

Chapter one

1-Introduction

1.1. Introduction

Urinary tract infections (UTIs) are one of the most common medical complications of pregnancy (Mittal & Wing, 2005). It is estimated that one in three women of childbearing age will have a UTI (Duarte *et al.*, 2008). Because of the normal physiologic changes induced by gestation, pregnant women are especially susceptible to these infections.

UTIs are characterized by the presence of infectious agents in the genito-urinary tract that cannot be explained by contamination. These agents have the potential to invade the tissues of the urinary tract and adjacent structures. The microbiological profile is well known and pathogens such as *Escherichia coli* have been present in the vast majority of cases (Sheffield & Cunningham, 2005). The infection may be limited to the growth of bacteria in the urine (which frequently don't produce symptoms) or can result in several syndromes associated with an inflammatory response to the bacterial invasion. Actually, the term UTI represent a wide variety of conditions, including asymptomatic forms of UTIs, urethritis, cystitis, acute pyelonephritis and pyelonephritis with bacteremia or sepsis (Joseph *et al.*, 2011).

Syphilis is a sexually transmitted infection (STI) caused by the *Treponemapalladium* spirochete. The route of transmission from mother to child in uterus. If not treated, syphilis can cause serious effects such as syphilis damage to the aorta, brain, eyes, and bones. In some cases these effects may be fatal (Olokoba *et al.*, 2009).

Sexually transmitted infections (STIs) are widespread in the developing countries and constitute a major public health problem in sub-Saharan Africa.

More recently, there has been a resurgence of syphilis (Olokobaetal, 2008). Testing for syphilis in pregnancy and labor is medically indicated because of the potential risk for congenital infection and fetal loss (Ratnamet al, 1982). Syphilis has also acquired a new potential for morbidity and mortality through association with increased risk for HIV infection (Olokobaetal, 2009).

Pregnant women should have serologic test for syphilis at the time of the first prenatal visit.

In women suspected of being at increased risk for syphilis or for populations in which there is a high prevalence of syphilis, additional tests should be performed during the third trimester at twenty eight weeks and again at delivery. Seropositive women should be considered infected and should be treated unless prior treatment with fall in antibody titer is medically documented (WHO, 2008).

Syphilis is believed to have infected 12 million people in1999, with greater than 90 in developingworld. It affects 16 million and 700,000 pregnant women a year, resulting in spontaneous abortion, stillbirth, and congenital syphilis (Tagnyetal, 2010).

1.2. Rationale

Syphilis is a serious bacterial infection caused by *Treponemapallidum* . The disease transmitted from person to person by direct contact with syphilis sore during vagina, and oral sexual contact. Pregnant women with disease can pass it to their unborn children. It can also be transmitted through blood transfusion and this will make it increasingly difficult to get safe blood because of this blood borne infection. Syphilis has adverse complication , it can cause rash , mild fever , fatigue , headache ,sore throat, patchy hair loss and swollen gland, through the body and most likely will affect the heart ,eye , brain , nervous system , bones and joint . It can cause mental illness, blindness, neurologic

problem, heart disease, and this greatly increase risk of miscarriage, stillbirth or newborn death within few days after birth, due to that the syphilis infection is problem in Sudan and worldwide. This study expected to highlight the problem in order to reduce the risk of infection.

1. 3.Objectives

1.3.1. General objective

To determine the frequency of syphilis among pregnant women in a Saudi hospital.

1.3.2. Specific objectives

1.3.2.1. To detect syphilis antibodies among pregnant women in a Saudi Hospital using Immune Chromatography Test (ICT) and Enzyme Linked Immune Sorbent Assay (ELISA) Technique.

1.3.2.2. To relate the prevalence of syphilis according to age group, miscarriage and number of miscarriages and trimesters.

1.3.2.3. To detect bacterial association with syphilis in UTI.

Chapter two

2. Literature review

2.1. History of syphilis

The exact origin of syphilis is unknown. Of two primary hypotheses were proposed, one proposed syphilis was carried to Europe by the returning crewmen from Christopher Columbus voyage to America, the other proposed syphilis existed in Europe previously, but went unrecognized. These are referred to as the “Columbian” and “pre-Columbian” hypotheses, respectively. The Columbian hypothesis is the best supported by the available evidence. The first written record of an outbreak of syphilis in Europe occurred in 1494/1495 in Naples, Italy, during a French invasion. Due to it being spread by returning troops, it was initially known as “French disease”, as it is traditionally referred. In his Latin poem in dactylic hexameter describing the ravages of the disease in Italy. It was also known historically as the “great pox” (Romanelli, 2008).

The causative organism *Treponemapalladium* was first identified by Fritz Schaudinn and Hoffmann in 1905. The first effective treatment (Salvarsan) was developed in 1910 by Paul Ehrlich, which was followed by trials of penicillin and confirmation of its effectiveness in 1943. (Knell, 2004). Before the advent of effective treatment, mercury isolation were commonly used, with treatment often worse than the disease. Many famous historical figures, including Franz Schurt, Arthur Schopenhauer, Edouard Manet and Adolf Hitler are believed to have had the disease (Farhi and Duppin, 2010).

