CHAPTER ONE

1. Introduction

1.1. Introduction:

Syphilis is a chronic infectious disease caused by the spirochaete *Treponema pallidum* subspecies *pallidum*. It is a systemic Sexually Transmitted Disease (STD) (Stamm, 2010). Syphilis is transmitted primarily by sexual intercourse (venereal syphilis) including genital, oral and/or anal contact with or without penetration or during pregnancy from mother to fetus, the spirochete is able to pass through intact mucous membrane or compromised skin (Kent and Eomanelli 2008, Stamm 2010). However, the disease can also be transmitted in vitro (via placenta) or via blood transfusion or via accidental inoculation from infectious material. Since the syphilis is almost always transmitted by sexual intercourse, it is thus classified as one of the Sexual Transmitted Diseases (STDs) (Abdelbagi *et al*; 2008).

Syphilis is found worldwide and an estimated 12 million cases of syphilis occurred worldwide in 1999 (WHO, 1999). In the United States, syphilis is the third most common sexually transmitted disease in contrast to a decline in the rate observed in Western Europe; the 1990s witness an alarming increase in syphilis infection in the newly independent states in the former Soviet Union (Wasserheit, 1992).

Untreated syphilis has a mortality of 8% to 58% with a greater death rate in males (Kent and Eomanelli, 2008). The symptoms of syphilis have become less severe over the 19th and 20th century in part due to widespread availability of effective treatment and partly due to decreasing virulence of the spirochete. (Mullooly and Higgins, 2010). With early treatment few complications result (Eccleston *et al*; 2008).
1.2 Rationale:

Studies amongst pregnant women in the North and North Eastern regions of Africa showed syphilis infection rates of 3.1% in Djibouti, 3% in Morocco and 2.4% in Sudan (WHO, 1999) many previous studies of syphilis were done in Sudan the prevalence of syphilis obtained in study among pregnant women in Tricapital Khartoum State in Sudan (9%) reported by (Abdelbagi et al; 2008).

It is of important to screening syphilis among pregnant women at different stages of pregnancy and at different ages. Because primary syphilis may be asymptomatic, the disease may pass unnoticed, Moreover, there is a risk of disease transmission from mother to her unborn child (Brillman et al; 1998) These prime factors necessitate routine screening of syphilis among pregnant women (CDC, 1993).
1.3 Objectives

1.3.1 General Objectives:

The main objective of this study was to detect the anti-Treponemal antibodies among pregnant women in Jebel Awlia Teaching Hospital.

1.3.2 Specific Objectives:

1. To detect the anti-Treponemal antibodies using Rapid Plasma Reagin test as screening test.
2. To confirm the result of rapid syphilis test by using Enzyme Linked Immune Sorbent Assay (ELISA).
3. To compare between Enzyme Linked Immune Sorbent Assay (ELISA) and Rapid Plasma Reagin (RPR) in the diagnosis of syphilis among pregnant women.
4. To determine risk factors associated with syphilis among pregnant women.
CHAPTER TWO
2. Literature Review

2.1. History of syphilis:

The origins of syphilis have been discussed for many centuries (Dennie, 1962, Oriel, 1994, Quetel, 1990). Two main theories have been proposed—the New World or Columbian theory and the Old World or pre-Columbian theory. The former holds that syphilis was endemic in the part of the world now known as Haiti and was then acquired and carried to Europe by Columbus in the 1400s. The pre-Columbian theory purports that syphilis originated in central Africa and was introduced to Europe prior to the voyage by Columbus. The causative organism, Treponema pallidum, was first identified by Fritz Schaudinn and Erich Hoffmann in 1905 (Franzen, 2008). The first effective treatment (Silverman) was developed in 1910 by Paul Ehrlich, which was followed by trials of penicillin and confirmation of its effectiveness in 1943 (Franzen, 2008). Before the advent of effective treatment, mercury and isolation were commonly used, with treatments often worse than the disease (Dayan, 2005). Many famous historical figures, including Franz Schubert, Arthur Schopenhauer, and Édouard Manet, are believed to have had the disease (Franzen, 2008).

2.2. Epidemiology of syphilis:

Syphilis is believed to have infected 12 million people in 1999 with greater than 90% of cases in the developing world (Stamm, 2010). It affects between 700,000 and 1.6 million pregnancies a year resulting in spontaneous abortions, stillbirths, and congenital syphilis (Woods, 2009). In Sub-Saharan Africa syphilis contributes to approximately 20% of perinatal deaths (Woods, 2009).

In the developed world, syphilis infections were in decline until the 1980s and 1990s due to widespread use of antibiotics. Since the year 2000, rates of syphilis have been
increasing in the US, UK, Australia and Europe primarily among men who have sex with men (Stamm, 2010). This is attributed to unsafe sexual practices. In Europe rates of syphilis are higher in eastern and southern countries than in western ones (Uusküla et al; 2010).

In the United States rates of syphilis have increased among men between 2000 and 2007 (Celum, 2010). Rates are currently six times greater in men than women and seven times greater in African Americans than Caucasians (Celum, 2010). More than 60% of cases are in men who have sex with men (Celum, 2010). It occurs most commonly in those between 15–40 years of age.

In the United States, rates of syphilis as of 2007 were six times greater in men than women while they were nearly equal in 1997 (CDC, 2007). Rates are also greater in African Americans and Hispanics than in Caucasians (CDC, 2007).

2.3. Causative Organism:

The Spirochaetae are large group of spiral, motile bacteria. One family (Spirochaetaceae) of the order Spirochaetales consist of three genera of free-living, large spiral organism. The other family (Treponemataceae) include three genera whose members are human pathogen, Treponema, Borrelia, and Leptospira (Pope et al; 2007).

The spirochaete have many structural characteristics, they are long, slender, helically, coiled, and spiral or corkscrew shaped, Gram negative bacilli. T. pallidum has an outer sheath or glycosaminoglycan coating inside the sheath is the outer membrane, which contain peptidoglycan and maintain the structural integrity of the organism. End flagella (axial filaments) are the flagella –like organelles in the periplasmic space encased by outer membrane. The end flagella begin at each end of the organisms and wind around it, extending to and overlapping at the midpoint. Inside the endo flagella the inner membrane (cytoplasmic membrane) that provide osmotic stability and covers the
proplasmic cylinder. A series of cytoplasmic tubules (body fibrils) are inside the cell near the inner membrane. Treponemes reproduced by transverse fission. (Pope et al; 2007).

2.3.1. Treponema:

Although given various names following its discovery, the causative organism of syphilis was finally named Treponema because of its resemblance to a twisted thread and pallidum because of its pale color (Quetel, 1990).

The pathogenic species are T. pallidum subsp. pallidum which causes venereal syphilis, T. pallidum subsp. endemicum, which causes endemic syphilis (bejel), T. pallidum subsp. pertenue, and, which causes yaws, and T. carateum, which is the etiologic agent of pinta and two non-venereal infections that occur in the tropics and subtropics (Kayser, 2005).

