11.9% versus 10.3%), early spontaneous abortions (67.1% versus 52.1%), stillbirths (62.5% versus 59.4%), prematurity (28.1% versus 21.7%) or pre-eclampsia (28.1% versus 23.1%). The study concluded that classic and SN-APS patients show similar clinical profiles and suggest that clinical management in patients with APS should not be based only on the presence of conventional APL.

2.7 Intervention Policies

2.7.1 Laboratory Tests for APS Diagnosis

There is no golden standard for the laboratory diagnosis of APS. The laboratory diagnosis of APS remains difficult because of the variable sensitivity and specificity of the tests. Two methods are currently used for APS diagnosis: (1) ELISA-based immunoassays for

the detection of ACL; and (2) clotting assays for determination of the LAs. However, the first method is limited by a low specificity and the second by low sensitivity. Furthermore, for both methods standardization is unsatisfactory. Therefore, a number of new assays have been proposed as alternative or supplementary to ACL and LAs tests. These include the β_2GPI or antiprothrombin ELISAs, an ELISA utilizing a PL mixture, clotting assays with varying activators and assays utilizing chromogen substrates (Passam and Krilis, 2004).

2.7.2 APS Treatment

Unfractionated heparin combined with aspirin significantly reduced pregnancy loss compared to aspirin alone, LMWH combined with aspirin compared with aspirin did not significantly reduce pregnancy loss. There was no advantage of high-dose, over low-dose unfractionated heparin. Three trials of aspirin alone showed no significant reduction in pregnancy loss. Prednisone and aspirin resulted in a significant increase in prematurity when compared to placebo, aspirin, and heparin combined with aspirin, and an increase in gestational diabetes, but no significant benefit. Intravenous immunoglobulin with or without unfractionated heparin and aspirin was associated with an increased risk of pregnancy loss or premature birth when compared to unfractionated heparin or LMWH combined with aspirin. When compared to prednisone and aspirin, intravenous immunoglobulin was not significantly different in outcomes (Empson *et al*, 2011).

Chapter 3:

Materials and Methods

3.1 Study Design

This study is a case-control study; depend on cases that were exposed to previous second-trimester abortion, in Gaza Governorates. It is the simplest variety of observational analytical study that can be conducted on representative samples of population at a particular time. It aims to assess the relationship between variables of the study.

3.2 Study Population

The study populations includes married women who had been exposed to at least one second-trimester abortion, live in Southern Gaza Governorates and still alive, compared with married women who had not exposed to previous abortion, live in Southern Gaza Governorates and still alive.

3.3 Inclusion Criteria

Subject who eligible to participate in the study were those who met the following criteria:

3.3.1 Inclusion Criteria for Cases Group

- Married women who had been exposed to at least one second-trimester abortion.
- Married women with recurrent abortion.
- Married women who had normal pregnancy.

3.3.2 Inclusion Criteria for Control Group

- Married women who had not been exposed to any type of abortion.
- Married women who had normal pregnancy.

3.4 Exclusion Criteria for Cases and Control Groups

- Sporadic abortion in the first-trimester.
- Pregnant women.
- Divorced and widow women.
- Married women take heparin, clexane, or aspirin treatment at the time of sample collection.
- Hypertensive women.
- Diabetic women.
- Women with thyroid disease.

3.5 Sample Size

According to previous studies the prevalence of APS abortion during the second-trimester is 25-30%. The sample size of the study calculated by using the formula of WHO/WFP/ UNHCR/IFRC with (95%) confidence interval, (3%) margin of error and (1%) prevalence of APS abortion. Therefore, the sample size consist of 50 cases and 50 of age matched control, obtained from Southern Gaza Governorates, especially those who have been exposed to previous second-trimester abortion (Surveysystem.com, 2012).

3.6 Data Collection

Convenient sample obtained from Southern Gaza Governorates, the sample obtained from Rafah and KhanYounis Governorates. We found cases and control through people who visited private laboratories. In the beginning of data collection we collected data of cases and control from Rafah Governorate who visited Abu El-Walid laboratory, then we collected data of cases and control from KhanYounis Governorate who visited El-Magaida laboratory.

3.6.1 Questionnaire

The researcher collected the study data through an interviewed questionnaire (Annex 4). The majority of the questions that were included in the questionnaire were yes or no questions. In addition to the attached explanatory form, the interviewer answered all questions inquires that were asked by the participants and explained to each participant the importance, aim and purpose of the study. All patients who agree to participate in this

study kindly (Annex 5), asked to complete a questionnaire previously prepared as shown in appendixes and filled by the researcher himself. The questionnaire consists of two parts: The first, personal, sociodemographic status such as mobile number, telephone number, birth place, employment status, number of births, number of abortions and abortion time. The second part, covered four categories of second-trimester abortion, which were: Chronic maternal factors, other maternal factors, environmental factors and uterine defect such as taken anticoagulant medications, suffering from blood pressure, suffering from DM, having kidney file, suffering from thyroid dysfunction, exposed to previous radiation, exposed to previous agriculture chemical pollutants, taken chemical medications, exposed to accidents and exposed to falling.

3.6.2 Validity and Reliability of the Questionnaire

The content of the questionnaire was discussed with two expert of medical technologist in addition to other three expert obstetrics and gynecology specialists and two experts of public health to ensure that content is highly valid and reliable. Also face validity and standardization of measurement was done.

3.6.3 Pilot Study

A pilot study was conducted before starting the real data collection, to make pre-test for the questionnaire. A pilot study on 16 participants included 8 cases and 8 control. Some slight changes were done on questionnaire in cooperation with the academic supervisors. Due to slightly changes of questionnaire, the numbers of participants in pilot study were a part of the total sample size.

3.6.4 Study Settings

The study conducted at Southern Gaza Governorates where cases or control present, data collected through Abu El-Walid laboratory in Rafah Governorate and El-Magaida laboratory in KhanYounis Governorate.

3.6.5 Sample Collection

For both members of the cases and control, 6ml venous blood collected into two tubes (4mls in plain tube and 2 mls in Na citrate additive tube), under quality control and safety procedure. Serum and plasma separated from whole blood immediately by centrifugation for all specimens and afterwards serum and plasma samples stored at +4°C in a refrigerator until analysis.

3.6.6 Laboratory Tests

ACL (IgM), ACL (IgG), LA test, dRVVT, toxoplasma (IgM), rubella (IgM), FBS and TSH were analyzed for both cases and control groups. ACL (IgM), ACL (IgG), toxoplasma (IgM), rubella (IgM) and TSH were analyzed by ELISA technique. LA test and dRVVT were measured using clot formation technique. FBS was tested using enzymatic colorimetric method.

3.7 Materials

3.7.1 Chemicals and Reagents

Table 3.1: Shows the Chemicals and Reagents that had been Used in this Study

Reagent	**Supplier**
LA Test	Technoclon GmbH, UK
dRVVT	Technoclon GmbH, UK
ACL (IgM)	Orgenic, France
ACL (IgG)	Orgenic, France
Toxoplasma (IgM)	Diagnostic Bioprobes Sr1, Italy
Rubella (IgM)	Diagnostic Bioprobes Sr1, Italy
Sugar	Diasys Diagnostic Systems, Germany
TSH	TECO Diagnostics,

3.7.2 Equipments

Table 3.2: Shows the Main Equipments Used in This Study

Equipments	**Supplier**
Spectrophotometer	Spectrumlab 23A-China
ELISA Reader	Stat Fax-3200
Centrifuge	Gemmy Industrial Corp-Thailand
Water Bath	Julabo-Germany
Refrigerator with Freezer-20C	XL2-Occupied Palestine
Micropipettes	Lab Mate-Finland
Stop Watch	China

3.8 Biochemical Analysis

3.8.1 Determination of LA Test

Principle of the Test

LA test are clot –based assays, which directly activate factor X to factor Xa in the presence of PLs and calcium, factor Xa cleaves prothrombin to thrombin, which converts fibrinogen to fibrin leading to clot formation.

Reagents

Vial contains Russell's viper venom, PLs anti heparin agents, calcium, buffers, stabilizers, sodium azide and dye.

Assay Procedure

1. Each test requires 100µl plasma and 100µl reagent, prewarm amount of reconstituted reagent at 37°C for at least 2 minutes.
2. Pipette 100µl of plasma into glass tube then100µl reagent of LAs.
3. Record the time for clot formation.

Interpretation of Results

LA test normal value: 28-45 second negative

LA test normal value: > 45 second positive

3.8.2 Determination of dRVVT

Principle

Both dRVVT and dRVV confirm are clot –based assays, which directly activate factor X to factor Xa in the presence of PLs and calcium, factor Xa cleaves prothrombin to thrombin, which converts fibrinogen to fibrin leading to clot formation.

Reagents

1. dRVVT vial contains Russell's viper venom, PLs anti heparin agents, calcium, buffers, stabilizers, sodium azide and dye.
2. dRVV confirm vial contains Russell's viper venom, PLs anti heparin agents, calcium, buffers, stabilizers, sodium azide and dye.

Assay Procedure

1. Each test requires 100µl plasma and 100µl reagent, prewarm amount of reconstituted reagent at 37°C for at least 2 minutes.
2. Pipette 100µl of plasma into glass tube then100µl reagent of dRVV test.
3. Record the time for clot formation.
4. Pipette 100µl of plasma into glass tube then 100µl reagent of dRVV confirms.

5. Record the time for clot formation.

Calculation

dRVV test Ratio = dRVV test time / dRVV confirm time

Interpretation of Results

dRVV test normal value: 28-45 second

dRVV confirm normal value: 30-40 second

dRVV test Ratio normal value: 0.8-1.2 negative

dRVV test Ratio normal value: 1.2-1.5 borderline

dRVV test Ratio normal value: > 1.6 positive

3.8.3 Determination of ACL (IgM and IgG)

Principle

Highly purified cardiolipin is bound to microwells saturated with β_2GPI Abs against these antigens, if present in diluted serum or plasma, bind to the respective antigen. Washing of the microwells removes unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human (IgG) and (IgM) immunologically detect the bound patient Abs forming a conjugate-Ab-Ag complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow color is measured photometrical at 450 nm. The amount of color is

directly proportional to the concentration of (IgG) resp. (IgM) Abs present in the original sample.

Reagents

Table 3.3: Shows the Concentrations are those in the Final Test Mixture for ACL

Components	Concentration
Microplate	Coated with Highly Purified Bovine Cardiolipin and Saturated with β_2GPI
Negative Control	Serum / Buffer Matrix (PBS, BSA, NaN3 < 0,1% (w/w) Positive for ACL
Negative Control	Serum / Buffer Matrix (PBS, BSA, NaN3 < 0, 1% (w/w) Negative for ACL
Calibrator	Combined Calibrators with (IgG) and (IgM) Class ACL
Wash Buffer	(PBS,NaN3 < 0,1% (w/w)
Enzyme Conjugate	Polyclonal Rabbit anti-human (IgG); Labeled with HRP
Serum/Buffer	PBS, BSA, NaN3 < 0,1% (w/w) Containing: (IgG): 0; 7.5; 15; 30; 60; and 120 GPL U/ml and (IgM): 0; 5; 10; 20; 40; 80 MPL U/ml.
Chromogen	50Mm Citrate Phosphate,0.03%Tetra- Methyl-Benzedine
Sulphoric Acid	0.3M H2SO4

Assay Procedure

1. Prepare a sufficient number of microplate modules to accommodate control and prediluted patient samples.

2. Pipette 100 µl of calibrators, control and prediluted patient samples in duplicate into the Wells.

3. Incubate for 30 minutes at room temperature (20-28 °C).

4. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.