2.2.Treponema palladium

2.2.1. Structure and biology

T.pallidum is Gram –negative bacteria. Treponemes are helically coiled, corkscrew-shaped organisms 6 to 15 µm long and 0.1 to 0.2 µm wide. The organisms stain poorly with aniline dyes. Treponemes in tissues can be visualized by silver impregnation methods. Live treponemes, which are too slender to be seen by conventional light microscopy, can be visualized by using dark-field microscopy (Holmes, 1990). *Treponemapallidum* exhibits characteristic motility that consists of rapid rotation about its longitudinal axis and bending, flexing, and snapping about its full length. *Treponemapallidum* is a fastidious organism that exhibits narrow optimal ranges of pH (7.2 to 7.4), and temperature (30 to 37°C). It is rapidly inactivated by mild heat, cold, desiccation, and most disinfectants. Traditionally this organism has been considered a strict anaerobe, but it is now known to be microaerophilic. Treponemes multiply by binary transverse fission. The in vivo generation time is relatively long (30 hours). Despite intense efforts over the past 75 years, *T.pallidum* has not been successfully cultured in vitro. Viable organisms can be maintained for 18 to 21 days in complex media, while limited replication has been obtained by co-cultivation with tissue culture cells. The other three pathogenic treponemes also have not been successfully grown in vitro. The composition of *T.pallidum* (dry weight) is approximately 70 percent proteins, 20 percent lipids, and 5 percent carbohydrates. This lipid content is relatively high for bacteria. The lipid composition of *T pallidum* is complex, consisting of several phospholipids, including cardiolipin, and a poorly characterized glycolipid which is biochemically and immunologically distinct from

lipopolysaccharide. While antigenic analysis of *T.pallidum* has been hampered by the inability to grow this organism in vitro, this situation has largely been circumvented through the use of modern molecular techniques, including monoclonal antibodies and recombinant DNA (Holmes,1990). During the course of infection, antibodies develop to a number of treponemal proteins, most notably the lipoproteins and flagella..Although treponemes possess both outer and cytoplasmic membrane, they differ considerably in structure from enteric Gram-negative bacteria. The organism has an outer membrane containing an extremely low density of surface-exposed transmembrane proteins (Holmes,1990). Typically, three flagella originate from each end of the bacterium, and, winding about the bacterium within the periplasmic space, overlap at the midpoint. The presence of peptidoglycan in the cell wall, originally surmised on the basis of the bacterium's exquisite sensitivity to penicillin, has been confirmed by biochemical analysis. Unlike Gram-negative bacteria in which the peptidoglycan underlies the outer membrane, in treponemes the murein layer overlies the cytoplasmic membrane. The cytoplasmic membrane covers the protoplasmic cylinder; this membrane contains the majority of the bacterium's integral membrane proteins and is particularly abundant in lipid-modified polypeptides (lipoproteins). (Holmes, 1990).

2.2.2. Genome structure

The complete genome for *T.pallidum* was sequenced in July of 1998. The genome was sequenced using “the whole genome random sequencing method “. The genome consists of a single double strand circular DNA chromosome 1,138,006 base pairs long. It contains approximately 1,090 genes which encode approximately 1,041 proteins. These open reading frames account for 92.9% of the genomic DNA. 55% of genes were assigned defined roles and 17% were

categorized based on similarities to other organisms. The main organism use in comparison was *Borrelia burgdorferi* which is another pathogenic spirochete which cause lyme disease. 28% of gene were considered novel (unique to *T. pallidum*) and placed in separate category. The average size of encoded proteins was estimated to range from 3235 to 172,869 Dalton (Fraser *et al.*, 1998).

2.3. Transmission

2.3.1. Sexual contact

In the direct sexual contact, About 95-98% of syphilis patients are infected in on. Syphilis, genital area outside the patient's skin and mucous membrane lesions over there, lesions of the ulcer surface and exudation of a large number of *Treponema pallidum*. The study found that *Treponema pallidum* on human skin and mucous membranes have affinity can penetrate normal skin and mucous membranes. (Heymann, 2004). Syphilis lesions on the genitals is pointless, without prejudice to sexual intercourse, if the patient continues to have sex with other healthy people, the latter can easily be infected with syphilis (Public Health Agency of Canada, 2006). In addition, sexual intercourse, genitalia, extreme hyperemia, and mutual friction, the number of skin and mucous membranes may cause slight abrasions; more invasion of *Treponema pallidum* has created favorable conditions. Kissing, homosexuality, oral-genital contact, hand-genital contact with a category of sexually transmitted syphilis can also damage can occur in the lips, anus, tongue, throat, fingers and other parts (Eccleston *et al.*, 2008).

2.3.2. Congenital Syphilis

The women suffering from syphilis without treatment, pregnant mother body *Treponema pallidum* into the fetal blood circulation through the body, so that the fetus infected with syphilis (Tsui, 1997).

2.3.3. blood-borne transmission

If the blood donor in patients with latent syphilis, he (she) may be provided by the blood with *Treponemapallidum*. Once the input into the recipient's body can produce infection, so patients do not produce a performance of syphilis, while the direct symptoms of syphilis occur. So, for blood donor screening tests for syphilis serological examination is very important(Gardella *et al.*,2002).

2.3.4.Period of communicability

Syphilis is infectious during primary, secondary and early latent stages and also in mucocutaneous recurrences. Congenital transmission is mostly likely during primary and secondary maternal syphilis, but can occur in the latent period (Public Health Agency of Canada,2006).