2.3.1.1. Treponema pallidum:

T. pallidum is a member of the order Spirochaetales, family Spirochaetaceae, and genus Treponema, which includes four human pathogens and at least six human nonpathogens (Norris and Larsen, 1995). Is a Gram–negative bacterium which is spiral in shape, It is an obligate internal parasite which causessyphilis, a chronic human disease. T. palladium subsp. species palladium is etiological agent of the Venereal Disease (Patrick et al; 2002).

2.3.1.1.1. Physiology and structure

The physiology, metabolism, and antigenic structure of T. pallidum is derived from the Nichols strain, which has been maintained in rabbits since 1912 (Nichols and Hough, 1913). T. pallidum is a spirochete varying from 0.10 to 0.18 μm in diameter and from 6 to 20 μm in length, making it invisible by light microscopy (Willcox and Guthe, 1996). Dark-field microscopy is generally used in clinical practice for visualization (Creighton, 1990). The average number of windings is 6 to 14, and the organism has pointed ends and lacks the hook shape seen in some commensal human spirochetes.
The bacterium exhibits characteristic corkscrew motility due to endoflagella, with rapid rotation about the longitudinal axis and flexing, bending, and snapping about the full length (Norris and Larsen, 1995). The spirochete is a strict human pathogen, natural syphilis is not found in any other species, and experimental syphilis has been established only in the rabbits. *T. pallidum* is a thin, coiled spirochete that cannot be grown in cell-free cultures. Limited growth of the organism has been achieved in cultured rabbit epithelial cell, but replication is slow (doubling time is 30 ours) and can be maintained for only a few generation. The spirochetes were once considered strict anaerobic; however, it is now known that they can use glucose oxidatively (Patrick et al.; 2002).

### 2.3.1.1.2. Morphology and culture:

These organisms are slender bacteria, 0.2 wide and 5–15 lm long; they feature 10–20 primary windings and move by rotating around their lengthwise axis. The organisms are actively motile, rotating steadily around their end flagella even after attaching to cells by their tapering end. The long axis of the spiral is ordinarily slight but may sometimes bend, so that organisms forms a complete circle for moments as the time, returning then to normal straight position. The spirals are so thin that they are not readily seen unless immune fluorescent stain or dark field illumination is employed (Jawetz et al.; 2010). Their small width makes it difficult to render them visible by staining. They can be observed in vivo using dark field microscopy. In-vitro culturing has not yet been achieved (Kayser, 2005).

### 2.3.1.1.3. The genome

The genome of *T. pallidum* subsp. *pallidum* has recently been sequenced by the whole pulse genome random sequencing method (Fraser et al.; 1998). The genome is a circular chromosome of 1,138,006 bp and contains 1,041 open reading frames (ORFs). Predicted biological roles were assigned to 55% of ORFs, while 17% match those encoding hypothetical proteins from other species and 28% represent novel genes. Physiologic studies have previously shown that the organism has limited biosynthetic capabilities,
requiring multiple nutrients from the host (Fraser et al; 1998). The *T. pallidum* genome confirms this by demonstrating the inability of the organism to synthesize enzyme cofactors, fatty acids, and nucleotides de novo and by encoding for a pathway for the conversion of phosphoenolpyruvate or pyruvate to aspartate. Given its limited biosynthetic properties, it is assumed that *T. pallidum* must have good transport proteins, and, indeed, the *T. pallidum* genome contains 57 ORFs (5% of the total) that encode 18 distinct transporters specific for amino acids, carbohydrates, and cations. Metabolic-pathway analysis shows that genes encoding all of the enzymes of the glycolytic pathway are present in *T. pallidum*, suggesting that it uses several carbohydrates as energy sources. The organism has previously been demonstrated to survive better in very low concentrations of oxygen and is therefore considered microaerophilic (Fieldsteel et al; 1979). This is confirmed by the lack of genes encoding superoxide dismutase, catalase, or peroxidase, which protect against oxygen toxicity. Motility-associated genes are highly conserved in *T. pallidum*, consistent with the importance of this activity; 36 genes encode proteins in the flagella structure. Freeze fracture studies of the outer membrane of *T. pallidum* show that it contains a small number of integral membrane proteins which may allow it to evade the host immune response (Walker et al; 1991; Radolf et al; 1989).

### 2.3.1.1.4. Antigenic Structure:

*T.pallidum subspecies pallidum* has hyaluronic acid in the ground substance of tissue and presumably enhanced the invasiveness of the organism. The protein profiles of *T.pallidum* (all subspecies) are indistinguishable; more than 100 protein antigens have been noted. The end flagella are composed of three core proteins, plus an unrelated sheath protein. Cardiolipin is an important component of treponemal antigen (Jawetz et al; 2010).

### 2.3.1.1.5. Pathogenesis:
*T. pallidum* initially enters from mucosal or cutaneous lesions of an infected through skin or mucous membranes abraded during sexual activity. The organisms reach local lymph nodes within 30 minutes after skin inoculation. Viable treponemes attach to cells, after which polymorph nuclear leukocytes accumulate in the area of infection (Musher *et al*; 1983). Both B and T lymphocytes accumulate in the area. Activated macrophages ingest treponemes. By the time a chancre appears, most patients have evidence of antibody formation to the organism. Although both humeral and cell-mediated immunity to *T. pallidum* have been demonstrated. The treponemes may actually suppress host immunity to facilitate their own survival (Lukehart *et al*; 1980; Soltain *et al*; 1978).

### 2.4. Signs and symptoms

Syphilis can present in one of four different stages: primary, secondary, latent, and tertiary (Kent and Eomanelli, 2008) and may also occur congenitally (Stamm, 2010).

#### 2.4.1. Primary syphilis

Primary syphilis is typically acquired by direct sexual contact with the infectious lesions of another person (Larry and Pickering, 2008). Approximately 3 to 90 days after the initial exposure (average 21 days) a skin lesion, called a chancre, appears at the point of contact (Kent and Eomanelli, 2008). This is classically (40% of the time) a single, firm, painless, non-itchy skin ulceration with a clean base and sharp borders between 0.3 and 3.0 cm in size. The lesion, however, may take on almost any form (Eccleston *et al*; 2008) In the classic form, it evolves from a macule to a papule and finally to an erosion or ulcer (Eccleston *et al*; 2008). Occasionally, multiple lesions may be present (~40%) (Kent and Eomanelli, 2008) with multiple lesions more common when coinfected with HIV. Lesions may be painful or tender (30%), and they may occur outside of the genitals (2–7%). The most common location in women is the cervix (44%), the penis in heterosexual men (99%), and anally and rectally relatively commonly in men who have sex with men (34%) (Eccleston *et al*; 2008). Lymph node enlargement frequently (80%) occurs around the area of infection (Kent and Eomanelli, 2008). Occurring seven to 10 days after
chancre formation (Eccleston et al; 2008). The lesion may persist for three to six weeks without treatment (Kent and Eomanelli, 2008).