5. Dispense 100 µl of enzyme conjugate into each well.

6. Incubate for 15 minutes at room temperature.

7. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.

8. Dispense 100 µl of TMB substrate solution into each well.

9. Incubate for15 minutes at room temperature.

10. Add 100 µl of stop solution to each well of the modules and incubate for 5 minutes at room temperature.

11. Read the optical density at 450 nm and calculate the results. Bi-chromatic measurement with a reference at 600-690 nm is recommended. The developed color is stable for at least 30 minutes. Read optical densities during this time.

Interpretation of Results

In a normal range study with serum samples from healthy blood donors the following ranges have been established with the ACL tests:

Table 3.4: Show the Normal Values of ACL

Interpretation of ACL (IgM)	**Normal Values**
Negative	< 7
Positive	> 7
Interpretation of ACL (IgG)	**Normal Values**
Negative	< 10
Positive	> 10

3.8.4 Determination of Toxoplasma (IgM)

Principle

The assay based on the principle of "(IgM) capture" where (IgM) class Abs in the

sample are first captured by the solid phase coated with anti (IgM) Abs. After washing out all the other components of the sample and in particular (IgG) Abs, the specific (IgM) captured on the solid phase detected by addition of a preparation of inactivated T. gondi lobed with a specific monoclonal Abs conjugated with POD. After incubation, microwells washed to remove unbound conjugated and then the chromogen added in the presence of POD, the colorless substrate hydrolyzed to a color end–product, whose proportional to the amount of (IgM) Abs to T gondi present in the sample.

Reagents

Table 3.5: Shows the Concentrations are those in the Final test Mixture for Toxoplasma (IgM)

Components	**Concentration**
Microplate	Coated with Anti Human (IgM)
Negative Control	1% Human Plasma Negative for T Gondi
Positive Control	1% Human Plasma Positive for T Gondi
Calibrator	Contain Anti T. Gondi (IgM)
Lyophilized T. gondi Ag	2% Bovine Protein
Wash Buffer	10Mm Phosphate Buffer
Enzyme Conjugate	T. Gondi-Specific Monoclonal Antibody.
Antigen Diluent	10mM Tris
Specimen Diluent	2% Casein, 10mm Citrate
Chromogen	50mM Citrate Phosphate, 0.03% Tetra-Methyl-Benzedine
Sulphoric Acid	0.3m H2so4

Assay Procedure

1. Dilute samples 1:101 by dispending first 10µl sample and then 1 ml specimen diluents into a dilution tube: Mix gently.

2. Place the required number of microwells in the microwells holder. Leave the well in position 1 empty for the operation of blanking.
3. Dispense 100µl of negative control and 100µl of calibrator in the proper wells. dispense100µl of positive control into the proper well. Control and calibrator ready to use.
4. Dispense 100µl diluted samples in the proper sample wells and then check that all the samples well.
5. Incubate the microplate for 60 min at + 37°C.
6. Wash the microplate 5 times with 300 µl of wash solution.
7. Pipette 100µl conjugate into each well except the blanking well.
8. Incubate the microplate for 60 min at + 37°C.
9. Wash the microplate 5 times with 300 µl of wash solution.
10. Pipette 100µl chromogen into each well.
11. Incubate the microplate for 15 min at 18-24°C.
12. Pipette 100µl sulphoric acid into each well.
13. Measure the color intensity of the solution in each well at 450nm.

Calculation

Cut Off = Negative control absorbance + 0.250

Interpretation of Results

Table 3.6: Show the Interpretation of Toxoplasma (IgM) Results

Interpretation	**Normal Values**
Negative	< 1.0
Borderline	1.0-1.2
Positive	> 1.2

3.8.5 Determination of Rubella (IgM)

Principle

The assay is based on the principle of " (IgM) capture" where (IgM) class Abs in the sample are first captured by the solid phase coated with anti rubella (IgM) Abs. After washing out all the other components of the sample and in particular (IgG) Abs, the specific (IgM) captured on the solid phase are detected by addition of a preparation of inactivated rubella lobed with a specific monoclonal Abs conjugated with POD. after incubation, microwells washed to remove unbound conjugated and then the chromogen added in the presence of POD, the colorless substrate hydrolyzed to a color end–product, whose proportional to the amount of (IgM) Abs to rubella present in the sample.

Reagents

Table 3.7: Shows Concentrations are those in the Final Test Mixture for Rubella (IgM)

Components	**Concentration**
Microplate	Coated with Anti Human (IgM)
Negative Control	1%Human Plasma Negative for Rubella
Positive Control	1%Human Plasma Positive for Rubella
Calibrator	Contain Anti Rubella (IgM)
Lyophilized Rubella Ag	2%Bovine Protein
Wash Buffer	10Mm Phosphate Buffer
Enzyme Conjugate	Rubella -Specific Monoclonal Antibody.
Antigen Diluent	10mm Tris
Specimen Diluent	2%Casein, 10mm Citrate
Chromogen	50Mm Citrate Phosphate,0.03% Tetra- Methyl-Benzedine
Sulphoric Acid	0.3m H2so4

Assay Procedure

1. Dilute samples 1:101 by dispending first 10µ sample and then 1 ml specimen diluents into a dilution tube: Mix gently.
2. Place the required number of microwells in the microwells holder. Leave the well in position 1 empty for the operation of blanking.
3. Dispense 100µl of negative control and 100µl of calibrator in the proper wells.dispense100µl of positive control into the proper well. Control and calibrator ready to use.
4. Dispense 100µl diluted samples in the proper sample wells and then check that all the samples well.
5. Incubate the microplate for 60 min at +37°C.
6. Wash the microplate 5 times with 300 µl of wash solution.
7. Pipette 100µl conjugate into each well except the blanking well.
8. Incubate the microplate for 60 min at +37°C.
9. Wash the microplate 5 times with 300 µl of wash solution.
10. Pipette 100µl chromogen into each well.
11. Incubate the microplate for 15 min at 18-24°C.
12. Pipette 100µl sulphoric acid into each well.
13. Measure the color intensity of the solution in each well at 450nm.

Calculation

Cut Off = Negative Control Absorbance + 0.250

Interpretation of Results

Table 3.8: Show the Interpretation of Rubella (IgM) Results

Interpretation	Normal Values
Negative	<1.0
Borderline	1.0-1.2
Positive	>1.2

3.8.6 Determination of Blood Sugar

Principle

Determination of glucose after enzymatic oxidation by GOD. The colorimetric indicator is quinonimine, which is generated from 4-aminoantipyrine and phenol by hydrogen peroxide under the catalytic action of POD.

Reagents

Table 3.9: Shows the Concentrations are those in the Final Test Mixture for Sugar

Concentration	Reagent
Phosphate buffer (pH 7.5)	250 mmol/l
Phenol	5 mmol/l
4-Aminoantipyrine	0.5 mmol/l
GOD	≥ 15 ku/l
POD	≥ 1 ku/l
Standard	100 mg/dl

Assay Procedure

Wavelength: 510 nm

Optical path: 1cm

Temperature: 37 ºC

Measurement: against blank reagent.

1. 10 µl of standard (sample or control) was added to 1 ml of reagent and mixed well.
2. The mixture was incubated for 10 min at 37 ºC.
3. The absorbance was measured within 60 min.

Calculation

Sugar (mg/dl) = A test* Conc. Standard / A standard

Reference Value: adults 80-120 mg/dl.

3.8.7 Determination of TSH

Principle

A certain amount of anti–TSH coated on microtiter wells, serum of patients and TSH conjugated with HRP are added to wells. after incubation, microwells are washed to remove unbound conjugated and then the chromogen is added in the presence of POD the colorless substrate is hydrolyzed to a color end–product, whose proportional to the amount of TSH present in the sample.

Reagents

Table 3.10: Shows the Concentrations are those in the Final Test Mixture for TSH

Components	Concentration
Microplate	Coated with Anti TSH Antibody
Standards	Different Concentration
Wash	(PBS,NAN3 < 0,1% (w/w)
Enzyme Conjugate	Anti TSH Antibody with HRP
Chromogen	50mm Citrate Phosphate,0.03%Tetra- Methyl-Benzedine
Hydrochloric Acid	0.3m HCL

Assay Procedure

1. Prepare a sufficient number of microplate modules to accommodate control and patient samples.
2. Pipette 50 µl of standards, and patient samples into the wells and then dispense 100 µl of enzyme conjugate into each well: Mix gently.
3. Incubate for 60 minutes at room temperature (20-28 °C).
4. Discard the contents of the microwells and wash 5 times with 300 µl of wash solution.
5. Dispense 100 µl of TMB substrate solution into each well.
6. Incubate for 20 minutes at room temperature.
7. Add 50 µl of stop solution to each well.
8. Read the optical density at 450 nm and calculate the results. Bi-chromatic measurement with a reference at 600-690 nm is recommended.

Interpretation of Result

TSH normal value: 0.5-5.0 μlU/Ml

3.9 Ethical Matters and Procedures

An official letter of approval to conduct the study obtained from the Helsinki Committee in Gaza Governorate (Annex 6). Also an official letter obtained from general directorate of hospitals (Annex 7). Every participant provided with a full explanatory form attached to blood withdrawal both verbally and written. This form included the general objective of the study, assurance about the confidentiality of the information, and the instructions how to respond to laboratory technicians and research. Also it included a statement indicating that the participant has the right to refuse or participate in this study. The participation optional, anonymity and confidentiality given and maintained. Consent form obtained from each participant and it attached to each blood withdrawal process to ensure their voluntary participation after signing the consent.

3.10 Study Instrument

A quantitative research used because it is the best way of assessment which was hold by measuring the tests biomarkers that reflect the relationship between APS and second-trimester abortion. According to the study objectives, a group of tests were used including: ACL (IgM), ACL (IgG), LA test, dRVVT, toxoplasma (IgM), rubella (IgM), TSH and FBS.

3.11 Data Management and Statistical Analysis

1. SPSS program version 13.0 was used in statistical data analysis. This includes: Data entry, data cleaning, data frequency and Chi-square test.
2. Chi-square test between APS and second-trimester abortion.
3. Chi-square test between LA test among aborted and non aborted women.
4. Chi-square test between dRVVT among aborted and non aborted women.
5. Chi-square test between APS among employee and non employee participants.
6. Chi-square test between APS and place.
7. Chi-square test between APS and age group.
8. Chi-square test between LA test and second-trimester abortion.
9. Chi-square test between dRVVT and second-trimester abortion.
10. Sensitivity formula and specificity formula for positive tests used in the diagnosis of APS.

3.12 Limitations

1. Poor documentation in MOH patient files, there were missed and interrelated data.
2. Poor computerized data and limited statistical data, patients full address was not available especially mobile or telephone number.
3. During the period of the study, the treatment of abortion (heparin) is not available at the MOH hospitals and thus aborted women were not visiting MOH hospitals at the time of research. Therefore accessible to aborted women was not easy which made it difficult to deal with patients in other parts of the Gaza Governorates except Southern Gaza Governorates where I live.