2.4 .Risk factors

- sex with multiple sex partners
- drugs addict
- sex workers

2.5. Pathology

2.5.1. Primary syphilis

The primary stage of syphilis typically begins with a sore (called a "chancre") on the area of your body that was initially exposed to the infection—usually the genitals, rectum, or mouth. The sore has been described by others as feeling like a button: firm, round, usually measuring half an inch across, and not tender to the touch (Committee on Infectious Diseases,2006).

2.5.2 .Secondary syphilis

After the primary stage, if left untreated, the infection moves into the secondary stage of syphilis. Secondary syphilis can often occur four to ten weeks after the chancre heals, once the bacteria have spread throughout the body. You may feel sick. Common symptoms include headache, achiness, loss of appetite, and/or a rash. The non-itchy rash that sometimes presents in the secondary stage of syphilis is usually reddish-brown in color. But the appearance of the rash's individual lesions can vary dramatically. They may be flat or raised, they may or may not be scaly, and pustules may or may not be present. It's partially due to the variability of this rash that syphilis became known as "the great imitator." It can often resemble many other conditions. The rash itself can last from a few weeks to several months. Other symptoms of secondary syphilis include sores in the mouth, nose, and throat, and on the genitals or folds of the skin. Lymph node swelling is common, and patchy hair loss can occur. All signs and symptoms of the second stage of syphilis will disappear without treatment between three weeks and nine months, but the infection will still be present in the body (Bhatti, 2007).

2.5.3. Latent syphilis

The latent stage of syphilis, which occurs after the symptoms of secondary syphilis have disappeared, can last anywhere from a few years to up to 50 years! There are no symptoms in this stage and, after about two years, you may cease to be contagious. However, if you are in the latent stage of syphilis, you are still infected, and the disease can still be diagnosed by a blood test (Edward *et al.*, 2003).

2.5.4. Tertiary Syphilis

The final stage of syphilis, which occurs in about one third of those who are not treated, is known as the tertiary stage. Many organs may be affected. Common symptoms include fever; painful, non-healing skin ulcers; bone pain; liver

disease; and anemia. Tertiary syphilis can also affect the nervous system (resulting in the loss of mental functioning) and the aorta (resulting in heart disease) (Romanelli,2008).

2.5.5. Syphilis in pregnancy and congenital syphilis

Syphilis can be transmitted transplacentally to fetus at all stages during course of untreated maternal disease from incubating syphilis to primary, secondary, tertiary and latent disease. Syphilis can also be transmitted during passage through the birth canal when the newborn infant contacts a genital lesion (Tsui, 1997). Breast feeding does not result in transmission of syphilis unless an infection lesion is present on the breast. Pregnancy has no known effect on the clinical course of syphilis. The rate of vertical transmission in untreated women is 70-100% in primary syphilis, 40% for early latent syphilis and 10% for latent disease.

The longer the interval between infection and pregnancy, the more benign is outcome in the infant. All pregnant women should be screened for syphilis and other STIs on their first prenatal visit. High seroconversion rates for syphilis in high risk population during pregnancy have led some experts to suggest repeat screening of women during late pregnancy and delivery (Qolohle, 1995).

Syphilis in pregnancy can cause wide widespread complication for both the mother and fetus. At least two-third of babies born to untreated women with syphilis with syphilis are infected .if evidence of syphilis is present treatment should be initiated immediately according to stage of the disease. Efficiency of syphilis treatment in pregnancy considers resolution of maternal infection and prevention of congenital syphilis (Zanker and Rlofs,1989).

CS can cause: miscarriage (losing the baby during pregnancy), Stillbirth (a baby born dead), or death shortly after birth. Up to 40% of babies born to women with untreated syphilis may be stillborn, or die from the infection as a newborn.

Babies born with Congenital Syphilis CS can have: Deformed bones, Enlarged liver and spleen, jaundice (yellowing of the skin or eyes) nerve problems, like blindness or deafness, meningitis, and Skin rashes. Babies who do not get treatment for CS and develop symptoms later on can die from the infection. They may also be developmentally delayed or have seizures (Tsui, 1997).

2.6. Diagnosis of syphilis

2.6.1. Dark Field Microscope and Direct or Indirect Fluorescent Antibody Test (DFA/IFA)

Definitive diagnosis of syphilis is complicated by the inability to cultivate *T. pallidum* in vitro. Clinical manifestations, demonstration of treponemes in lesion material, and serologic reactions are used for diagnosis. In many cases, clinical manifestations are highly characteristic. If manifestations include one or more cutaneous exudative lesions, motile treponemes can often be visualized within lesion exudate by dark-field microscopy. Specimen must appropriately be collected and quickly examined within 5-20 minutes. Positive test on these material for immunofluorescent (DFA) testing are diagnostic (Fiumara, 1995).

2.6.2. Histochemistry and Immunohistochemistry

The direct fluorescent antibody tissue test detects *T.pallidum* in tissue sections and can be used in combination with a histological staining(Larsenet *al.*,1998). *T.pallidum* antibody-based nonfluorescent stain such as the IHC stain (Dako Corporation, California) can also be use (Norris *et al.*,2003)The latter offers the advantage of hematoxylin counterstain to simultaneously examine tissue structures. Silver staining, while useful, is nonspecific.