### 2.4.2. Secondary syphilis

Typical presentation of secondary syphilis with a rash on the palms of the hands. Reddish papules and nodules over much of the body due to secondary syphilis. Secondary syphilis occurs approximately four to ten weeks after the primary infection (Kent and Eomanelli, 2008). While secondary disease is known for the many different ways it can manifest, symptoms most commonly involve the skin, mucous membranes, and lymph nodes. (Mullooly and Higgins, 2010). There may be a symmetrical, reddish-pink, non-itchy rash on the trunk and extremities, including the palms and soles (Kent and Eomanelli, 2008; Dylewski and Duong, 2007). The rash may become maculopapular or pustular. It may form flat, broad, whitish, wart-like lesions known as condyloma latum on mucous membranes. All of these lesions harbor bacteria and are infectious. Other symptoms may include fever, sore throat, malaise, weight loss, hair loss, and headache. (Kent and Eomanelli, 2008). Rare manifestations include hepatitis, kidney disease, arthritis, periostitis, optic neuritis, uveitis, and interstitial keratitis (Kent and Eomanelli, 2008; Bhatti, 2007). The acute symptoms usually resolve after three to six weeks, (Bhatti, 2007) however, about 25% of people may present with a recurrence of secondary symptoms. Many people who present with secondary syphilis (40–85% of women, 20–65% of men) do not report previously having had the classic chancre of primary syphilis (Mullooly and Higgins, 2010).

### 2.4.3. Latent syphilis

Latent syphilis is defined as having serologic proof of infection without symptoms of disease (Larry and Pickering, 2008). It is further described as either early (less than 1 year after secondary syphilis) or late (more than 1 year after secondary syphilis) in the United States (Bhatti, 2007). The United Kingdom uses a cut-off of two years for early and late latent syphilis (Eccleston et al; 2008). Early latent syphilis may have a relapse of
symptoms. Late latent syphilis is asymptomatic, and not as contagious as early latent syphilis, (Bhatti, 2007)

2.4.4. Tertiary syphilis

Tertiary syphilis may occur approximately 3 to 15 years after the initial infection. Without treatment, a third of infected people develop tertiary disease (Bhatti, 2007). People with tertiary syphilis are not infectious (Kent and Eomanelli, 2008).

2.5. Identification of *T. pallidum*:

Syphilis is a curable sexually transmitted infection caused by the bacterium *Treponema pallidum*; the infection can also be passed from mother to her fetus during pregnancy.

Diagnosis of syphilis is based on clinical evaluation, detection of the causative organism, and confirmation of the disease by serodiagnosis.

*T. pallidum* cannot be cultured in the laboratory, but can be identified in lesions using dark-field or fluorescence microscopy or by molecular techniques. Most infected individuals have no symptoms or have transient lesions and therefore a serological test must be used to screen for infection. Blood tests are more commonly used, as they are easier to perform (Kent and Eomanelli, 2008). Diagnostic tests are, however, unable to distinguish between the stages of the disease (Farhi and Dupin, 2010).

2.5.1. Direct detection of *Treponema pallidum*:

There are number of methods available for direct detection of intact organisms or *T. pallidum* DNA. Dark ground microscopy of serous fluid from a chancre may be used to make an immediate diagnosis. However, hospitals do not always have equipment or experienced staff members, whereas testing must be done within 10 minutes of acquiring the sample. Sensitivity has been reported to be nearly 80%, thus can only be used to confirm a diagnosis but not rule one out. Two other tests can be carried out on a sample from the chancre: direct fluorescent antibody testing and nucleic acid amplification tests.
Direct fluorescent testing uses antibodies tagged with fluorescein, which attach to specific syphilis proteins, while nucleic acid amplification uses techniques, such as the polymerase chain reaction, to detect the presence of specific syphilis genes. These tests are not as time-sensitive, as they do not require living bacteria to make the diagnosis (Eccleston et al.; 2008)

2.5.1.1. Dark field microscopy:
The oldest method still remains one of the simplest and most reliable for the direct detection of *T. pallidum*. An experienced microscopist can identify *T. pallidum* from lesions based on the characteristic morphology and motility of the spirochete. This method is suitable when the lesions are moist, and the examination can be done immediately after specimen collection. Exudates and fluids from lesions are examined as a wet mount using dark-field microscopy. During the primary stage, serous fluid from the lesion contains numerous treponemes and, therefore, this approach is particularly useful in patients with immunodeficiency or in early syphilis when antibodies are not yet detectable. Success is dependent on a number of factors, including too little or too much fluid on the slide, the presence of retractile elements in the specimen, improper thickness of the slide or cover slip, etc. Treatment with antibiotics may result in a false-negative finding. Therefore, although the demonstration of *T. pallidum* is the definitive method of diagnosis, dark-field microscopy has limited sensitivity, and failure to detect *T. pallidum* by this test does not rule out syphilis (Larsen et al.; 1990).

2.5.1.2. Direct fluorescent antibody test for *T. pallidum* (DFA-TP) in the blood fluids:
The direct fluorescent antibody test for *T. pallidum* is easier to perform than dark-field microscopy; it detects antigens and does not require the presence of motile treponemes. DFA-TP test use fluorescein isothiocyanate-labelled anti-*T. pallidum* antibody to identify the organism. However, this test does not differentiate between *T. pallidum* and other pathogenic treponemes causing yaws, endemic syphilis and pinta specific to pathogenic treponemes.
The number organisms in the fluid or tissue that can be detected by fluorescent antibody tests is similar to that for darkfield microscopy. The sensitivity of these methods is only slightly better than darkfield microscopy (Larsen et al; 1990).

2.5.3. **Antitreponemal antibody responses:**
Serum immunoglobulin (IgM and IgG) antibody responses to *T. pallidum* have been studied extensively in experimentally infected animals and humans (Holmes et al; 2008). Antitreponemal IgM antibodies are produced ~2 weeks after exposure, followed by IgG antibodies 2 weeks after IgM production, late latent syphilis have faint IgM responses and greater variability in the number of antigen reactive with IgG compared with those with early latent syphilis (Young et al; 1988). Both antitreponemal IgM and IgG antibody may be detectable within 3 days of lesion onset in primary syphilis. IgG antibodies appear to increase in patients with longer duration of symptoms (Baker-Zander et al; 1986).

In secondary syphilis, there is a disproportionate increase in antitreponemal IgG3-species responses. Host antibody responses can lead to immune-complex formation consisting of either IgG1 or IgG3 antibodies coupled to spirochete antigens (Baughn et al; 1988). In untreated late syphilis, both faint –to- moderate IgM and strong IgG and reactivity are evident, person with AF therapy in primary and secondary syphilis, *T.Pallidum* IgM antibodies decrease rapid becoming undetectable within 6-12 months (Baker –zander et al; 1986). Several studies sugger that decreasing IgM levels indicate adequacy of treatment (Baughn et al; 1988). IgM antibody does not cross the placenta and have been primarily evaluated for diagnosis of congenital syphilis (Schmitz et al; 1994).