4. Limited time: the time used in finding appropriate cases and control was one a month.
5. Limited fund: the cost price of material used for research was about 3000$ (Annex 8) .

Chapter 4:

Results

4.1 Introduction

In this chapter, we will represent the main study results based on statistical analysis. The first part of the result is related to the distribution of the study population, sample frequency and descriptive statistics. The second part is related to the results of the studied questions, Chi-square test and confidence interval (CI) analysis ($P \leq 0.05 = 95\%$ CI) was used to compare mean values.

4.2 Sample Distribution

4.2.1 Sample Distribution According to Place, Age Group and Employment Status

Table 4.1 shows the number and percentage of samples according to place, age group and employment. The total studied samples were distributed as (50%) from Rafah Governorate (25% cases and 25% control) and (50%) from KhanYounis Governorate (25% cases and 25% control). The unemployed were (90%) and (10%) were employed among cases and control. The mean age of cases group were 28.8yrs and the mean age of control group were 26.3yrs, the predominant age of APS are young to middle-age adults .

Table 4.1: Frequency and Percentage of Place, Age Group and Employment

		Cases (n=50)		Control (n=50)	
		N	**%**	**N**	**%**
Place	Rafah	25	50	25	50
	KhanYounis	25	50	25	50
Employment	Employed	5	10	5	10
	Unemployed	45	90	45	90
Age Group	18-24 Yrs	12	24	22	44
	25-31Yrs	22	44	19	38
	32-37Yrs	10	20	7	14
	38-44Yrs	6	12	2	4

4.2.2 Sample Distribution According to the Number of Abortions among Cases

Table 4.2 shows the number and percentage of samples according to the number of the second-trimester abortions. The largest number of abortions was 1-3 times which represented (74%) of all cases, (16%) had aborted 4-7 times and (10%) had 8-12 times of abortions.

Table 4.2: Frequency and Percentage of Abortion Group

Type	**Abortion Group**	**Frequency**	**%**
Cases	1-3	37	74
	4-7	8	16
	8-12	5	10
	Total	50	100

4.2.3 Sample Distribution According to the Number of Births among Cases and Control

Table 4.3 shows the number and percentage of samples according to births group among cases and control. The largest group was 0-4 births which represented (80%) of cases and (92%) of control.

Table 4.3: Frequency and Percentage of Birth Group

Type	Birth Group	Frequency	%
Cases	0-4	40	80
	5-8	10	20
	Total	50	100
Control	0-4	46	92
	5-8	4	8
	Total	50	100

4.2.4 Sample Distribution According to Tests for the Exclusions Criteria

All samples of cases and control were investigated for TSH and FBS, to ensure that all participants were negative for these tests (Table 4.4). The mean concentrations of TSH was 3.5 μlU/mL and the mean concentrations of FBS was 98mg/dl.

Table 4.4: Frequency and Percentage of Tests for Exclusions Criteria

Tests	Mean	Cases (n=50)		Control (n=50)	
		N	%	N	%
TSH*	3.5	50	100	50	100
FBS*	98	50	100	50	100
Hypertension*	105/74	50	100	50	100

* TSH normal value: 0.5-5 μlU/mL
* FBS Normal values: 80-120 mg/dl
* Hypertension Normal values: 110-130/70-90

Only one participant was excluded due to abnormal result of TSH. Also we measured blood pressure for all sample population, to confirm that they are free of hypertension, the mean of blood pressure was 105/74. All were done to avoid any interference that could affect our main study objectives.

4.3 Results of the Studied Questions and Objectives

4.3.1 The Relationship between APS among Cases and Control

According to APS diagnosis criteria, APS positive cases required at least one clinical criteria such as second-trimester abortion and another laboratory criterion such as positive dRVVT and/or positive LA test (Gaufberg *et al*, 2010). To determine the rate of APS among cases and control, we used Chi-square test between APS among cases and control. Crosstabulation was performed to identify if there is any difference of APS among cases and control.

Table 4.5: APS versus Sample Population Crosstabulation

APS	**Sample Population**				**Total**	**P Value**
	Cases (n=50)		**Control (n=50)**			
	N	**%**	**N**	**%**		
Positive	11	22	0	0	11	0.000*
Negative	39	78	50	100	89	
Total	50	100	50	100	100	

*Statistically significant

Table 4.5 and figure 4.1 revealed that (22%) of cases were APS positive and (100%) of control was APS negative. There is a relationship between APS and cases, the difference

reach statistically significant because (p value < 0.05). Our study deals with APS compared with other studies through number of tests carried out for sample population to diagnose APS for example in Jordanian study (ACL was only found with recurrent abortion) (Daboubi, 2001) and USA study research (ACL was only found with recurrent abortion) (Damoiseaux *et al*, 2009). Our study evaluated important and sensitive markers of APS which is an important cause of abortion worldwide. The result compatible with that APS is characterized by venous or arterial thromboses and fetal losses (Rai *et al*, 2010).

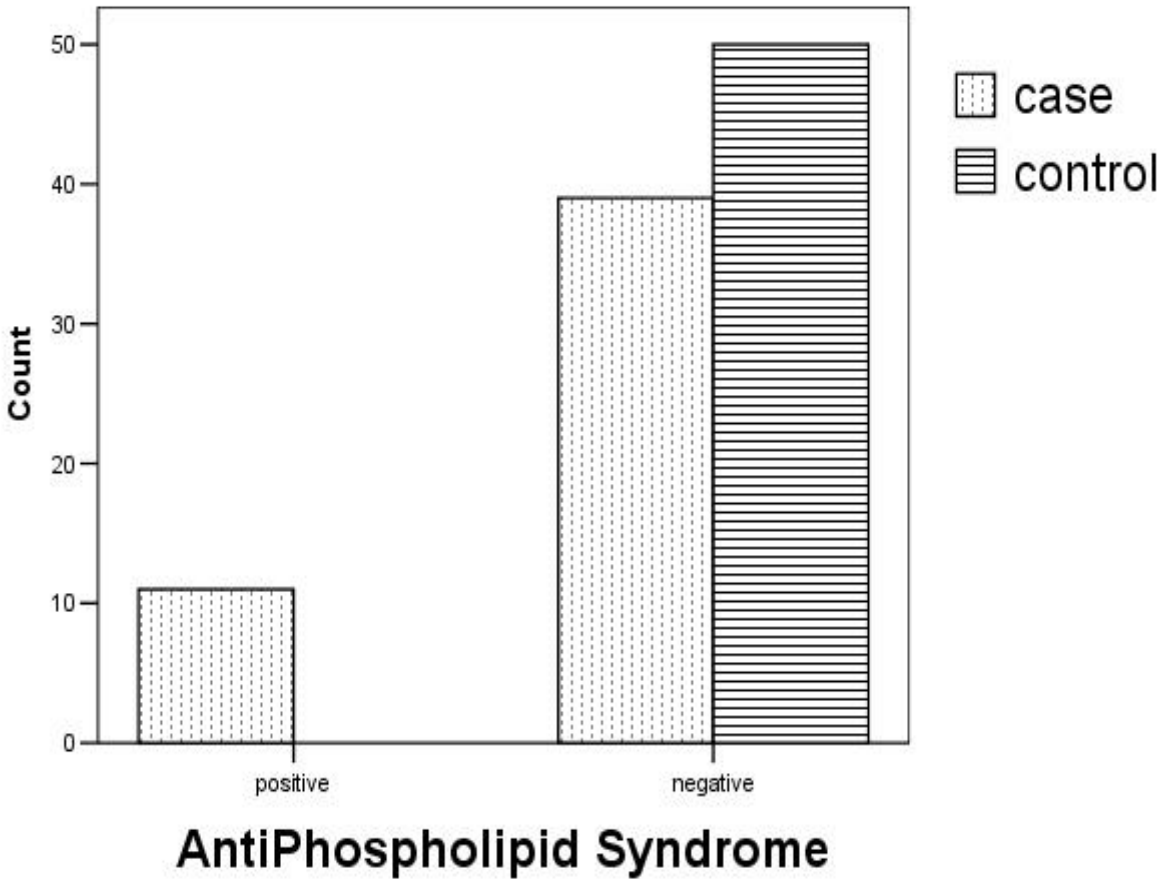

Figure 4.1: The Distribution of APS among Cases and Control

4.3.2 The Relationship between LA Test among Aborted and Non Aborted Women

To determine the relationship between LA test among aborted and non aborted women, we used Chi-square test between LA test among aborted and non aborted women. Crosstabulation was performed to identify if there is any difference of LA test among aborted and non aborted women.

Table 4.6: Frequency and Percentage of LA Test among Aborted and Non Aborted Women

	Results	**Mean**	**F**	**Sample Population**			**Total**	**P Value**
				Cases (n=50)		**Control (n=50)**		
				APS +ve Aborted	**APS -ve Aborted**	**Non Aborted**		
LA Test*	Positive	51	Count	11	0	4	15	0.000*
			% of Total	11%	0%	4%	15%	
	Negative	35	Count	0	39	46	85	
			% of Total	0%	39%	46%	85%	
Total		38	Count	11	39	50	100	
			% of Total	11%	39%	50%	100%	

*Statistically significant
* LA test normal value: 28-45 second

The percent of positive LA test of total sample population were (11%) which are considered as APS +ve since they have the clinical criteria of being aborted in the second-trimester. Also (4%) positive for LA test of total sample population and considered as non aborted women. Moreover, (39%) of APS -ve aborted and (46%) of non aborted women were negative for LA test, therefore they were considered as APS –ve, the difference

between the positive and negative means is statistically significant (p value ≤ 0.05) as shown in Table 4.6 and Figure 4.2.