2.6.3. Nucleic acid amplification methods:

A number of PCR-based methods have been developed for the detection of *T pallidum* in clinical specimens . Although these methods are not standardized, they have been found to be highly sensitive, able to detect as low as one to 10 organisms per specimen with high specificity. These methods are also the most practical in certain settings (Wicheret *al*,1999). PCR undoubtedly holds promise as a test of choice for congenital syphilis, neurosyphilis and early primary syphilis when traditional tests have limited sensitivity. This method could be used to monitor treatment, and there is also potential to use it to differentiate new infections from old infections (Larsen *et al*,1998). While there are no commercially available PCR-based test kits at the time of writing, this service may be available through select laboratories.

2.6.4. Demonstration of antibodies

2.6.4.1.Nontreponemal Serological Tests

Centers for Disease Control and Prevention (CDC)-approved standard tests include the VDRL slide test, the rapid plasma reagin (RPR) card test, (Larsenet *al.*, 1998).Nontreponemal tests are rapid, simple and inexpensive. They are the only tests recommended to monitor the course of disease during and after treatment. Nontreponemal tests can also serve to detect reinfection. The main limitations of nontreponemal tests are their reduced sensitivity in primary syphilis and late latent syphilis, false-positive results due to crossreactivity, and

the potential for false-negative results due to prozone reactions. Prozone reactions are false-negative reactions that occur due to interference by high concentrations of target antibodies in a specimen. The disproportionate antibody-to-antigen ratio results in a 'rough' nonreactive or a very weakly reactive reaction. Such specimens will give a clearly positive reaction when diluted and retested, a process that brings the antibody-to-antigen ratio within the optimal range (Larsen *et al.*, 1998).

The VDRL test is microflocculation tests and is read under a microscope. A disadvantage of the VDRL test is that the antigen suspension must be prepared fresh daily. However, the VDRL test is the only nontreponemal test that can be used to test CSF due to the limited sensitivity and specificity of the other nontreponemal tests. The RPR test is macroscopic flocculation test and requires no microscope. The RPR test uses a stabilized suspension of VDRL antigen to which charcoal particles are added to aid in the visualization of the test reaction. The RPR test is one of the most commonly used nontreponemal tests, and is a simplified version of the VDRL test. Each of the above tests can be used as a quantitative test. Quantitative tests allow for the establishment of a baseline titre, which allows evaluation of recent infection and response to treatment. This also allows for the detection of reinfection or relapse in persons with a persistently reactive titre. However, the numerical values obtained may vary between tests; thus, when a patient is being followed with serial titres, the same test and preferably the same laboratory should be used (Larsen *et al.*, 1998).

2.6.4.2. Specific Treponemal Tests

The fluorescent treponemal antibody absorption (FTA-ABS) test is an indirect fluorescent antibody technique. In this procedure, serum samples are pretreated with an absorbent to remove nonspecific antibodies. The FTA-ABS double staining test is a modification of the FTA-ABS test using a double staining

procedure with the addition of a contrasting counterstain. While these tests are highly sensitive and specific, they may produce variable results due to variation in equipment, reagents and interpretation (Tramont, 2005).

The *Treponemapallidum* particle agglutination (TP-PA) test is a qualitative assay for the detection of antibodies to *T pallidum* in serum or plasma. This test is based on the agglutination of coloured particle carriers sensitized with *T pallidum* antigen and has replaced its predecessor, the microhemagglutination assay T.pallidum. The TP-PA test uses the same treponemal antigen as the MHA-TP test but offers the advantage of gelatin particles instead of erythrocytes, thus eliminating nonspecific reactions with plasma samples. The TP-PA test is less expensive and less complicated than the FTA-ABS tests, and the results are read with the unaided eye. It is one of the more commonly used treponemal tests. A positive TP-PA test in conjunction with a positive nontreponemal test is indicative of current or past infection with *T pallidum*. The TP-PA test - found to be an appropriate substitute for the MHA-TP test (Pope *et al.*,2001).Is as sensitive as the FTA-ABS test in primary syphilis and as useful as the RPR test in monitoring therapy(Castro *et al.*,2001).

2.6.5. Application of Enzyme Linked Immunosorbent Assay

The ELISA are widely used in the field of infectious disease owing to possibility of automation and the broad potential to detect antibodies ,antigens and haptens . After the first use of the indirect **ELISA** in the serodiagnosis of syphilis, several related tests in which detergent extracted or sonicated *T.pallidum* (Nichols) were used as antigen have been reported. Indirect ELISA in which components from non-pathogenic treponems or modified VDRL antigen have been developed. Most ELISA designed to detect antibodies against treponemal antigens perform well when used for screening. Theoretically, ELISA that detect both IgG and IgM should be more sensitive in early disease than those that detect

only IgG. There appears to be a benefit of having multiple recombinant antigen rather than antigen obtained from whole cell lysates. (Sambriet *et al.*,2001).

2.6.6. Western blot

This is similar to the Western blot test used for confirmation of HIV antibodies, and provides a molecular characterization of the antibody response through the visualization of characteristic banding patterns. Western blot methods using whole cell lysate and recombinant antigens have been developed(Sambriet *et al.*,2001). Antibody reactivity to some of the treponemal antigenic bands is highly specific for syphilis. This test can detect either IgG or IgM antibodies and is considered a very useful adjunct confirmatory test (Byrne *et al.*,1992). This technique is used in some laboratories to resolve questionable results obtained with other treponemal tests; this service may be available through select laboratories. Details of this technique are described elsewhere(Larsen *et al.*,1998).