2.5.4. **Serological tests:**
Serology is still the most reliable method for laboratory diagnosis of syphilis, regardless of the stage of infection. Serologic test are divided into **nontreponemal** and **treponemal tests**, neither alone is sufficient for diagnosis. Conventional serologic diagnosis used a two-step approach, of first screening with nontreponemal method, and then using a confirmatory test that uses treponemal antigens based methods to confirm a positive
screening test result. Nontreponemal test are useful also to monitoring treatment response. 

The first serologic test for syphilis was the Wassermann test developed in 1906. It was a complement fixation test and the antigen used was an extract of liver from newborn who had died of congenital syphilis. Landsteiner demonstrated that other tissues, such as beef heart extracted in alcohol, could be used equally well as antigens. Cholesterol and lecithin were added to increase the sensitivity of antigens. In 1922, Kahn introduced a flocculation test without complement that could be read macroscopically in a few hours. In 1941, Pang bornisolated from the beef heart the active antigenic component cardiolipin. The pure phospholipid cardiolipin combined with lecithin and cholesterol could be standardized chemically and serologically, ensuring greater reproducibility of test results both within and between laboratories. In 1946, Harris, Rosenberg and Riedel developed the Venereal Disease Research Laboratory (VDRL) and the Rapid Plasma regain (RPR) was developed in 1957; both are still in use currently. The addition of choline chloride and EDTA to the VDRL antigen enhanced the reactivity of the test and stabilized the antigen suspension (Larsen et al; 1995).

The T. pallidum was identified in 1905, and the first test identifying treponemal antibodies was developed in 1949 by Nelson and Meyer. The T. pallidum immobilization test (TPI) uses T.pallidum (Nichols strain) grown in rabbit testes as the antigen and is based on the ability of patient’s antibody and complement to immobilize living treponemes, as observed by darkfield microscopy. The fluorescent treponemal antibody (FTA) test was developed in 1957, which was later improved by the absorption procedure (FTA-ABS) in 1964 (Larsen et al; 1995).

2.5.4.1. Non-treponemal tests:

The most common nontreponemal screening tests include the Veneral Disease Research Laboratory (VDRL) and the Rapid Plasma Reagin (RPR) which detect IgM and IgG antibodies against cardiolipin that is present in the sera of patients with syphilis. The VDRL test is a slide microfloculation test. The antigen, which is an alcohol solution containing 0.03% cardiolipin, 0.21% lecithin, and 0.9% cholesterol, is suspended in a
buffered saline solution. When combined with antibodies, it forms flocculates that are visible using microscope’s low magnification (Larsen et al; 1995).

The RPR is a modification of VDRL test, the antigen for RPR contain choline chloride (to eliminate the inactivation of tested serum), ethylenediaminotetraacetic acid – EDTA (to enhance the stability of the suspension), and charcoal particle for visualization of the suspension. This macroscopic flocculation test is done on plastic cards having multiple 18-mm circles onto which serum and modified VDRL antigen are placed and gently rotated. In the presence of antibodies a flocculation reaction takes place, and the charcoal particles are entrapped in the antigen-antibody aggregates, causing visible agglutination.

The mean sensitivities of the VDRL during primary syphilis, secondary, latent and late latent are 78%, 100%, 95% and 71%, respectively; while sensitivities of RPR are 86%, 100%, 98% and 73%. The mean specificities of both tests are 98% (Larsen et al; 1995).

These tests are widely available, relatively inexpensive and important for monitoring treatment. Only VDRL is the test of choice for examination of cerebrospinal fluid (CSF) unsuspected neurosyphilis.

Limitation of the nontreponemal serologic tests include: lack of sensitivity in early primary and late latent syphilis, the possibility of prozone reaction or false positive results (Larsen et al; 1995).

A prozone reaction occurs when antibody is in excess and it is occasionally demonstrated in the nontreponemal serologic tests. Prozone reactions occur in 1 to 2% of patients with secondary syphilis (Jurado et al; 1993).

False positive reactions are associated with increased age, pregnancy, drug addition, malignancy, and auto-imune diseases, such as lupus erythematosus or rheumatoid arthritis, as well as with viral (hepatitis, infectious mononucleosis, viral pneumonia, measles and others), protozoal (malaria) or mycoplasma infection (Hook and Marra, 1992; Larsen et al; 1995).

Nontreponemal test results must be interpreted according to the stage of syphilis disease. Also, the interpretation of these results depends on the population being tested. The
predictive value of the nontreponemal tests is increased when combined with reactive treponemal tests (Larsen et al; 1995).

2.5.4.2. Treponemal tests:
Treponemal tests which are based in antigens derived from *T. pallidum*, allow detection of specific anti-treponemal antibodies. These tests have higher sensitivity and specificity than nontreponemal and were used as confirmatory tests for syphilis after a reactive nontreponemal on screening. Treponemal tests include the fluorescent treponemal antibody-absorbed test (FTA-ABS), *Treponema pallidum* hemaglutination assay (TPHA), *Treponema pallidum* particle agglutination (TPPA) and enzyme immunoassay (EIA).

Treponemal tests may remain reactive for years with or without treatment. Therefore, these tests should not be used to evaluate response to therapy, relapse or re-infection in previously treated patients. Also, it do not differentiate venereal syphilis from endemic syphilis (yaw and pinta). However, one treponemal IgM test, the Captia Syphilis-M EIA showed high sensitivity in primary syphilis (Lefevre et al; 1990) and also useful in monitoring treatment response of early syphilis (McMillan and Young, 2008).

2.6. Rapid tests:
Development of rapid treponemal-based syphilis tests was driven by the need for simple, point-of-care tests in resource-poor countries. Although no rapid syphilis test has received FDA clearance, 120 are commercially available worldwide using serum, plasma, or whole blood specimens (WHO, 2003). Most rapid tests detect IgM, IgG, and IgA antibodies and involve immunochromatographic strips (ICSs) in which 1 or multiple *T. pallidum* recombinant antigens are applied to nitrocellulose strips as capture reagents (Zarakolu et al; 2002). Syphilis Fast (Disease Diagnostics) is a latex agglutination test in which the recombinant antigens are bound to latex particles (Young et al; 1998). Overall, rapid tests are highly sensitive and specific. The World Health Organization compared the performance of 8m rapid syphilis tests to a combined reference standard of TPHA/TPPA, reporting sensitivities of 84.5%–97.7% and specificities of 92.8%–98% (Herring et al; 2006). Comparison of rapid tests among US sexually transmitted disease clinic patients demonstrated that finger stick specimens were at least as good as venous samples
for detection (Siedner et al; 2004). The advantages of rapid syphilis tests include their costs at $1–$3 per kit and availability of results within 5–20 minutes (WHO, 2003). Rapid tests require minimal equipment and training, which is ideal for nonclinical settings. However, they cannot distinguish between active and treated syphilis, and false-positive reactions can occur. Positive results need confirmation with quantitative nontreponemal testing to determine recent infection and response to therapy. (Van Dommelen et al; 2008)

2.7. ELISA:

Veldkamp and Visser recognized the potential for an automated T. pallidum enzyme-linked immune Sorbent assay in the 1970s (Veldkamp and Visser, 1975). Since then, several EIA using either native or recombinant T. pallidum antigens have been developed and numerous are commercially available. EIA reported in the literature have used different approach to determine sensitivities and specificities. Some studies had used a panel of anti-treponemal positive specimens from patients whose disease stage and treatment status was known and a negative serum from health blood donors. Other studies had evaluated the performance of new test by comparing with results of conventional laboratorial tests used for diagnosis of syphilis. In general, EIA presents higher sensitivity and specificity.