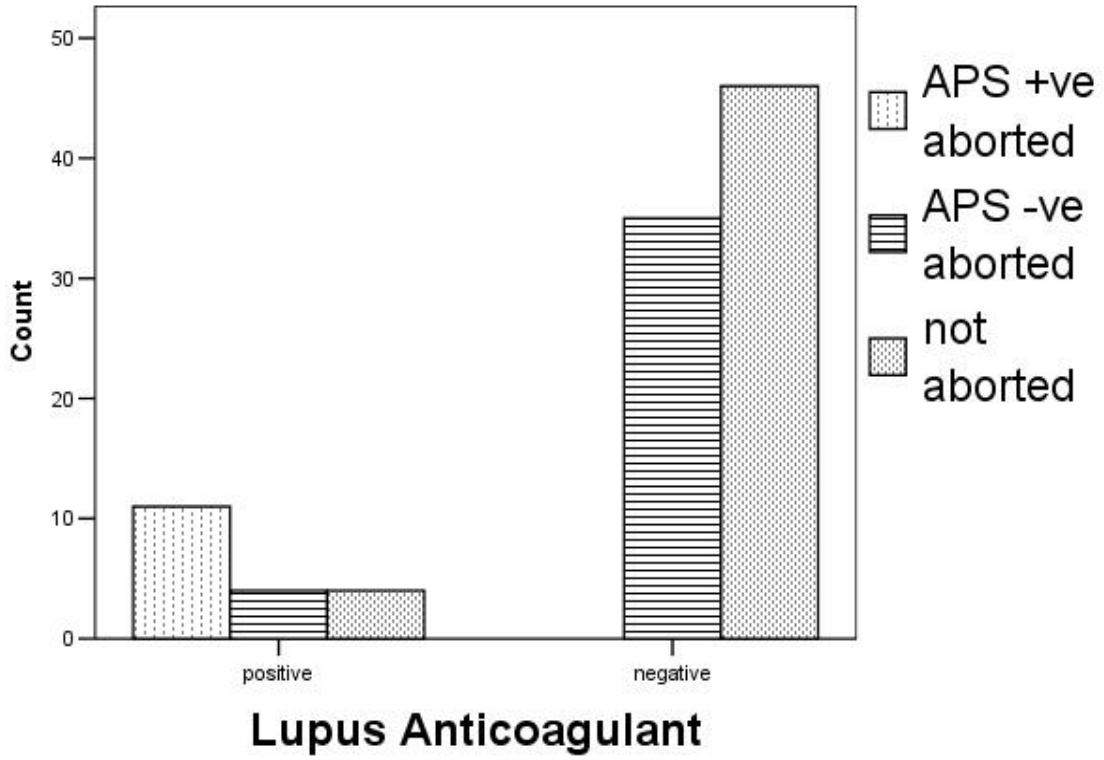

Figure 4.2: The Distribution of LA Test among Aborted and Non Aborted Women

4.3.3 The Relationship between dRVVT among Aborted and Non Aborted Women

To determine the relationship between dRVVT among aborted and non aborted women, we used Chi-square test, Crosstabulation was performed to identify if there is any difference in dRVVT results among aborted and non aborted women.

Table 4.7: Frequency and Percentage of dRVVT among Aborted and Non Aborted Women

Test	Results	Mean	F	Sample Population			Total	P Value
				Cases (n=50)		Control (n=50)		
				APS +ve Aborted	APS -ve Aborted	Non Aborted		
dRVVT*	Positive	1.8	Count	11	0	0	11	0.00*
			% of Total	11%	0%	0%	11%	
	Negative	1.1	Count	0	39	50	89	
			% of Total	0%	39%	50%	89%	
Total		1.2	Count	11	39	50	100	
			% of Total	11%	39%	50%	100%	

*Statistically significant
* dRVV test Ratio normal value: > 1.6

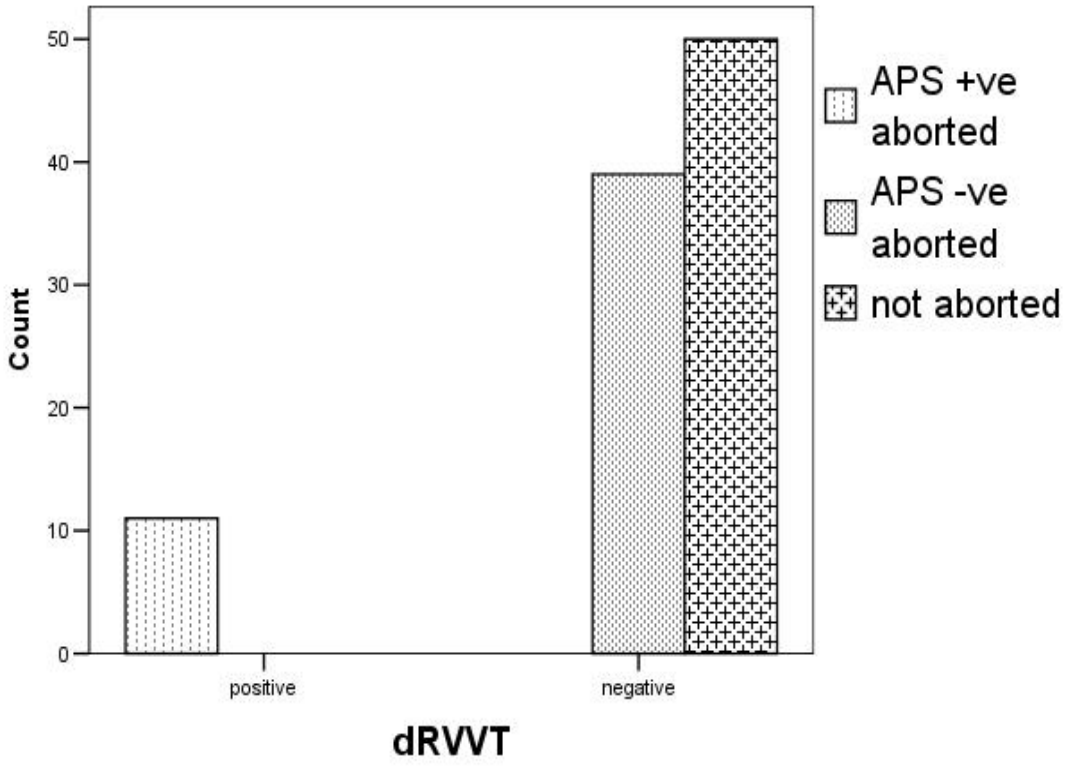

Figure 4.3: The Distribution of dRVVT among Aborted and Non Aborted Women

The percent of positive dRVVT of total sample population were (11%) and considered as APS +ve since they have the clinical criteria of being aborted in the second-trimester. Moreover, (39%) of APS -ve aborted and (50%) of non aborted women were negative for dRVVT, therefore they were considered as APS –ve, the difference between the positive and negative means is statistically significant (p value ≤ 0.05) as shown above in Table 4.7 and Figure 4.3.

4.3.4 The Relationship between APS and Employment Status

To determine whether APS increase in non employee women or not, Chi-square test has been used. Crosstabulation was performed to identify if there is any difference of APS among employee and non employee.

Table 4.8: APS versus Employment Status Crosstabulation

APS	**Employment Status**				**P Value**
	Employee		**Non Employee**		
	N (10)	%	**N (90)**	%	
Positive	1	10	10	11	0.915
Negative	9	90	80	89	
Total	10	100	90	100	

Non statistically significant

Table 4.8 and Figure 4.4 revealed that (10%) of employed cases were positive for APS and (11%) of none employed cases were positive for APS. The difference was not statistically significant. The sample of participants matched with the employment status, the result was incompatible with the economic status and played a role in the abortion rate in spite of that abortion rate is higher in developing countries than developed countries according to previous study (Stanley *et al*, 2010).

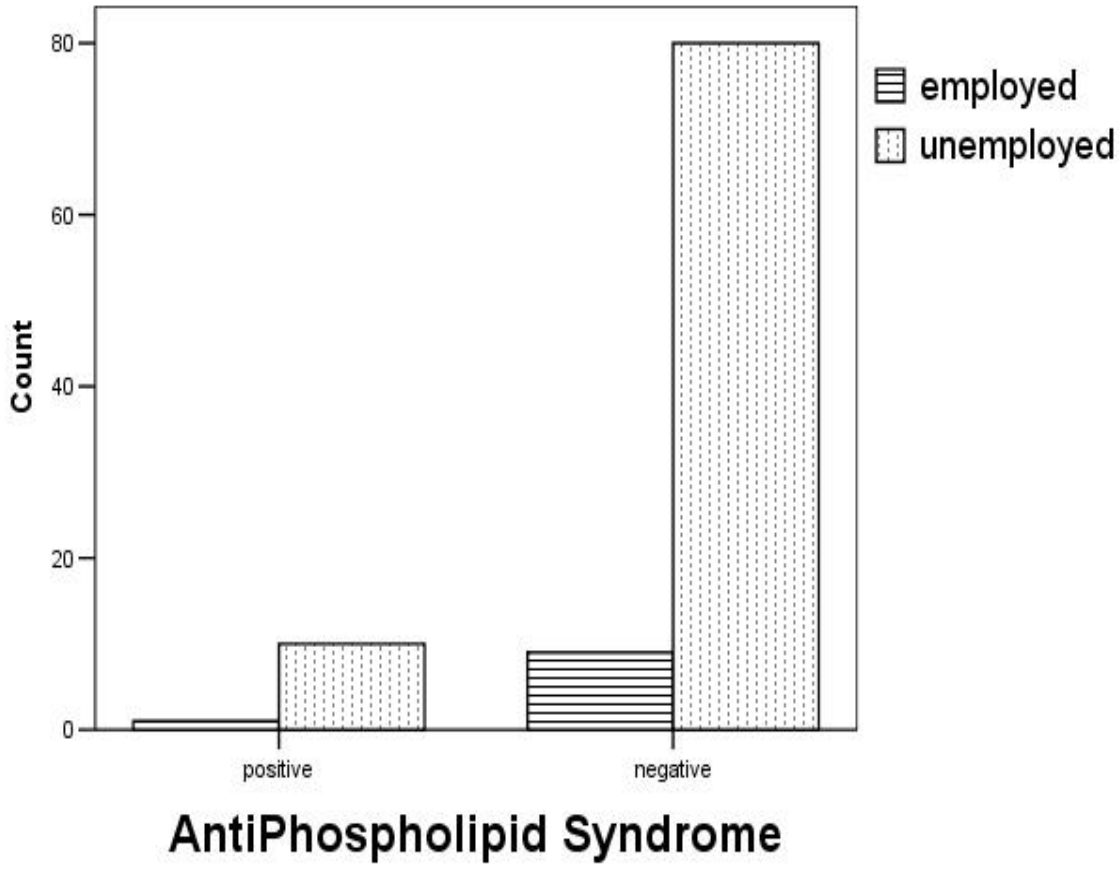

Figure 4.4: The Distribution of APS among Employed and Unemployed

It may be that the small size of the employed participants may cause this confusion, or even the difference between employee and unemployee in income is relatively similar, so its effects similar. The participant's income may be need classification to show a difference in abortion rate between employee and non employee participants.

4.3.5 APS Rate in Rafah and KhanYounis Governorates

To determine whether APS rate differs from Rafah Governorate than KhanYounis Governorate, we used Chi-square test between APS and place. Crosstabulation was performed to identify if there is any difference of APS among Rafah Governorate and KhanYounis Governorate participants.