2.6.7. Rapid tests

Treponemal tests are also commercially available in formats that can be performed at the point of care. They are available either as agglutination tests using latex particles coated with treponemal antigen or as immunochromatographic strips on which a positive reaction appears as a coloured line. Most of these tests can be used with whole blood, serum or plasma. They can be stored at room temperature, are simple to perform, require minimal training and no equipment, and the results can be read visually in less than 30 min. Over 20 such tests are commercially available but none are approved for use in North America. The World Health Organization has evaluated a number of rapid tests (Peeling *et al.*,2004).

2.7. Best Practice Considerations

Best practice considerations are summarized in; as stated, these considerations should be guided by the indications for, and limitations of, the various tests. The direct detection of *T pallidum* by microscopy has diagnostic and practical limitations, and may be technically challenging. Due to a low prevalence of syphilis in North America, most diagnostic laboratories are unlikely to have the necessary technical expertise to perform this testing. Therefore, testing should be limited to those laboratories with expertise and, where possible, the direct detection of *T pallidum* by microscopy and PCR-based tests for direct detection should be obtained from appropriate laboratories. The use of only one type of serological test is insufficient for diagnosis; both nontreponemal and treponemal serological tests should be carried out in all clinically suspected cases. Nontreponemal serological tests are amenable to being performed in the hospital laboratory setting, and if testing volume demands, syphilis screening using an approved test should be performed. However, a strict quality control program should be maintained to ensure reliability and reproducibility of the tests. If strict quality control cannot be met, it is prudent to obtain syphilis screening services from a reference laboratory. All reactive nontreponemal results must be confirmed by a treponemal test. In some instances, the patient's clinical history may require that treponemal tests are performed on samples with nonreactive nontreponemal results. In Europe, syphilis screening with both nontreponemal and treponemal tests has been widely used for many years. Although this combination provides an excellent screen for all stages of syphilis with the exception of very early primary infection when the treponemal test may not yet be positive, the cost-benefit of this approach should be considered. The other option might be to use one of the newer EIA tests, which can serve as both a screening and a confirmatory test. Rapid treponemal tests such as the Syphilis fast (Diesse, Italy) may be useful for point-of-care confirmation of screen

results(Fears and Pope,2001). Regardless, unless the testing volume demands an on-site treponemal testing service, such testing should be limited practical reference

2.8. Treatment:

Penicillin G, administered parentally is the preferred drug for treatment all stage of syphilis.

Injected benzathine penicillin G is viable for the treat incubating, primary, secondary, latent, tertiary's syphilis.(Katsambaset *al.*,1987)

Chapter three

3. Materials and Methods

3.1-Study design

3.1.1. Study type

The is work was a cross- sectional study.

3.1.2. Study area

This study was carried out in Khartoum State. Blood samples were collected from pregnant women attending Saudi maternal Hospital.

3.1.3. Study duration

The study was conducted from January to march 2016.

3.1.4. Study population

Hundred pregnant women were involved in the study.

3.1.4.1. Inclusion criteria

Individuals who were included in the study were pregnant women, aged between 15-55 years.

3.1.4.2. Exclusion criteria

Pregnant women with current history of medication.

3.2. Data collection

After explaining the purpose of the study, data were collected from each subject by interviewing questionnaire (appendix). The data included demographic information (age), history of miscarriage and number of miscarriage and trimesters.

3.3. Sample size

One hundred blood and hundred urine samples were collected.

3.4. Specimen collection and preparation

3.4.1 .blood collection

Using sterile syringes, about 5 ml of blood were drawn from antecubital vein under aseptic condition. The blood samples were collected in sterile containers without any additives, and let to clot at room temperature for 15 minutes, each blood sample was then centrifuged at 1500rpm for 5 minutes, and each serum was separated in another sterile plain container. Sample was labeled by giving laboratory number. Serum samples were kept frozen at -20degree centigrade without addition of preservatives, until the time of analysis.

3.4.2.urine collection

The mid-stream urine samples was collected in sterile container, and then cultured immediately in sterile bacteriological culture media in CLED medium.

3.5. Laboratory examination

All the specimens were tested for syphilis using two screening serological tests, Rapid Syphilis Test (RST), using ICT and Enzyme Linked immunosorbent Assay (ELISA).

3.5.1. Immune Chromatography Test (ICT)

The sample was considered positive if it showed two red lines in the test strip.

3.5.1.1. Principle of test

The One site syphilis Ab combo rapid test is a lateral flow chromatographic immunoassay. The test cassette consist of :1)a burgundy colored conjugate pad containing recombinant *Tp* antigens conjugated with colloidal gold (*Tp*conjugates) and a control antibody conjugated with colloidal gold. 2) a nitrocellulose membrane strip containing a test line (T line) and a control line(C line). The T line is pre-coated with non-conjugated recombinant *Tp* antigens, and the c line pre-coated line antibody.

When an adequate volume of test specimen is dispensed in to the sample well of the test cassette. Anti-*Tp*antibody, if present in the specimen. Will bind to the *Tp*conjuates. The immuocomplex is then captured on the membrane by the pre-coated *Tp* antigen forming a burgundy colored T line indicated a*Tp* antibody positive test result. Absence of the T line suggests a negative result.