Captia Syphilis G (Trinity Biotech, former Centocor) is an indirect test for detection of treponemal antibodies. This test uses microtitration plates or strips coated with sonicated T. pallidum antigen. The reacting human IgG treponemal antibodies are detected by antihuman IgG monoclonal antibodies labeled with biotin and horse radish peroxidase (HRP) labeled streptavidin and revealed by tetramethylbenzidine (TMB) substrate. The sensitivity has ranged from 92.4% to 100% and specificity from 98.2% to 99.3%. (Halling et al; 1999, Silletti, 1995, Young et al., 1989, Young et al., 1998). The newer Captia select Syph-G EIA (Trinity Biotech) using anti-human IgG monoclonal antibodies labeled with HRP as a conjugate instead of biotin-streptavidin system had sensitivity and specificity of 99.0% and 98.0%, respectively (Woznicova and Valisova, 2007).

Enzygnost Syphilis (Dade Behring) is a one-step competitive EIA with T. pallidum Nichols strain detergent extract antigen. T. pallidum specific antibodies, IgG and/or IgM
contained in the sample and the conjugate (HRP labeled anti-Tp antibodies) compete for the binding sites of _T.pallidum_ antigen coated onto wells of microtitration plates. The reaction is revealed with substrate TMB. The intensity of the resultant color is inversely proportional to the concentration of anti-treponemal antibodies in the sample. Enzygnost Syphilis showed sensitivities varying from 98.2% to 100% and specificities from 96.8% to 100% (Cole _et al._, 2007; Gutierrez _et al._, 2000; Maidment _et al._, 1998; Marangoniet _et al._, 2009; Viriyataveekul _et al._, 2006).

**Bioelisa Syphilis** (Biokit) is a competitive assay using _T.pallidum_ whole antigen to coat the well of a plate. The treponemal antibodies in the test serum compete with HRP labeled human anti-treponemal antibodies. In this assay, the binding of the conjugate to the specific antigen, determined by measuring the intensity of substrate (TMB) color, is inversely proportional to the amount of specific antibodies in the test sample. Compared with FTAABS and TPHA, this assay had sensitivity of 99.5% and specificity of 99.4% (Ebel _et al._; 1998). Another version of this assay, Bioelisa Syphilis 3.0 (Biokit) is a two step recombinant EIA using recombinant antigen (TpN15 and TpN17) to coat the solid phase and HRP conjugated recombinant antigen for detection of anti-treponemal IgG and IgM. This assay showed sensitivity of 97.4%, and specificity of 100%. However, lower detection rate was observed in samples from patients with untreated primary syphilis (Cole _et al._; 2007).

**ICE Syphilis** (Murex) is a two-step recombinant sandwich EIA using three _T. pallidum_ recombinant antigen (TpN15, TpN17 and TpN47) coated onto the wells of microtiter platestrips; the wells are also coated with anti-human immunoglobulin G (IgG) and M (IgM). If the antibodies to _T.pallidum_ are present in the specimens (serum or plasma) they are captured by the antigen on the plate. In addition, a proportion of a total IgG and IgM of tested specimens are captured by the anti-human antibodies. The anti-treponemal components of the captured antibodies is detected by recombinant antigens (TpN15, TpN17 and TpN47) labeled with HRP. The intensity of the enzyme substrate TMB color is proportional to the concentration of antibodies reacting with recombinant _T.pallidum_ antigens. The range of sensitivity and specificity for the ICE Syphilis assay were 98.2%
to 100% and 99.2% to 100%, respectively (Cole et al., 2007; Lam et al., 2010; Viriyataveekul et al., 2006; Young et al., 1998). Other studies for evaluation of ICE Syphilis (Murex) as a screening test for syphilis, the sensitivity in primary syphilis were 84% (48/50) (Manavi and McMillan, 2007) and 77.2% (61/79). (Young et al., 2009).

Trep-Chek (Phoenix) is an indirect test for detection of anti-treponemal antibodies. The microplates wells is coated with specific recombinant treponemal antigens. Anti-treponemal antibodies present in the serum samples binds to the immobilized antigens. Anti-human IgG antibodies labeled with HRP and substrate TMB are used to detect specific antitreponemal antibodies present in the patient´s samples. When compared with results of conventional serological tests, the sensitivity and specificity for Trep-chehk was 85.3% and 95.6%, respectively (Tsang et al., 2007); other study found sensitivity of 98.9% and specificity of 95.6% in comparison with results of FTA-ABS. (Binnicker et al., 2011).

Trep-Sure(Phoenix) is a two-step recombinant sandwich EIA for detection of antitreponemal antibodies IgG and IgM. This assay uses specific recombinant treponemal antigens immobilized on the microplate wells. Anti-treponemal antibodies from patient´s samples bind to the immobilized antigen, which is detected with HRP conjugated treponemal antigens and substrate TMB. Trep-Sure had sensitivity and specificity of 98.9% and 94.3%, respectively (Binnicker et al., 2011).

Captia Syphilis M (Trinity, former Centocor) is a capture ELISA using a microtitration plates coated with anti-human μ chain specific antibodies, which bind IgM present in serum. A tracer complex is used to detect anti-treponemal specific IgM antibody captured on the plate. The tracer complex consisted of T. pallidum antigens, a biotinylated antiaxial filament monoclonal IgM antibody, and streptavidin conjugated with horseradish peroxidase. The enzyme substrate TMB yields a colored product and the intensity of the color is proporcional to the concentration of antibodies (Lefevre et al., 1990); newer version of this assay employ a HRP conjugated recombinant antigen instead of the tracer complex (Rotty et al., 2010). This EIA was specifically designed for diagnosis of congenital syphilis, but may be applied for detection of primary infection.
The sensitivity was 94% for primary syphilis, 85% for secondary, and 82% for early latent syphilis (Lefevre et al., 1990). This IgM capture EIA is also useful for monitoring treatment response in early syphilis. (McMillan and Young, 2008; Rotty et al., 2010).