Table 4.9: APS versus Place Crosstabulation

APS	Place				P Value
	KhanYounis Governorate		Rafah Governorate		
	N (50)	%	N (50)	%	
Positive	2	4	9	18	0.025*
Negative	48	96	41	82	
Total	50	100	50	100	

* Statistically significant

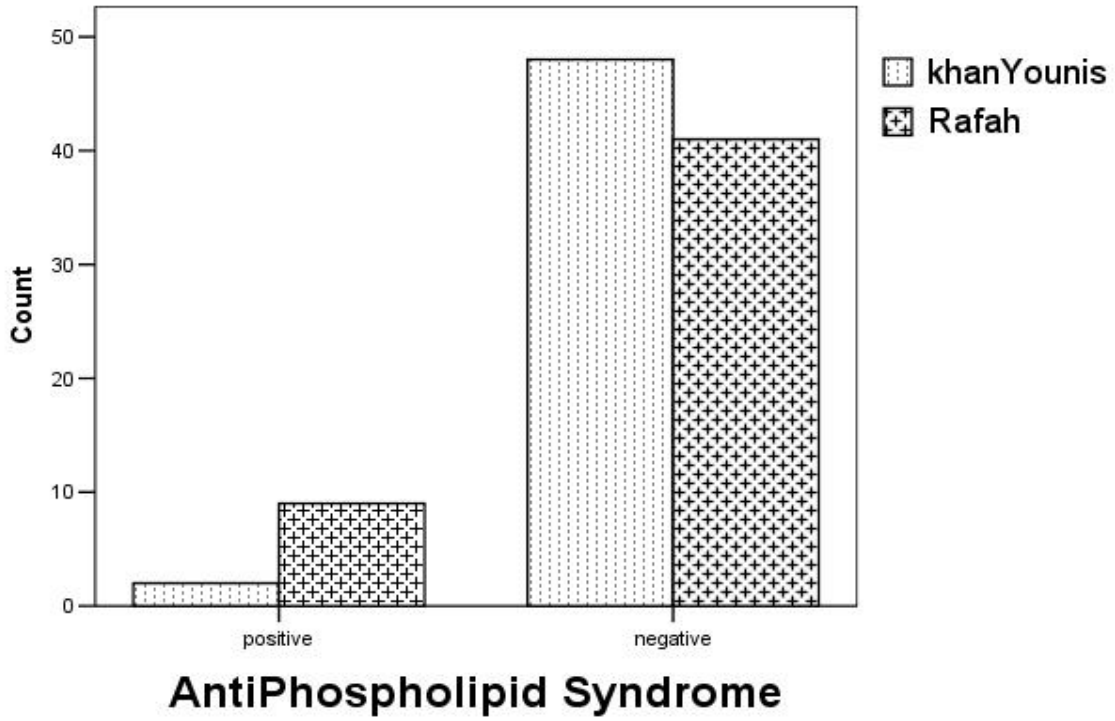

Figure 4.5: The Distribution of APS in Rafah and KhanYounis Governorates

As shown in table 4.9 and figure 4.5 (4%) of APS were from KhanYounis Governorate and (18%) of APS from Rafah Governorate. The difference reached statistically significant difference (p value = 0.025). The socioeconomic structure of the two Governorates relatively the same but this difference could be due to maternal factors such as severe emotional shock, trauma and infections, which could be different from one Governorate to another. Further research to study APS in all Gaza Governorates is suggested to know if there is a difference or no.

4.3.6 The Relationship between APS and Age Group

To determine the relationship of APS and age group we used Chi-square test between APS and age group. Crosstabulation was performed to identify if there were any difference of APS among age group.

Table 4.10: APS versus Age Group Crosstabulation

APS	Age Group								P Value
	18-24 Yrs	%	25-31 Yrs	%	32-37 Yrs	%	38-44 Yrs	%	
Positive	3	8.8	7	17	0	0	1	12.5	0.281
Negative	31	91.2	34	83	17	100	7	87.5	
Total	34	100	41	100	17	100	8	100	

Non statistically significant

As shown in Table 4.10 and figure 4.6 the highest percent of APS was among age group 25-31yrs and the median age is 28 yrs, but the difference does not reach statistically significant (p value > 0.05). Our results correlates with the international median age of APS age group which is approximately 30 years (Riley, 2005).

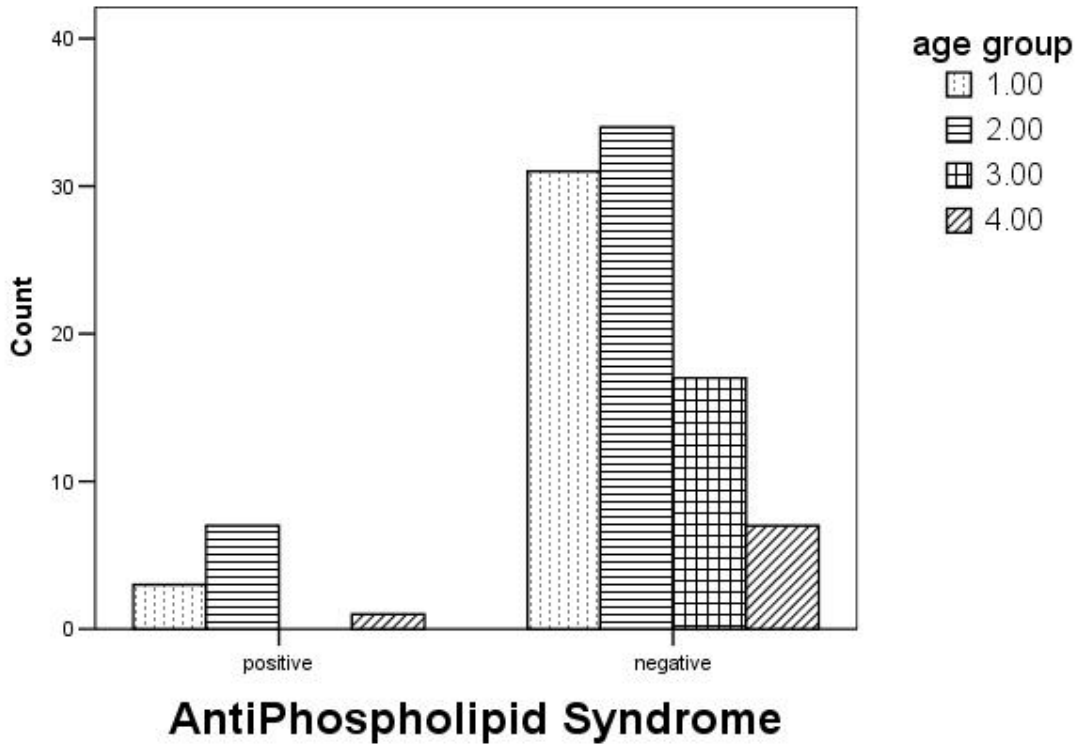

Figure 4.6: APS versus Age Group

4.3.7 The Relationship between LA Test among Cases and Control

We used LA test to distinguish between LA test and LAs as a group of Abs. LA test was investigated for cases and control and positive cases for LA test was investigated again after 6 weeks to ensure the persistent of the positivity of LA test. If test after 6 weeks gave negative result, it done after further 6 weeks to confirm positive result. To determine the relationship between LA test among cases and control, we used Chi-square test between LA test among cases and control. Crosstabulation was performed to identify if there is any difference of LA test among cases and control.

Table 4.11: LA Test versus Sample Population Crosstabulation

LA Test*	Mean	Sample Population				P Value
		Cases		Control		
		N (50)	%	N (50)	%	
Positive	59	11	22	4	8	0.050*
Negative	35	39	78	46	92	
Total	38	50	100	50	100	

*Statistically significant
* LA test normal value: > 45 second

Table 4.11 and Figure 4.7 revealed that (22%) of cases were positive for LA test and (8%) from negative control were LA positive, the difference was statistically significant (p value ≤ 0.05). This result may reveal that LA test is not an acceptable evidence to evaluate APS among second-trimester aborted women and can give false positive results.

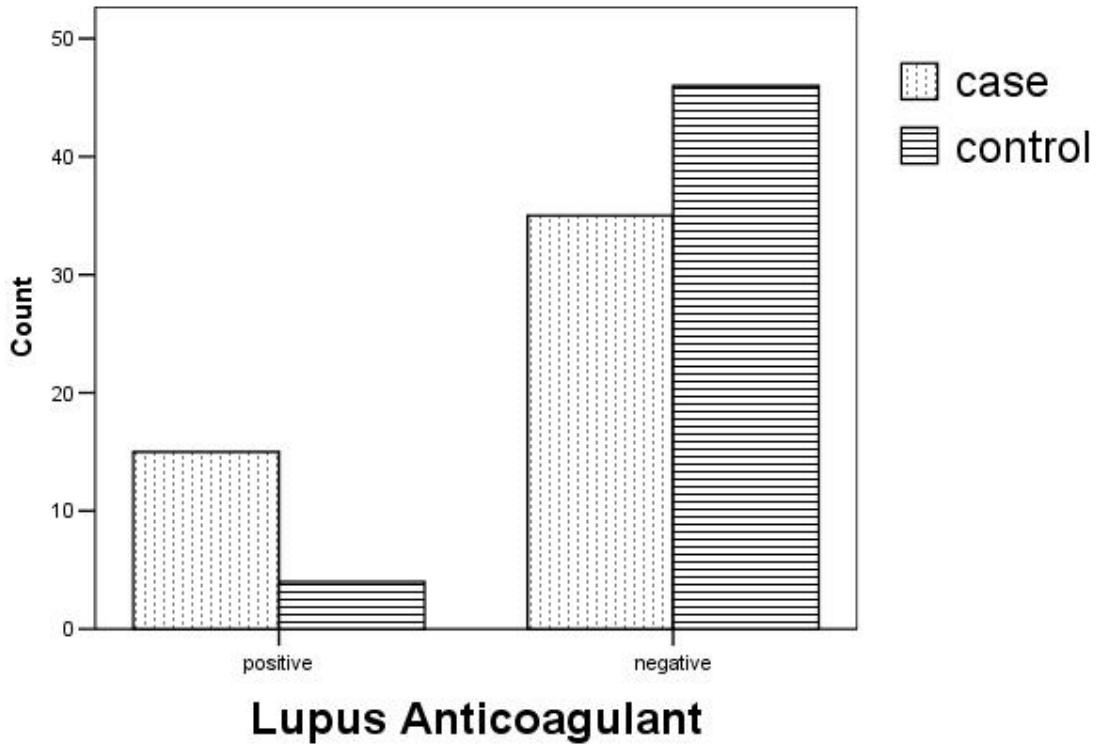

Figure 4.7: LA Test versus Sample Population

Positive results for LA test among control reflect poor specificity comparable with other tests. This result compatible with other study reported that LA test ranging from 15 to (34%) among aborted women (Gezer, 2003) but our results add an input on the poor specificity of LA test for APS diagnosis. However LA test is popularly used in APS diagnosis and considered as a strong risk factor for thromboembolism than ACL (Shoenfeld *et al*, 2006) ACL (IgM and IgG) tests that may be negative among cases.

4.3.8 The Relationship between dRVVT among Cases and Control

dRVVT test was investigated among cases and control, dRVVT was tested in the blood of cases and control on two or more occasions at least 6 weeks apart according to the guidelines of the International Society on Thrombosis and Hemostasis (Gezer, 2003). To determine the relationship between dRVVT among cases and control, we used Chi-square test between dRVVT among cases and control. Crosstabulation was performed to identify if there is any difference of dRVVT among cases and control.

Table 4.12: dRVVT versus Sample Population Crosstabulation

dRVVT*	Mean	Sample Population				P Value
		Cases		Control		
		N (50)	%	N (50)	%	
Positive	1.8	11	22	0	0	0.000*
Negative	1.1	39	78	50	100	
Total	1.2	50	100	50	100	

*Statistically significant
* dRVV test Ratio normal value: > 1.6

Table 4.12 and figure 4.8 revealed that (22%) of cases were dRVVT positive and (100%) of control were negative and the difference is statistically significant (p value < 0.05).

These results revealed good specificity of dRVVT test for APS diagnosis compared with LA test. Our study introduced dRVVT in diagnosis of APS but several studies did not use this test, for example Rai et al, (2010) used LA test, ACL and anti-β_2GPI for APS diagnosis. Also, we found that dRVVT is very specific and sensitive test compared to other tests. These results may prove that dRVVT test is not affected by any interference like LA test.