The test contains an internal control (C line) which should exhibit a burgundy colored line of the immunocomplex of the control antibodies regardless of color development on the T line. If the C line does not develop, the test result is invalid, and the specimen must be retested with another device.

3.5.1.2. Procedure:

- The specimen and test components were brought to room temperature if frozen. Mix the specimen well prior to assay once thawed.
- The pouch was opened at the notch and the device was removed. The test device was placed on a clean, flat surface
- The plastic dropper was filled with the specimen and the dropper was held Vertically, 1 drop serum was dispersed into the sample well.

- 1 drop of sample diluent was immediately added to the sample well with bottle positioned vertically and the timer was set.
- Result was read in 15 minutes. Positive result was visible in as soon as 1 minute.

3.5.2. Enzyme Linked Immunosorbent assay ELISA

3.5.2.1. Determination of ELISA

The reagents were supplied by EUROIMMUNE, German, ELISA gen, fourth generation ELISA for in vitro determination of specific antibodies to syphilis in human plasma and serum.

3.5.2.2. Principle of the test

The ELISA test kit provides a semiquantitative or quantitative in vitro assay for human antibodies of the IgG class against *Treponemapallidum* in serum or plasma. The test kit contains microtiter strips each with 8 break-off reagent wells coated with purified recombinant antigens of *Treponemapallidum*. In the first reaction step, diluted patient sample are incubated in the well. In the case of positive sample, specific IgG antibodies (also IgA and IgM) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme –labelled anti-human IgG (enzyme conjugate), catalyzing a colour reaction.

3.5.2.3. Procedure

3.5.2.3.1. Control and calibrators

For quantitative analysis incubate calibrators 1, 2, and 3 along with the positive and negative controls and patient samples.

3.5.2.3.2. Sample incubation

Hundred microliter of the calibrators, positive and negative controls or diluted patient samples were transferred into the individual microplate well according to the pipetting protocol.

For manual processing of microplate wells, the finished test plate was covered with protective foil, follow the instrument manufacturer recommendations with incubate 60 minutes at 37°C.

3.5.2.3.3. Washing

The protective foil was removed and emptied the wells and subsequently washed 3 times using 300 µL of strength wash buffer for each wash.

The wash buffer was left in each well for 30 to 60 seconds / washing cycle, and then emptied the wells. All liquid from the microplate was disposed by tapping it on absorbent paper with opening facing downwards to remove all residual wash buffer.

3.5.2.3.4. Conjugate incubation

100µL of enzyme (peroxidase-labelled anti-human IgG) were pipetted into each of the microplate wells and Incubated for 30 minutes at room temperature.

3.5.3.3.4. Washing

the wells were emptied and washed.

3.5.2.3.5. Substrate incubation

Hundred microliter of chromagen /substrate solution were pipetted into each of microplate wells and Incubated for 15 minutes at room temperature.

3.5.2.3.6. Stopping the reaction

100µL of stop solution (0.5M sulphuric acid) was pipetted into each of the microplate wells in the same order and the same speed as the chromagen/substrate solution was introduced.

3.5.2.3.7. Measurement

Photometric measurement of color intensity was be made at a wavelength of 450nm a reference wavelength between 620nm and 650 nm within 30 minutes of adding stop solution. Prior to measuring, the microplate was shaken to ensure a homogeneous distribution of the solution.

3.5.2.4. Calculation and interpretation of result

The standard curve from which antibodies concentration in the patient samples was taken to obtain point-to-point plotting of the extinction values measured for 3 calibration sera against corresponding unites. Point-to-point for calculation the standard curve by computer. If the extinction of a serum samples lies above the value of calibrator 1 (200 RU/ml). The result should be given as >200 RU /ml. it is recommended that the sample be re-tested at a dilution of 1:400. The result in RU/ml read from the calibration curve of this sample was multiplied by a factor 4.

The upper limit of the normal range of non-infected person (cut-off value) recommended by EUROIMMUN is 20 relative unite (RU)/ml. EUROIMMUN recommends interpreting result as follows:

Negative: <16 RU/ml

Borderline: ≥ 16 to < 22RU/ml

Positive : >22

3.6.CLED culture medium

Cystin Lactose Electrolyte Deficiency use for urine sample for detection of bacterial isolate contain indicator bromothymolo blue in acidic medium give yellow color and in alkaline medium give blue color (Cheesbrough,2006).

3.7. Gram stain

The Gram stain reaction was used to help identify pathogen in specimen and culture by their gram reaction (gram positive or gram negative) and morphology. Gram positive bacteria stain dark purple with crystal violet and are not decolorized by alcohol and gram negative bacteria stain red because after being stained with crystal violet decolorized by alcohol (Cheesbrough, 2006).

3.8.Indole test

Use for identification of gram negative bacteria tryptophan is broken down with the release of indole which reacts with dimethylaminobenzaldehyde to give a red ring in a positive result (Cheesbrough, 2006).

3.9. Urease test

Use for identification of gram negative bacteria the enzyme urease hydrolyzes urea, producing ammonia which changes the color of the indicator to give a red pink medium (Cheesbrough, 2006).

3.10. Citrate test

Use for gram negative bacteria organisms use citrate as its only source of carbon, producing an alkaline reaction with a color change of indicator blue turbid medium (Cheesbrough, 2006).

3.12. Catalase test

To differentiate staphylococci from streptococci the enzyme catalase breaks down hydrogen peroxide to oxygen and water, releasing oxygen bubbles (Cheesbrough, 2006).