Schmidt et al performed a comparative evaluation of different EIAs for determination of antibodies against T. pallidum in patients with primary syphilis by testing 52 sera, all negative in TPHA. The sensitivity for Captia Syphilis M was 86.5% (45/52) and other assays for detection of IgG and IgM, such as ICE Syphilis (Murex), Enzygnost Syphilis (Berhing) and Bioelisa Syphilis (Biokit) showed sensitivities of 75.0% (39/52), 69.2% (36/52) and 67.3 (24/36), respectively (Schmidt et al., 2000).

2.8. Syphilis in pregnancy:

Syphilis remains a major cause of reproductive morbidity and poor pregnancy outcomes in developing countries. Syphilis in pregnant woman can result in adverse outcomes of pregnancy in up to 80% of cases, such as still birth and spontaneous abortion (40%), perinatal death (20%), and serious neonatal infections and low –birth weight babies (20%) (WHO, 2005). Syphilis has also acquired anew potential for morbidity and mortality through association with increased risk for HIV infection. A round 340 million cases of curable new STI occur every year.

Of these, syphilis accounts for an estimated 12 million cases, 2 million of them among pregnant women (Olokoba et al., 2008).

2.8.1. Complication of syphilis in pregnant women:

Syphilis can seriously complicate pregnancy and result in spontaneous abortion, stillbirth, nonimmune hydrops, intrauterine growth restriction, and perinatal death, as well as serious sequelae in liveborn infected children. While appropriate treatment of pregnant women often prevents such complications, the major deterrent has been inability to identify the infected women and get them to undergo treatment (Genc and Ledger, 2009).

2.8.2. Risk factors of syphilis in pregnant women:

Women at higher risk for syphilis infection, such as pregnant women who already have another STD, are single, or are using drug during the pregnancy, should have a repeat syphilis serology in the third trimester. Women with non-immune hydrops or stillbirth
should also undergo syphilis testing. Adults with sexually transmitted syphilis or other genital ulcers have an estimated two- to fivefold increased risk of contracting HIV. A syphilis sore can bleed easily, providing an easy way for HIV to enter blood stream during sexual activity (Hook, 1992).

2.9. Congenital syphilis:
Syphilitic pregnant women are likely to infect their unborn children with congenital syphilis who then have an increased likelihood of stillbirth or becoming victim to major birth defects such enlarged liver and spleen, rash, fever, extreme blistering, rhinorrhea and edema of the face (Woods, 2009). Hematogenous passage through the placenta can lead to widespread fetal lesions. The spectrum of congenital syphilis ranges from spontaneous abortion and stillbirth through prematurity to sever neonatal disease and death. Neonates become infected by blood-borne organisms, and all neonatal disease manifestation is more common during the early stage of maternal disease, in which bacteremia is more common than in latent maternal infection. Dark field examination of the amniotic fluid for spirochetes also may be helpful in the evaluation of stillbirth when syphilis is suspected (Walter, 1982).

2.10. Treatment:
Penicillin is effective in treatment of all stages of syphilis. A single injection of benzathine penicillin G (2.4 million units) can eradicate T. pallidum and cure early syphilis. If the patient is allergic to penicillin, tetracycline or erythromycin can be used but must give for prolonged period to effect a cure. In neurosyphilis, high dose of aqueous penicillin G are administrated. No resistance to penicillin has been observed (Warren, 2002).

2.10.1 Jarisch-Herxheimer reaction
One of the potential side effects of treatment is the Jarisch-Herxheimer reaction. It frequently starts within one hour and lasts for 24 hours, with symptoms of fever, muscles
pains, headache, and tachycardia (Kent and Eomanelli, 2008). It is caused by cytokines released by the immune system in response to lipoproteins released from rupturing syphilis bacteria (Radolf and Lukehart, 2006)

CHAPTER THREE

3. Material and Methods

3.1. Study design:
The study design was a cross-sectional, analytical study.

3.2. Study Population:

The study population included the pregnant women, attending Jebel Awlia Teaching Hospital.

3.3. Study area:

The study was conducted in Jebel Awlia Teaching Hospital.

3.4. Sample size:

A total 100 serum samples were collected from Jebel Awlia Teaching Hospital.

3.5. Study duration:

The study was conducted during the period from May to July 2014.

3.6. Inclusion criteria:

Pregnant women

3.7. Exclusion criteria:

Nonpregnant women.

3.8. Data collection

After explaining the purpose of the study, data were collected from each subject by interviewing questionnaire with informed consent. The data include the demographic information (age and residence), history of previous abortion and genital ulcer.
3.9. Methods:

Hundreded serum samples were collected from pregnancy women has different age range from 18-41 year in the department of gynecology and obstetrics of Jebel Awlia Teaching Hospital to detect seroprevelence of syphilis among pregnant women. All specimens were tested by Rapid Plasma Reagin test (RPR) and then confirmed by enzyme linked immune sorbent assay (ELISA)

3.10. Ethical consideration:

A apparently healthy pregnant woman, attending the antenatal clinics in Jebel Awlia Teaching Hospital. A total of 100 pregnant women were recruited from this hospital, irrespective of age, ethnic group and gestational period. Before questionnaires were administered to any eligible women, the latter was provided with a consent form to sign or thumbprint after the study was explained to them in detail.

3.11. Specimen collection and preparation

Using sterile disposable syringes, about 3ml of blood were withdrawn from the anticubital vein under aseptic conditions. The site of collection was disinfected using alcohol.

The blood samples were collected in sterileplain containers without any additives, and left to clot at room temperature. Each blood sample was then centrifuged at 1500 rpm for 5 minutes, and each serum was separated in another sterile plain container .Samples were labeled by giving laboratory numbers. Serum samples were collected in another plain sterile container with corresponding laboratory number,and then the samples were transported in an ice bag, from the site of collection to the lab, where they stored, foranalysis. The samples kept frozen at -20ºc without addition of preservatives, until the time of analysis (no more than 3 months).

3.12. Laboratory examination:
All the specimens were tested for syphilis; Rapid Plasma Reagin Test (RPR) and enzyme immune sorbent assay (ELISA).

### 3.12.1. Rapid Plasma Reagin test (RPR):

The Rapid plasma Reagin (RPR) test is a macroscopic, nontreponemal flocculation card test used to screen for syphilis.

The kits used were manufactured by spin react (Spain).

**Rapid:** Its rapid test that can be done within few minutes. **Plasma:** The sample is plasma (or serum). **Reagin:** The term “reagin” means that this test does not look for antibodies against the actual bacterium, but rather for antibodies against substances released by cells when they are damaged by *Treponema pallidum*.

#### 3.12.1.1 Principle of the method:

The RPR-carbon is non-treponemal slide agglutination test for the qualitative and semi-quantitative detection of plasma regains in human serum.

Carbon particles coated with a lipid complex are agglutinated when mixed with samples containing regains of the patients affected by syphilis.