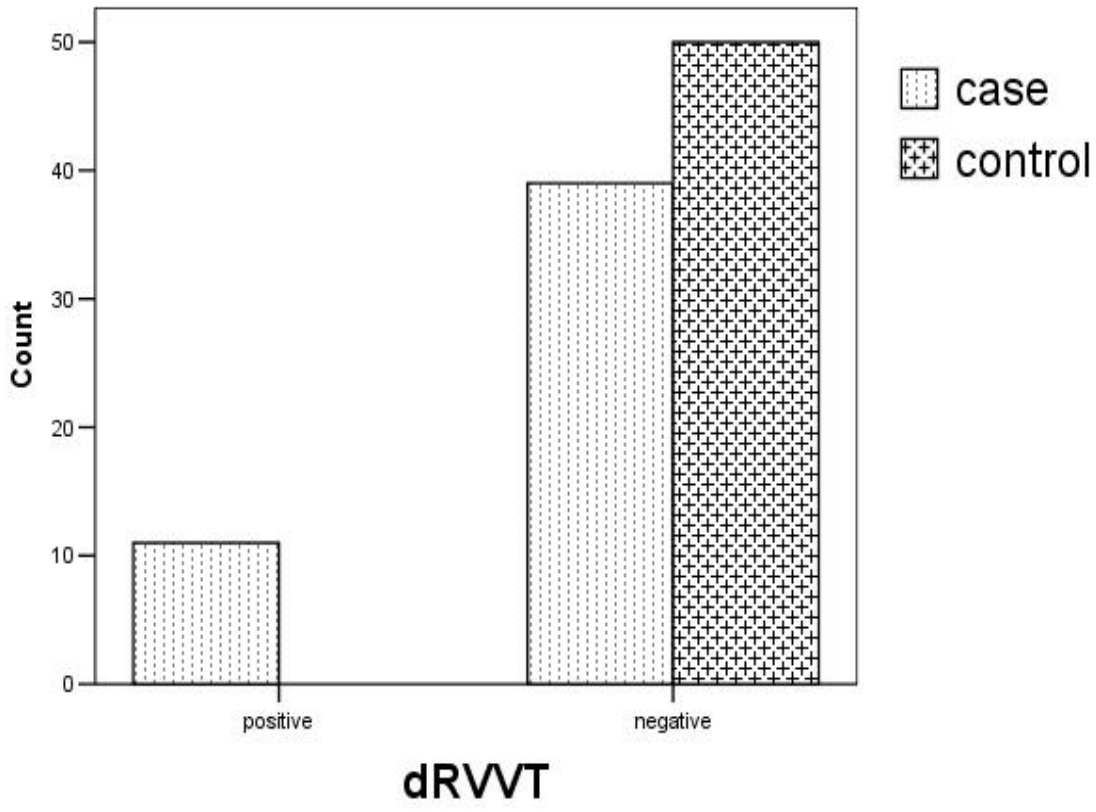

Figure 4.8: dRVVT versus Sample Population

Therefore, dRVVT test could be preferred for the diagnosis of APS and our results are correlated with other studies (Gezer, 2003). Moreover, dRVVT test is reported as more

sensitive than the APTT for the detection of LAs, because it is not influenced by deficiencies or inhibitors of clotting factors VIII, IX or XI (Kaul *et al*, 2007). APLs is an important marker for recurrent thrombosis (Kaul *et al*, 2007). Therefore, our results added impute for the important of dRVVT for APS diagnosis and evaluation.

4.3.9 The Relationship between ACL among Cases and Control

All cases and control of our study were ACL (IgM and IgG) negative, which excluded ACL (IgM and IgG) as the cause of APS in our cases. Shoenfeld et al, (2006) said that LAs is a stronger risk factor for thromboembolism than ACL that all ACL (IgM and IgG) negative for sample population.

4.3.10 Sensitivity and Specificity of LA Test in APS Diagnosis

To determine the highest sensitive and specific test for APS diagnosis, we calculated the results of LA test by the following equations.

Sensitivity = true positive (T.P)/all diseased (TP+FN)*100

Specificity = true negative (T.N)/all healthy (TN+FP)*100

As shown in Table 4.11 the sensitivity of LA test = 11/50*100 = (22%)

It means LA test positivity is 22% from cases

The specificity of (LA test) = 46/50*100 = (92%)

LA test negativity was (92%) in control, which means that (8%) of normal control have APS positive which is false. These results confirmed the poor specificity of LA test and can't be taken alone with clinical correlation as an evidence of APS diagnosis. This could be explained by previous findings which found that APS is present in (1-5%) of the general

population, which can explain why we have 4 positive LA test among the non aborted women which was used as a negative control.

4.3.11 Sensitivity and Specificity of dRVVT in APS Diagnosis

To determine the highest sensitive and specific test for APS diagnosis, we calculated the results of dRVVT test by the following equations.

Sensitivity = true positive (T.P)/all diseased (TP+FN)*100

Specificity = true negative (T.N)/all healthy (TN+FP)*100

As shown in Table 4.12 the positive dRVVT among cases was 11 and the positive dRVVT among control was nil

Therefore, sensitivity of (dRVVT) test =11/50*100 = (22%)

This means the dRVVT positivity is 22% among cases

The specificity of (dRVVT) test =50/50*100 = (100%)

This means that dRVVT negativity is (100%) among control. These results reflected very high specificity and sensitivity of dRVVT for APS diagnosis compared with LA test.

4.3.12 The Relationship between Toxoplasma (IgM) among Cases and Control

We found that (100%) of our cases and control are toxoplasma (IgM) negative. Infections are viewed as a rare cause of recurrent abortion (Gaufberg *et al*, 2010). Our findings are in agreement with those reported in previous studies (Kimball et al., 1971; Southern, 1972; Giorgino and Mega, 1981; Zargar et al., 1999 and Qublan, *et al*, 2002).

4.3.13 The Relationship between Rubella (IgM) among Cases and Control

We found that (100%) of our cases and control was rubella (IgM) negative, which excluded rubella (IgM) as the cause of APS in our cases. Our results are in agreement with others that infections are a rare cause of recurrent abortion (Gaufberg *et al*, 2010). Rubella is the most important and best known of fatally harmful viral infections, the fetus usually becomes infected and is therefore in great danger when rubella occurs during the first 8 weeks of pregnancy (Taina *et al*, 1985). However, the high rate of vaccination in Gaza Governorates plays an important role in diminishing rubella viral infection among pregnant women.

4.4 Summary of the Main Findings

4.4.1 The Distribution of Laboratory Tests Related to APS Diagnosis

Table 4.13 showed the main tests and results for cases and control. The tests results showed a strong relationship between LA test and dRVVT among cases, no relationship between ACL (IgM and IgG), toxoplasma (IgM) and rubella (IgM) among cases. TSH, FBS and hypertension were measured to exclude any interference from thyroid insufficiency, hypertension and diabetes as a proper cause of abortion among our cases and control.

Table 4.13: The Distribution of Laboratory Tests Related to APS among Cases and Control

Laboratory Tests	Results	Mean	Sample Population			
			Cases		Control	
			N	%	N	%
LA Test*	Positive	51	11	22	4	8
	Negative	35	39	78	46	92
dRVVT*	Positive	1.8	11	22	0	0
	Negative	1.1	39	78	50	100
ACL (IgM)*	Positive	5.2	0	0	0	0
	Negative	5.0	50	100	50	100
ACL (IgG)*	Positive	6.7	0	0	0	0
	Negative	6.0	50	100	50	100
Toxoplasma (IgM)*	Positive	0.4	0	0	0	0
	Negative	0.4	50	100	50	100
Rubella (IgM)*	Positive	0.3	0	0	0	0
	Negative	0.3	50	100	50	100
TSH*	Positive	7.0	1	2	0	0
	Negative	3.5	50	98	50	100
FBS*	Negative	98	50	100	50	100
Hypertension*	Negative	74/105	50	100	50	100

* LA test normal value: 28-45 second
* dRVV test ratio normal value: < 1.6
* ACL (IgM) normal value: < 7
* ACL (IgG) normal value: < 10
* Toxoplasma (IgM) normal value: < 1.0
* Rubella (IgM) normal value: < 1.0
* TSH normal value: 0.5-5 µlU/mL
* FBS normal values: 80-120 mg/dl
* Hypertension normal values: 110-130/70-90

4.4.2 The Distribution of Laboratory Tests Related to APS among Aborted and Non Aborted Women

Table 4.14 showed the main tests and results for aborted and non aborted women. The tests showed strong relationship between LA test and dRVVT among APS +ve aborted women.

No relationship was found between ACL (IgM) and ACL (IgG) among aborted and non aborted women.

Table 4.14: The Distribution of Laboratory Tests among Aborted and Non Aborted Women

Laboratory Tests	Results	Mean	Sample Population					
			APS +ve Aborted		APS -ve Aborted		Not Aborted	
			N	%	N	%	N	%
dRVVT	Positive	1.8	11	11	0	0	0	0
	Negative	1.1	0	0	39	39	50	50
LA Test	Positive	51	11	11	0	0	4	4
	Negative	35	0	0	39	39	46	46
ACL (IgM)	Positive	5.0	0	0	0	0	0	0
	Negative	5.0	11	11	39	39	50	50
ACL (IgG)	Positive	6.0	0	0	0	0	0	0
	Negative	6.0	11	11	39	39	50	50

4.4.3 Risk Factors among Cases and Control

Table 4.15 showed risk factors studied for cases and control and the strong relationship between APS among cases. No relationship was found between toxoplasma (IgM) and rubella (IgM) among cases.

Table 4.15.a: Risk Factors among Cases and Control

Risk Factors	Results	Mean	Sample Population			
			Cases (n=50)		Control (n=50)	
			N	%	N	%
APS	Positive		11	22	0	0
	Negative		39	78	50	100
Toxoplasma (IgM)	Positive	0.4	0	0	0	0
	Negative	0.4	50	100	50	100
Rubella (IgM)	Positive	0.3	0	0	0	0
	Negative	0.3	50	100	50	100
LA Test	Positive	51	11	22	4	8
	Negative	35	39	78	46	92

Table 4.15.b: Risk Factors among Cases and Control

Risk Factors	Results	Mean	Sample Population			
			Cases (n=50)		Control (n=50)	
			N	%	N	%
dRVVT	Positive	1.8	11	22	0	0
	Negative	1.1	39	78	50	100
ACL (IgM)	Positive	5.2	0	0	0	0
	Negative	5.0	50	100	50	100
ACL (IgG)	Positive	6.7	0	0	0	0
	Negative	6.0	50	100	50	100
Toxoplasma (IgM)	Positive	0.4	0	0	0	0
	Negative	0.4	50	100	50	100
Rubella (IgM)	Positive	0.3	0	0	0	0
	Negative	0.3	50	100	50	100
TSH	Positive	7.0	1	2	0	0
	Negative	3.5	50	98	50	100
FBS	Negative	98	50	100	50	100
Hypertension	Negative	74/105	50	100	50	100

Chapter 5:

General Discussion Conclusion

In spite of good diagnosis and treatments (heparin and aspirin medication) of the second-trimester abortion, abortion rate in Gaza Governorates is still high. There are many risk factors that lead to second-trimester abortion as mentioned in the theoretical framework, which need further investigation as a group or in separate. Chronic maternal health factors account for the majority of second-trimester abortion such as severe hypertension, renal disease, hypothyroidism, hyperthyroidism, DM, toxoplasmosis and rubella considered as confounder variables, therefore any case and control having one or more of these variables were excluded from our study

5.1 APS among Cases and Control

APS is an autoimmune disease responsible for (1%) of second-trimester abortion. APS is an important marker for recurrent thrombosis, and often warrants indefinite anticoagulant (blood thinner) therapy, the criteria were defined in 1999 and revised in 2006 (Kaul *et al*, 2007). Our research was concentrated on the second-trimester to avoid any other factors responsible for abortion. We found a strong relationship between APS and second-trimester abortion $p < 0.05$. There are several risk factors needs to be investigated related to abortion especially inherited thrombophilia, which is responsible for (90%) of abortion especially in the first-trimester. However, other studies handled the relationship between preeclampsia and APS (Mitsue *et al*, 2005). The sensitivity of any test used to diagnose APS depends on the kind of activator, the nature and the concentration of the PLs in the

reagent and the clot detection technique that is used. There is no golden standard for the laboratory diagnosis of APS. The laboratory diagnosis of APS remains difficult because of the variable sensitivity and specificity of the tests. The idea of our question was similar to a question in other study that said "Which are the Best Biological Markers of the Antiphospholipid syndrome?" (Daboubi, 2001). Our study compared between the most possible tests that can be used for APS diagnosis such as LA test, dRVVT and ACL (IgM and IgG) and concluded that dRVVT is sensitive and more specific than LA and ACL for the APS diagnosis. Other study compared between LA test and ACL and concluded that LA test is less sensitive but more specific than ACL for the APS diagnosis (Carreras *et al*, 2000). Our results is in agreement with Gezer study who confirmed that dRVVT is the most sensitive assay of the LAs tests (LA , dRVVT, APTT and KCT test) (Gezer, 2003).

5.2 LA Test among Cases and Control

LAs are a mixture of immunoglobulin's (IgG, IgM and IgA) which interferes with one or more of the PL-dependent of coagulation tests. LAs are the most significant risk factor among various APLs that form an important diagnostic tool in the diagnosis of APS. There are many tests to detect the LAs such as LA test, dRVVT, APTT, dilute prothrombin time (Dpt), KCT, textarin time (TT), taipan snake venom time (TSVT), silica clotting time, dilute activated partial Thromboplastin time (DAPTT), tissue thromboplastin inhibition test (TTIT) and activated seven lupus anticoagulant (ASLA). These tests vary in there sensitivity and specificity. LA test may give falsely positive results which was found in elderly patients or if diagnosed for the first time. In this study we used LA test as APS diagnostic test and we found a significant relationship between LA test and second-trimester abortion. Our results are in correlation with study of Galli et al. (2003). An

overview of other clinical studies indicated that the presence of LAs correlates better with thromboembolic complications than the presence of ACL Abs (Devreese, 2006). Therefore, LAs activity should be quantitatively expressed to indicate the strength and to make the possible differentiation between high and low titers in a numerical manner. In parallel with the quantification of ACL and anti β_2GPI Abs, it will help to stratify patients into risk groups (Devreese *et al*, 2010). However, our results reflected a poor specificity of LA test for APS diagnosis compared with dRVVT.

5.3 dRVVT among Cases and Control

Russell's viper venom (RVV) is the most widely used clinical laboratory assay for the confirmation of the presence of a PL-dependent Abs. It causes massive thrombosis when injected *in vivo* (Riley, 2005). This *in vitro* diagnostic test is based on the ability of the venom of the Russell's viper to induce thrombosis. In the dRVVT assay, low, rate-limiting concentrations of both Russell's viper venom and PL are used. This makes the test sensitive to the presence of LAs, because these Abs interfere with the clot-promoting role of PL *in vitro* and their presence results in a prolonged clotting time. The dRVVT test is more sensitive than the APTT test for the detection of LAs, because it is not influenced by deficiencies or inhibitors of clotting factors VIII, IX or XI. A number of studies have shown that commercial dRVVT reagents vary in their sensitivity for LAs detection. The differences in performance considered to be predominantly due to Abs heterogeneity, a wide variation in PL content , the techniques and clot detection methods employed (Moore *et al*, 2004). Analyzing results with normalized ratios and the use of integrated test systems like dRVVT can contribute to a better interpretation of results of APS diagnosis consists of clotting assays, sometimes with low sensitivity and an unsatisfactory standardization. We

found a strong relationship between dRVVT and second- trimester abortion, which is statistically significant with (p value < 0.05). Despite of the multiple tests available, the dRVVT found to be the most specific and sensitive test for APS diagnosis more than other tests. These results are in agreement with Thiagarajan et al. (1986) who was the first to describe the use of a modified Russell viper venom time (RVVT) for APS diagnosis, where they found that dRVVT to be a reproducible, sensitive and relatively specific method for identification of LA test (Triplett, 2000).

5.4 Conclusion

1. According to our study results we concluded that there is an association between APS and the second-trimester abortion.
2. Diagnosis of APS require both clinical and laboratory criteria which need a cooperation between obstetrics and gynecologist.
3. Our results found that dRVVT is the most specific and sensitive laboratory test for APS diagnosis.
4. dRVVT and LA test are more sensitive than other tests and can be used beside each other.
5. We advise to use dRVVT for APS diagnosis according to the good specificity and sensitivity more than LA test.
6. The largest number of abortions was found 1-3 times which represented (74%) of all cases and the median age of second-trimester abortion was at 28 years old
7. It requires good attention for pregnant women at this median age.

5.5 Recommendations

To help in the diagnosis of APS we recommend the following:

1. It's advisable to use dRVVT as a specific and a confirmatory test for APS diagnosis.
2. The positive tests for APLs should be repeated after 6 weeks. Diagnosis of APS should follow international diagnosis, especially about the reference value of tests, most literature review show the reference value of ACL > 20U/L.
3. There is a need to study other causes of abortion especially inherited thrombophilia.
4. There is a different in APS percentage among cases from Rafah Governorate than KhanYounis Governorate which need further investigation.
5. Further research needed to control chronic maternal risk factors.
6. Screening programs to determine pregnant women at high risk for abortion is needed in Gaza Governorates which could decrease abortion rate.
7. Training programs are required for obstetrics and gynecologists about psychological support for pregnant women.
8. MOH should consider a computerized system in data programming especially for patients with heparin lists in Gaza Governorates.
9. Encourage private clinics and laboratories to computerized their data.

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Annex 1

Prevalence of APS and Abortion

No	Country	No of Articles	Assessments in >1Province	Studies with > 50 Cases
1	Occupied Palestine	3	-	2
2	Jordan	1	-	0
3	Oman	1	-	0
4	Kuwait	1	-	0
5	Bahrain	1	-	0
6	USA	6	-	0
7	Spain	2	1	2
8	UK	2	-	1
9	Brazil	1	-	1
10	Japan	1	-	0
11	Italy	1	-	1

Annex 2

Summary of the Studies Conducted in the World on the APS and Abortion

No	Country	Ref. No	Year of Publication	Sample Size	Region	Target Age
1	Occupied Palestine	(Marai, 2001)	2001	90	Sheba Medical	
2	Occupied Palestine	(Shoenfeld *et al*, 2008)	2008			
3	Jordan	(Daboubi, 2001)	2001	26		
4	Oman	(Naqdy *et al*, 2005)	2005	44		16-64
5	Kuwait	(Diejomaoh *et al*, 2002)	2002	43	2 Clinics	21-45
6	Bahrain	(Naqdy *et al*, 2005)	2005	44		16-64
7	Spain	(Cervera, 2010)	2010	280		11-60
8	Spain	(Cervera *et al*, 2010)	2009	1000	European Countries	
9	UK	(Bramham *et al*, 2010)	2009	67	Thomas Hospital	28.9-39.7
10	Brazil	(Danowski *et al*, 2009)	2009	122		

Annex 3

Reported Prevalence of Abortion among APS

No	Country	Ref. No	%	Standard
1	Occupied Palestine	(Marai, 2001)		
2	Occupied Palestine	(Shoenfeld *et al*, 2008)		
3	Jordan	(Daboubi, 2001)	19.2	
4	Oman	(Naqdy *et al*, 2005)	27	
5	Bahrain	(Naqdy *et al*, 2005)	27	
6	Spain	(Cervera, 2010)	30	
7	Spain	(Cervera *et al*, 2010)	17.1	High Titer APLs
8	UK	(Bramham *et al*, 2010)	31.3	Sapporo Criteria
9	Brazil	(Danowski *et al*, 2009)		ACL (IgG) >40, +ve LA
10	Kuwait	(Diejomaoh *et al*, 2002)		

Annex 4

Arabic Questionnaire

أنا الباحث: محمود الحاج أحمد (طالب ماجستير بجامعة القدس- أبو ديس). أرجو المساعدة في إتمام هذه الدراسة، وذلك من خلال تعبئة هذا الاستبيان وتبرعك بعينة دم لإجراء بعض الفحوصات من أجل عمل بحث حول علاقة APS مع الإجهاض في الفصل الثاني من الحمل في قطاع غزة.

و لكِ منا جزيل الشكر مع تمنياتنا بالصحة والعافية.

الإستبانة:

١	رقم الإستبانة:	.....	
٢	الاسم(اختياري):	.....	
٣	رقم التلفون(اختياري):	.....	
٤	رقم الجوال(اختياري):	.....	
٥	مكان السكن:	.....	
٦	هل أنت حامل؟	نعم ☐	لا ☐
٧	العمر بالسنة؟	.....	
٨	هل أنت موظفة؟	نعم ☐	لا ☐
٩	عدد الولادات؟	.....	
١٠	عدد مرات الإجهاض؟	.....	
١١	زمن الإجهاض؟	نعم ☐	لا ☐
١٢	هل تتناولين علاجات سيولة الدم الآن؟	نعم ☐	لا ☐
١٣	هل تعانين في هذه الفترة من ضغط الدم المزمن ؟	نعم ☐	لا ☐
١٤	هل تعانين في هذه الفترة من مرض السكري النوع الثاني؟	نعم ☐	لا ☐
١٥	هل لديك ملف لمرضى الكلي؟	نعم ☐	لا ☐
١٦	هل تعانين من زيادة أو نقص في نشاط الغدة الدرقية؟	نعم ☐	لا ☐
١٧	هل تعرضت للتصوير الإشعاعي خلال فترة الحمل السابقة؟	نعم ☐	لا ☐
١٨	هل تعرضت للتلوث بمواد كيميائية زراعية أو غيرها خلال فترة الحمل السابقة؟	نعم ☐	لا ☐
١٩	هل تناولت علاجات كيميائية؟	نعم ☐	لا ☐
٢٠	هل تعرضت لحوادث سيارات أو سقوط؟	نعم ☐	لا ☐
٢١	هل تعرضت لصدمة عاطفية حادة؟	نعم ☐	لا ☐
٢٢	هل تعانين من عيوب خلقية في الرحم؟	نعم ☐	لا ☐

شكرا لحسن تعاونك

الباحث

محمود سليم الحاج أحمد

Annex 5

Arabic Consent Form

الأخت المشاركة:

أنت مدعوة للمشاركة في الدراسة البحثية المذكورة أعلاه. هذه الدراسة جزءً من متطلبات الحصول علي درجة الماجستير في الصحة العامة بجامعة القدس أبو ديس. الغرض من هذه الدراسة هو تحديد العلاقة بينAPS، والإجهاض في الفصل الثاني من الحمل في قطاع غزة، وهذا من شأنه أن يساعد في تشخيصAPS، و بالتالي ضبط الإجهاض.