3.12. DNase

To identify *S. aureus* the enzyme deoxyribonuclease hydrolyzes DNA (Cheesbrough, 2006).

3.13. Mannitol salt agar

Use for gram positive bacteria bacteria ferment mannitol gives yellow golden color (Cheesbrough, 2006).

3.14. Kligler Iron Agar

Use for gram negative bacteria bacteria ferment glucose and lactose give yellow color and not ferment lactose give yellow red and the production of blackening is different according to ability of bacteria to break the ferric iron or iron (Cheesbrough, 2006).

Chapter Four

4. Results

4.1. Results:

The individuals in this study were females with an age ranging from 15-45 years most of them between 26-35 years (Table 1).

Table 1: Distribution of females according to age group (n=100)

Age	Frequency	Percent
15-25	41	41%
26-35	47	47%
36-45	12	12%
Total	100	100%

The majority of pregnant women were in the third trimester (Table 2)

Table (2): Distribution of female according to trimester (n=100)

Trimester	Frequency	Percent
First	16	16%
Second	12	12%
Third	72	72%
Total	100	100%

Three (3%) of them were syphilis seropositive by two methods (Table 3).

Table 3: Total syphilis positive cases tested by ICT and ELISA (n=100)

Results	ICT	ELISA	Percent
Positive	3	3	3%
Negative	97	97	97%
Total	100	100	100%

All the seroprevalence female well observed among age group of 26-35 years (table 4). **Table 4: Syphilis positive cases according to age group (n=100)**

Age	Positive frequency	percent
15-25	-	0%
26-35	3	100%
36-45	-	0%
Total	3	100%

All of the examined women positive syphilis has history of miscarriage (Table 5).

Table 5: syphilis positive cases according to miscarriage (n =100)

Female	Positive frequency	Percent
With miscarriage	3	100%
Without miscarriage	-	0%
Total	3	100%

The female with UTI were 1(33.3%) and without UTI 2(66.7%) (Table 6).

Table 6: Related risk factor (UTI infection) among syphilis positive cases (n=100)

Female	Positive	Percent
With UTI	1	33.3%
Without UTI	2	66.7%
Total	3	100%

Four bacterial species were isolated from the pregnant women urine (Table7)

Table 7: Type and number of bacterial species isolate from urine samples

Species	Number (%)
<i>E. coli.</i>	5 (33.3%)
<i>K. pneumonia</i>	4 (26.7%)
<i>S. saprophitics</i>	4 (26.7%)
<i>P. mirabilis</i>	2 (13.3%)

Chapter five

5. Discussion

5.1. Discussion

The seroprevalence of syphilis obtained in this study among pregnant women in a Soudy hospital was (3%) The seroprevlence of syphilis obtained in this study was lower when to compered to study conducted by Sana (2014) among Ethiopian resident in Khartoum State (8%) and that other reported by Atif and Magazoub (2010) among Sudanese blood donors in south Western sudan (23.5%), and this result is considered slightly similar in to the result reported for syphilis among pregnant Nigerian women (4%) (Olokoba *etal.*, 2008).

In this study the higher seroprevlence in age group 26-35 (3%) in comperisa Nigerian pregnant women the higher age group 30-35 (4%) (Olokoba *etal.* , 2009).

Urinary tract infections (UTIs) are one of the most common medical complications of pregnancy (Mittal & Wing, 2005). It is estimated that one in three women of childbearing age will have a UTI (Duarte et al., 2008). Because of the normal physiologic changes induced by gestation, pregnant women are especially susceptible to these infections.

In this study the UTI among pregnant women were (15%) is lower than UTI infection among pregnant women in Can Fam (20%) (Lee *etal.*, 2008).

The most common isolated agent *E. coli* (33.3%), *K.pneumonia* (26.7), *S.epidermids* (26.7), and *P. mirabilus* (13.3) in compered to research center of Canada *E. coli* (85%), *K. pneumonia* (10%), *S. saprophiticus* (10%),*P. mirabilis* (10%) (Mittal and Wing, 2005), this percentage in bacterial infection is high.

This variation may be attributed to the open sexual relationship and low hygiene in south Sudan.

Further studies required with large sample size to validate the result.

5.2. Conclusion

It isconcluded that,Theseroprevalence of syphilis obtained in this study among pregnant women in a Saudi hospital 3% it is percentage. The prevalence of syphilis among age group 26-35 highlighted the need to target women to prevent transmission of infection. There is direct relationship between the miscarriage and high susceptibility to disease. The co infection between UTI and syphilis is high.

3.5. Recommendations:

1. Screening all pregnant women in maternity hospital for syphilis and managing those infected would have clinical and epidemiological importance.
2. Provision of many centers for voluntary screening for sexual transmitted disease before pregnant.