#### 3.12.1.2 Reagents:

**Antigen used in RPR Test:**

RPR Antigen contains cardiolipin, lecithin, cholesterol, 10% choline chloride, EDTA, charcoal etc in Buffer.
The antigen is prepared from a modified Venereal Disease Research Laboratory (VDRL) antigen suspension containing choline chloride to eliminate the need to heat inactivate serum, ethylenediaminetetraacetic acid (EDTA) to enhance the stability of the suspension, and finely divided charcoal particles as a visualizing agent.

Positive Control: Prepared from human serum samples containing antibodies to *Treponema pallidum* (regain). Serum is ready to use (artificial). Bring to room temp before use. (titr more or equal ¼).

Control negative: Animal serum preservative.

**Basic Test Procedure:**

The Rapid plasma Reagin (RPR) antigen is mixed with unheated or heated (to inactivate complement) serum or with unheated plasma on a plastic-coated card.

If antibodies are present, they combine with the lipid particles of the antigen, causing them to agglutinate. The charcoal particles coagglutinate with the antibodies and show up as black clumps against the white card (macroscopically visible flocculation-type precipitation if the patient’s sera contain reagin).

**Preparation for the RPR assay:**

Rapid plasma Reagin Antigen Suspension, Positive Control, Negative control and samples were left at room temperature (20–30°C) before use to worm.

The reagents were gently mixed before used.
3.12.1.3. Qualitative RPR Test Procedure:

1. The Rapid plasma Reagin card was labeled according to sample number, being careful not to interfere with the test areas of the card.
2. Disposable serum dispensers or droppers was used, one free-falling drop (0.05 ml) of serum sample was dispensing onto a circle on the test card.
3. One free-falling drop of positive control and negative control were also dispended in situ, new dispenser for each sample were used.
4. The sample was smoothly across the circle area using the paddle side of the dispenser as shown by instructor, the same for positive and negative control.
5. After mixing the antigen solution by swirling, one drop of the antigen suspension was added to each sample / control testing area.
6. The card was placed on an automatic rotator and then covered to maintain humidity, then rotated at 100 ± 5 rpm for 8 minutes (7 minutes 50 seconds to 8 minutes 30 seconds). Following rotation, a brief hand rotation and tilting of the card (3–4 times) was performed to aid in differentiating nonreactive from minimally reactive results.
7. Results were immediately read macroscopically in the “wet” state under a high intensity light source.

3.12.1.4 Interpretation of RPR Test

1. Non-reactive (NR) – smooth suspension, no clumping or slight roughness
2. Reactive (R) – any degree of clumping

3.12.2. Enzyme linked immunosorbent assay ELISA:
3.12.2.1. Determination of ELISA:

The reagent was supplied by Biorex, united kingdom, ELISA gen, fourth generation ELISA for in vitro determination of specific antibodies to syphilis in human plasma and serum.

Fortress Syphilis ELISA is an in vitro diagnostic kit for the detection of antibodies to *T.pallidum* in human serum or plasma.

3.12.2.2. Principle of the Assay:

The detection of anti-TP antibodies is achieved by antigen sandwich enzyme linked immune sorbent assay, where the micro wells are coated with recombinant *Treponema pallidum* antigens expressed in *E.coli*. The sample is incubated in the micro wells together with recombinant TP antigens conjugated to HRP. The pre-coated antigens express the same epitopes as hosts. In case of presence of anti-TP in the sample, during incubation the pre-coated and conjugated antigens will be bound to the two variable domains of the antibody and the specific antigen-antibody immune complex is captured on the solid phase. After washing to remove sample and unbound conjugates, chromogen solution containing TMB and urea peroxidase are added into the wells. In presence of the antigen-antibody sandwich complex, the colorless chromogen hydrolyzed by the bound HRP conjugate to a blue colored product, which turn yellow upon addition of the stop solution. This color is then read photometrically and is directly proportional to the amount of the antibody in the sample. Wells containing samples negative for anti-TP remain colorless.

3.12.2.3. Procedure:

3.12.2.3.1. Control:

3x negative control and 2x positive controls included with each batch of samples to be tested. and the controls were treated as though they are patient samples.
3.12.2.3.2. Conjugate and samples incubation:

Twenty microliter of negative control (x3) and positive control (x2) and sample were added into their respective wells.

Fifty microliter of conjugate HRP was added to each well. Then incubated (covered) at 37°C for 60 minutes.

3.12.2.3.3. Wash

The plate was washed 6 times with diluted washing buffer, soaked for about 30 seconds between each cycle. Excess liquid was tapped out.

3.12.2.3.4. Substrate incubation:

Fifty microliter of the TMB chromogen solution was added to each well then mixed by tapping the plate gently and incubated at 37 for 15 minutes avoiding light. The in the presence of positive sample, the color turned to blue in positive control and anti-TP positive sample wells, due to enzymatic reaction between the chromogen solution and the HRP-conjugate.

3.12.2.3.5 Stop color development:

Fifty microliter stop solution (H₂SO₄-0.5M) were added to each well, mixed gently blue color changed to yellow in positive control and anti-TP positive sample wells.

3.12.2.3.6. Reading:

The plate bottom was carefully wiped and OD was read at 450 nm using a plate reader within 5 minutes after stopping the reaction.

3.12.2.4. Calculation and Interpretation of results:
3.12.2.4.1. Assay validation:

The OD value of the positive control must be equal to or greater than 0.0800 at 450nm.

The OD value of the negative control must be less than 0.100 at 450nm.

Calculation of cut-off value:

Cut-off value (C.O.) = MNC+0.18.

The mean absorbance value for three negative controls+0.18

3.12.2.4.3. Interpretation:

(S= the individual absorbance (OD) of each specimens).

Negative Results (S/C.O.<1): samples giving an absorbance less than the Cut-off value are considered negative, which indicate that no anti-TP antibodies have been detected with this anti-TP ELISA kit and there are no serological indications for past infection with TP.

Positive Results (S/C.O.>/=1): samples giving an absorbance greater than or equal to the cut-off value are considered initially reactive, which indicates that anti-TP antibodies have been detected with this anti-TP ELISA kit.

Borderline (S/C.O=0.9-1.1): Samples with absorbance to cut-off ratio between 0.9 and 1.10 are considered borderline samples and retesting is recommended.

CHAPTER FOUR

4. Results

4. RESULTS:
Three hundred serum blood samples were collected from pregnant women have different age in the department of gynecology and obstetrics of Jebel Awlia Teaching hospital to detect seroprevalence of syphilis among pregnant women. All specimens were tested by two different methods first by Rapid Plasma Reagin test (RPR) and then confirmed by Enzyme Linked Immune Sorbent Assay (ELISA). The result 6% of positive cases with syphilis was detected by ELISA where 5% was detected by RPR.

Table 1 showed that the highest frequency of population range between 18-35 years only one patient with age >35 years old.