مشاركتك طوعية، لكِ الحق في معرفة النتيجة إن كانت غير طبيعية، البيانات ستكون سرية.

بيان الموافقة:

لقد قرأت المعلومات الواردة أعلاه، فهمت الأسئلة، وموافقة على المشاركة في البحث بما فيها سحب عينات دم.

شكراً لكِ على حسن تعاونك

الباحث

محمود سليم الحاج أحمد

Annex 6

Helsinki Committee Approval

Palestinian National Authority
Ministry of Health
Helsinki Committee

السلطة الوطنية الفلسطينية
وزارة الصحة
لجنة هلسنكي

التاريخ : 07/03/2011

Name: **Mahmoud El Hajj Ahmed**

الاسم: محمود الحاج احمد

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

نفيدكم علماً بأن اللجنة قد ناقشت مقترح دراستكم حول:-

" Anti-phospholipid syndrome among aborted women in their second trimester in Gaza: case control study."

In its meeting on March 2011

و ذلك في جلستها المنعقدة لشهر 3 2011

and decided the Following:-

و قد قررت ما يلي:-

To approve the above mention research study.

الموافقة على البحث المذكور عاليه.

Signature
توقيع

Member
عضو

Member
عضو

Chairperson

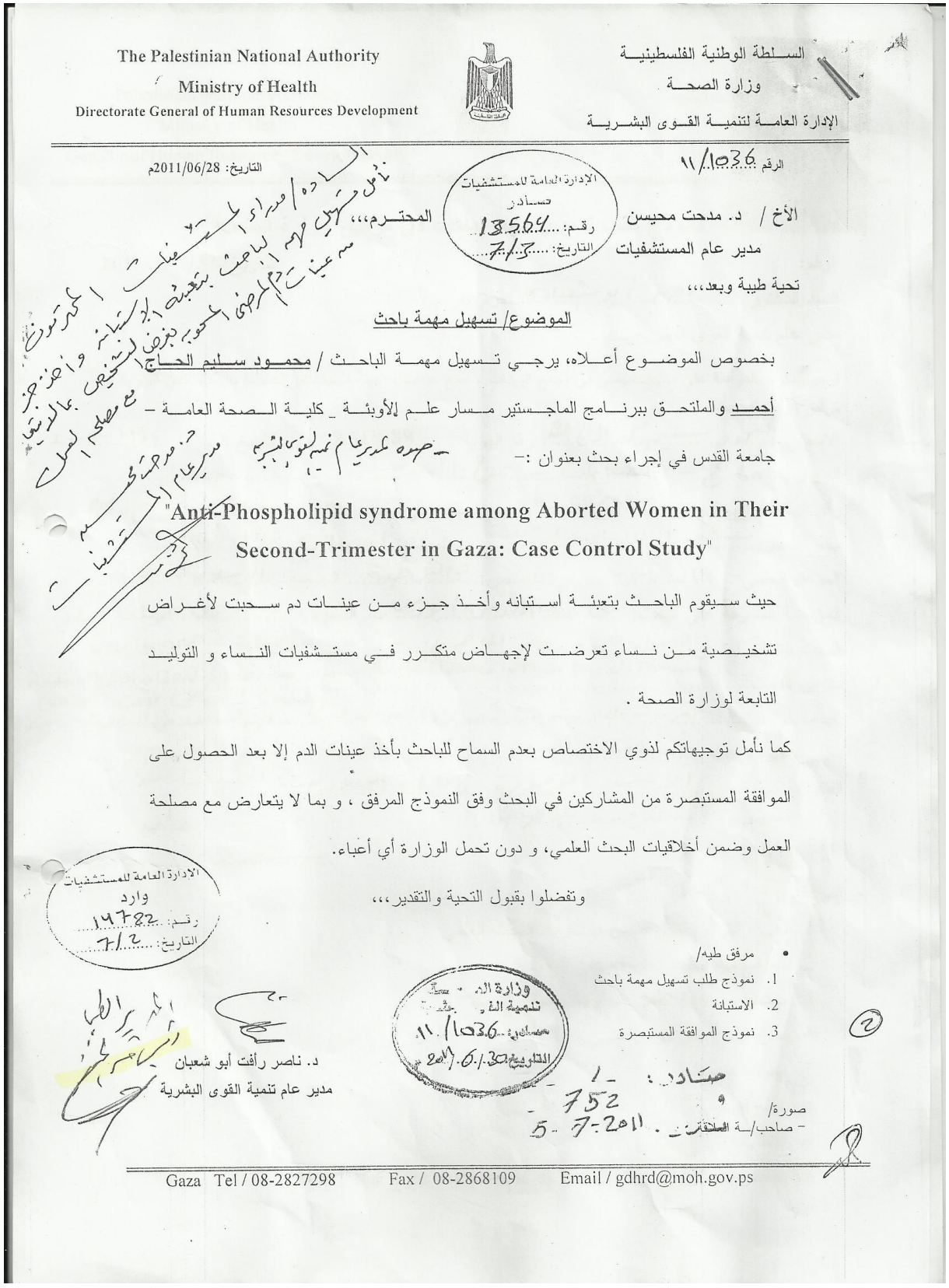
Conditions:-

- 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 appreciate receiving one copy of your final research when it is completed.

33

Annex 7

MOH Approval



The Palestinian National Authority
Ministry of Health
Directorate General of Human Resources Development

السلطة الوطنية الفلسطينية
وزارة الصحة
الإدارة العامة لتنمية القوى البشرية

الرقم ١١/١٥٣٦

التاريخ: 2011/06/28م

الإدارة العامة للمستشفيات
صادر
رقم: 13564
التاريخ: 7/7/2011

الأخ / د. مدحت محيسن
مدير عام المستشفيات

المحترم،،،

تحية طيبة وبعد،،،

الموضوع/ تسهيل مهمة باحث

بخصوص الموضوع أعلاه، يرجى تسهيل مهمة الباحث / محمود سليم الحاج أحمد والملتحق ببرنامج الماجستير مسار علم الأوبئة _ كلية الصحة العامة – جامعة القدس في إجراء بحث بعنوان :-

"Anti-Phospholipid syndrome among Aborted Women in Their Second-Trimester in Gaza: Case Control Study"

حيث سيقوم الباحث بتعبئة استبانه وأخذ جزء من عينات دم سحبت لأغراض تشخيصية من نساء تعرضت لإجهاض متكرر في مستشفيات النساء و التوليد التابعة لوزارة الصحة .

كما نأمل توجيهاتكم لذوي الاختصاص بعدم السماح للباحث بأخذ عينات الدم إلا بعد الحصول على الموافقة المستبصرة من المشاركين في البحث وفق النموذج المرفق ، و بما لا يتعارض مع مصلحة العمل وضمن أخلاقيات البحث العلمي، و دون تحمل الوزارة أي أعباء.

وتفضلوا بقبول التحية والتقدير،،،

الإدارة العامة للمستشفيات
وارد
رقم: 14782
التاريخ: 7/7/2011

وزارة الصحة
تنمية القوى البشرية
صادر
رقم: ١١/١٥٣٦
التاريخ: 2011/6/30

د. ناصر رأفت أبو شعبان
مدير عام تنمية القوى البشرية

- مرفق طيه/
1. نموذج طلب تسهيل مهمة باحث
2. الاستبانة
3. نموذج الموافقة المستبصرة

صادر: 752
5-7-2011

صورة/
- صاحب/ـة العلاقة

Gaza Tel / 08-2827298 Fax / 08-2868109 Email / gdhrd@moh.gov.ps

Annex 8

Estimated Budget

No	Item	Quantity	Estimated cost
1	ACL (IgM)	100	300$
2	ACL (IgG)	100	300$
3	LA Test	100	300$
4	dRVVT	100	600$
5	Toxoplasma (IgM)	100	300$
6	Rubella (IgM)	100	300$
7	Syringes	2 boxes	15$
8	Plain tubes	100	15$
9	Na citrate tubes	100	20$
10	TSH	100	200$
11	Sugar reagent	100	50$
12	Miscellanies		600$
		Total	3000$

Annex 9

ملخص الدراسة

متلازمة مضادات الفوسفوليبيد (APS) هو فرط الخثورية المكتسبة (acquired thrombophilia) وهي تسبب التخثرات الوريدية، والشريانية المتكررة، والإجهاض المتكرر الذي يحدث في حوالي (١%) من النساء حول العالم.

هدف الدراسة: تهدف هذه الدراسة إلي تقييم العلاقة بين متلازمة مضادات الفوسفوليبيد والإجهاض في الفصل الثاني من الحمل في محافظات غزة.

منهجية الدراسة: هذه الدراسة هي دراسة وصفية تحليلية تدرس الحالات المرضية وتقارنها مع عينة ضابطة، أخذت العينة من محافظات جنوب غزة، مائة من المشاركين (٥٠ حالة و٥٠ ضابطة)، جمعت البيانات بطريقتين: الطريقة الأولي باستخدام استبيان شمل البيانات الاجتماعية والاقتصادية ومجموعة من عوامل الخطر وذلك لكي تستثني من الدراسة، الطريقة الثانية شملت سحب عينات دم من المشاركين وقيست مجموعة من الفحوصات المتعلقة بمتلازمة الفوسفوليبيد والإجهاض. في هذه الدراسة استخدمت مجموعة من التقنيات مثل ELISA وspectrophotometer و Ag-Ab reaction وقيست المتغيرات المختلفة مثل فحوصات: Lupus Anticoagulant (LA) test وdRVVT و Anticardiolipin (ACL) (IgM and IgG) و Rubella (IgM) وFBS وToxoplasma (IgM) وTSH.

النتائج: أشارت الدراسة إلي وجود علاقة بين APS والإجهاض في الفصل الثاني من الحمل، كذلك أشارت الدراسة إلي وجود علاقة بين فحصdRVVT والإجهاض في الفصل الثاني من الحمل وأن فحص dRVVT يعتبر أدق فحوصات تشخيصAPS، أيضاً هناك علاقة بين فحص LA والإجهاض في الفصل الثاني من الحمل، وكل ما سبق ذكره من العوامل أكدت الدراسة أنه ذو دلالة إحصائية. لا يوجد علاقة بين(IgM) Toxoplasma والإجهاض في الفصل الثاني من الحمل وأخيرا لا يوجد علاقة بين Rubella (IgM) والإجهاض في الفصل الثاني من الحمل.

التوصيات:

١. فحصdRVVT يعتبر من أدق الفحوصات في تشخيصAPS.

٢. الفحوصات الموجبة في تشخيص APS يجب أن يعاد الفحص بعد ٦ أسابيع حيث أن APLs يبقي معدلها مرتفعاً.

٣. هناك حاجة لدراسة فرط الخثورية الوراثية (acquired thrombophilia) وعلاقته مع الإجهاض.