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Appendix (1)

1.1. Equipmemts:

Centrifuge	hettich-Germany
Automatic	axiom-German
Alarm clock	quartz-Japan
Microwell reader	state –fax USA
Automatic pipette(multichannel)	axiom-Germany
Dry incubator	torrpicenardi-Italy

Appendeix (2)

Reagents:

Deionized or distilled water

Alcohol

ELISA kit

EUROIMMUNE-

German

Concentrated wash solution (20x)

Negative control (human) green

Positive control (human) red

Conjugate ready –to-use

Peroxidase –lablled anti-human IgG (rabbit)

Substrate pink

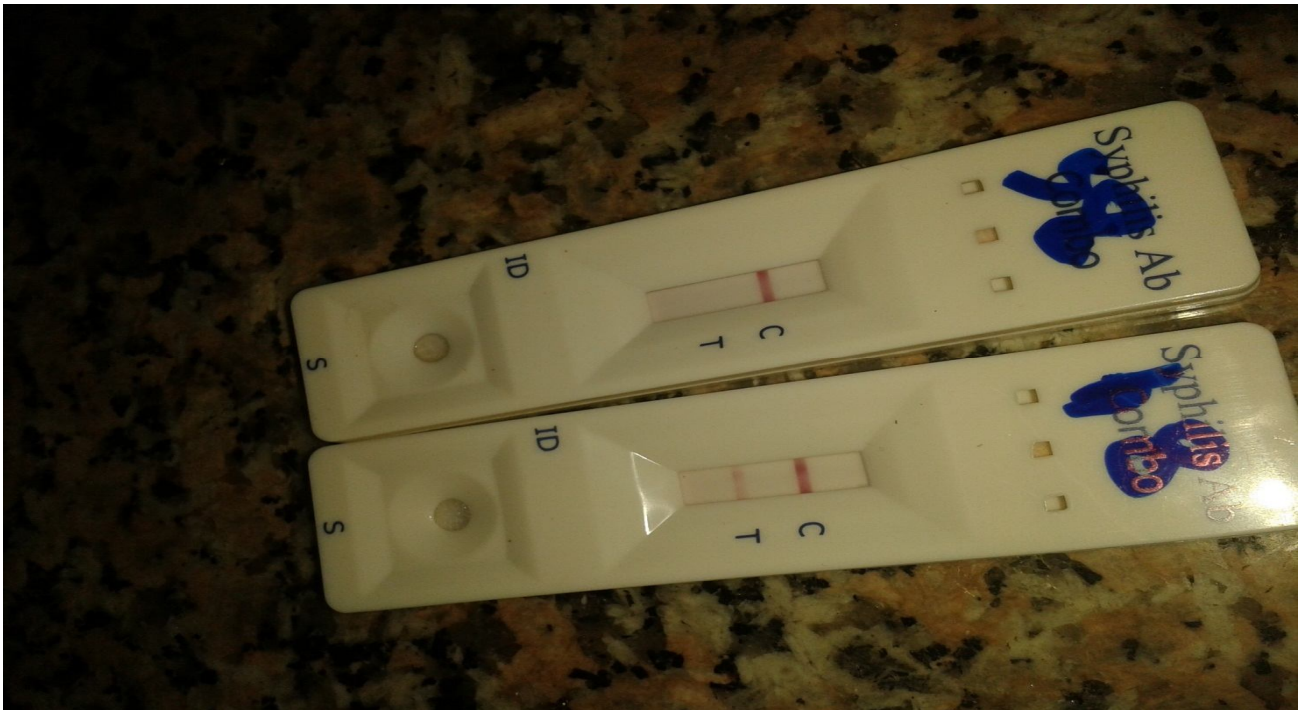
Stop solution 0.5 sulphuric acid

Appendix (3)

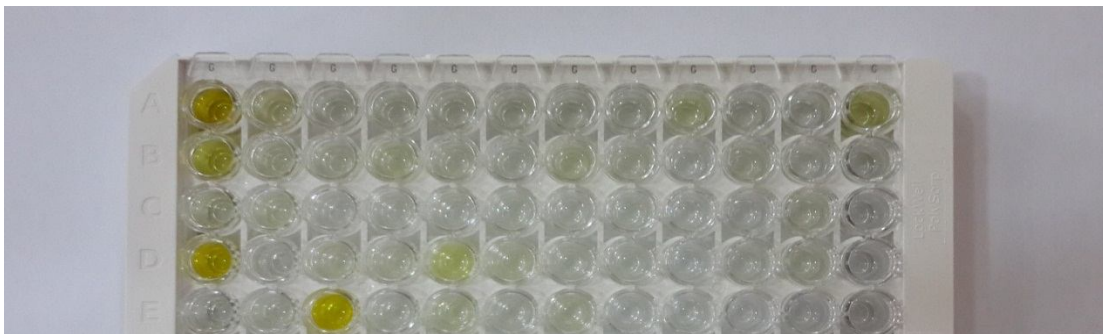
Other materials:

VDRL ICT	CTK-German
Cotton medical- sanitary	
Disposable pipette tips	China
Vacuum tube	China
Syringestuttlingen	Germany
Alcohol swab	Saudi Saches-Services-KSA-saudi
Plan containers	China
Disposable gloves	India
Absorbent tissue	
Sudan	
Microplate: 12 strips of eight wells coated with <i>T.pallidum</i> Ag	
Erlenmeyer flasks: 100 ml	
Automatic microtiter plate washer or squirt bottle	
Bag for storage unused wells	
Test tubes and racks	

Appendix (4)
ICT test



Appendix (5)
ELISA test



Appendix (6)

Sudan University of Science and Technology

Collage of Graduate Studies

Questionnaire

Name:

Serial No:

Age:

Trimester:

Miscarriage:

Number of miscarriage:

Previous infection (s) or disease (s):

Treatment (s):

Yes.....

No.....

Type of treatment(s)