**Table 1: Distribution of the population according to the age group (n=100)**

<table>
<thead>
<tr>
<th>Age</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-23</td>
<td>33</td>
<td>33%</td>
</tr>
<tr>
<td>24-29</td>
<td>36</td>
<td>36%</td>
</tr>
<tr>
<td>30-35</td>
<td>30</td>
<td>30%</td>
</tr>
<tr>
<td>36-41</td>
<td>1</td>
<td>1%</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 2 showed that syphilis cases were reported in pregnant women with history of previous abortion when tested by ELISA.

**Table 2: Detection of anti-*Treponema pallidum* antibodies in pregnant women with history of abortion.**

<table>
<thead>
<tr>
<th>Abortion</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>4</td>
<td>36</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>No</td>
<td>2</td>
<td>58</td>
<td>60</td>
<td>3.3</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>94</td>
<td>100</td>
<td>6</td>
</tr>
</tbody>
</table>
Table 3 showed that only 5 out of hundred samples were positive for syphilis when tested by RPR.

**Table 3:** Detection of anti-*Treponema pallidum* antibodies using RPR.

<table>
<thead>
<tr>
<th>RPR</th>
<th>Frequency</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Negative</td>
<td>95</td>
<td>95</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 4 showed that 6 out of hundred samples tested were positive for the disease when examined by ELISA.

**Table 4:** Detection of anti-*Treponema pallidum* antibodies using ELISA.

<table>
<thead>
<tr>
<th>ELISA</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Negative</td>
<td>94</td>
<td>94</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 5: The highest numbers (3) of positive cases were found in the age 24-29 years. No positive cases were found at age >35 year.

**Table 5:** Positive syphilis cases detected by ELISA in different age group (n=100)

<table>
<thead>
<tr>
<th>Age</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-23</td>
<td>1.0</td>
<td>32.0</td>
<td>33.0</td>
<td>33</td>
</tr>
<tr>
<td>24-29</td>
<td>3.0</td>
<td>33.0</td>
<td>36.0</td>
<td>36</td>
</tr>
<tr>
<td>30-35</td>
<td>2.0</td>
<td>28.0</td>
<td>30.0</td>
<td>30</td>
</tr>
</tbody>
</table>
Table 6: No positive cases were found in the age 18-23 and 36-41. All the positive cases were in the age 24-35 years.

Table 6: Positive syphilis cases detected by RPR in different age group (n=100)

<table>
<thead>
<tr>
<th>Age</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-23</td>
<td>0</td>
<td>33.0</td>
<td>33.0</td>
<td>33</td>
</tr>
<tr>
<td>24-29</td>
<td>3.0</td>
<td>33.0</td>
<td>36.0</td>
<td>36</td>
</tr>
<tr>
<td>30-35</td>
<td>2.0</td>
<td>28.0</td>
<td>30.0</td>
<td>30</td>
</tr>
<tr>
<td>36-41</td>
<td>0</td>
<td>1.0</td>
<td>1.0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>5.0</td>
<td>95.0</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

CHAPTER FIVE

5.1 Discussion

The aim of this study was done to detect seroprevalence of syphilis among pregnant women in Jebel Awlia Teaching Hospital. Hundred serum samples were collected from pregnant women in different trimesters. And different age in department of gynecology
and obstetrics at Jebel Awlia Teaching Hospital. All specimens were tested using two different methods first by (RPR) and then confirmed by Enzyme linked immunosorbent Assay (ELISA).

The seroprevalence of syphilis obtained in this study among pregnant women in Jebel Awliaa Teaching Hospital was (6%) this results relatively, high in comparison to the previous reports by the WHO (1.6%) (WHO, 1999) however, this prevalence was considered nearly similar to the result reported by Kadiga who reported seroprevalence of syphilis among pregnant women in Khartoum Teaching Hospital (7%) (Kadiga, 2012).

Percentage of syphilis with in age group 24-29 years (3%) and 30-35 years (2%) respectively was higher more than those in the other age. These findings was similar to result reported by, (Abdelbagi et al; 2008).

By employing the two serological techniques in this study, (5%) was positive by (RPR) test, (6%) was positive when confirmed by enzyme linked immunosorbent Assay, (1%) negative by (RPR) test and positive when confirmed by ELISA Showed no significant difference between the two technique.

The disease was shown to be risk factor for habitual abortion (table5). These finding is agreement with previous study (Abdelbagi et al; 2008).

5.2. Conclusion

The results of this study conclude that:

- The seroprevalence of syphilis obtained in this study among pregnant women in Jebel Awlia Teaching Hospital was (6%)
The disease was shown to be an important risk factor for habitual abortion.

There is a relationship between the age of the patient and susceptibility to the disease.

Strongly revealed the importance of performing screening serological tests for syphilis during pregnancy for better protection, for prevention of possible congenital transmission and for avoiding probable habitual abortion.

For diagnosis of syphilis all results must be confirmed by more specific and sensitive methods.

ELISA is better than RPR in diagnosis of syphilis.

5.3. Recommendations

1. All pregnant women should be screened to detect syphilis during pregnancy.

2. All finding cases should be treated to prevent further complication in mother and transmission of these diseases to new born infants.

3. Health education in rural area is necessary for women in all ages.

4. Provision of many centers for voluntary screening for sexual transmitted disease to detect sexually transmitted diseases before marriage for both male and female.

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56. Schmitz JL, Gertis KS, Mauney c, stamm LV, folds JD. (1994) laboratory diagnosis of congenital syphilis by Immune globulin M (IgM) and IgA immunoblotting clin diagan lab Immunol ;32-37


College Of medical Laboratory science

Questionnaire about syphilis in pregnancy

Date:......................................................................................

Sample number:............................................................................

Name:..............................................................................................

Age:...............................................................................................

Did you have history of previous abortion?

A) Yes          B) No

Appendix(1)

1.1 Equipment’s:
Centrifuge hettich –Germany
Automatic micropipette axiom-Germany
Alarm clock quartz-Japan
Micro well reader (450nm+-2nm) Stat-fax-USA
Micro well washer system Stat-fax-USA
Automatic pipette (multi channel) axiom-Germany
Dry incubator(37c+/−2c) torr picenardi-Italy

Appendix(2)

2.1. Reagents:
Deionized or distilled water
Alcohol
ELISA kit
Concentrated washing solution
Negative control (human) yellow
Positive control(human ) red
Conjugate ready-to- use blue rAg conjugated to horseradish peroxidase
Substrate pink
Stop solution H2SO4-0.5M

Appendix(3)

3.1 Other materials:
Cotton    Medical-sanitary
Disposable pipette tips    china
Vacuum tubes    china
Syringes    tuttligen -Germany
Alcohol swap    Saudi sashes - services KSA -Saudi
Plane containers    afma-Jordon
Microplates:12 strip of 8 wells coated with *T.pallidum rAg*
Automatic microtiter plate washer or squirt bottle
Bag for storing unused wells
Test tubes and racks
RPR test cards and reagents.( Spin)