CHAPTER ONE

INTRODUCTION

1.1 Introduction

The congenital syphilis (CS) is still a major public health concern, even after the implementation of intervention protocols in several countries (Broutet *et al*., 2007). Syphilis is a sexual transmitted disease caused by the spirochete *Treponema pallidum* and manifests as a systemic infectious process. Syphilis may be transmitted vertically, usually through the placenta, the risk for fetal infection increases with gestational age. Vertical transmission may also occasionally occur during delivery if maternal genital lesions are present (Majeroni and Ukkadam, 2007).

The consequences of fetal syphilis include prematurity, low birth weight, non immune hydrops, and intrauterine death (Chakraborty and Luck, 2008). Serological screening and treating mothers for syphilis during pregnancy can prevent adverse pregnancy outcomes and vertical transmission associated with maternal infection (Watson *et al*., 2002).

Syphilis is common in most parts of the world; those who suffer from it are plagued with rash and boils. If left untreated the disease can eventually lead to death (Kent and Eomanelli, 2008).

In 1999 WHO estimated an annual rate for syphilis of 12 million active infections. The risk of contracting syphilis through a sexual contact with a person with primary or secondary syphilis is 30 –50%. More than 80% of women with syphilis are in reproductive age; therefore, there is a serious risk of vertical transmission to the fetus (Values *et al*., 2000). Worldwide, a million pregnancies are adversely affected each year by syphilis, because of maternal infection. About 270,000 babies are born with congenital syphilis, 460,000 pregnancies end in abortion or prenatal death, and 270,000 babies are born prematurely or with low birth weight (Walker, 2002). Most affected infants are asymptomatic at birth with two-thirds developing symptoms by 3–8 weeks. Almost all exhibit symptoms by 3 months of age (Gurlek *et al*., 2005). Recent studies of pregnant women in Africa have revealed rates of 17.4 % in Cameroon, 8.4 % in South Africa, 6.7% in Central African Republic and 2.5 % in Burkina Faso (WHO, 1999). In 1997, 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).

A recent report from Tanzania estimates that up to 50% of stillbirths are caused by syphilis (Watson *et al*., 2002).

Penicillin has been used successfully for the treatment of syphilis for over 50 years but the need for intramuscular administration and patient hypersensitivity to penicillin has led to the development and use of second-line antimicrobials (Katz and Klausner, 2008).

It is of importantance to screening syphilis among pregnant woman at different stage of pregnancy and at different ages. Because primary syphilis may be asymptomatic, the disease may pass unnoticed (Brillman and Quenzer, 1998). CDC recommends that all women should be screened serologically for syphilis at the first prenatal visit and, for patients at high risk, during the third trimester and at delivery. Moreover, CDC recommends that all persons who have syphilis should be tested for HIV infection (Workowski and Berman, 2010).

1.2 Objectives:

1.2.1 General objective:

To determine the seroprevalence of syphilis amongst pregnant women in the Khartoum State.

1.2.2 Specific objectives:

- To detect seroprevalence of syphilis by using Plasma Regain Test and Syphilis Antibody Rapid Test**.**

- To confirm the positive results of both screening tests by using enzyme linked immunosorbent assay (ELISA).

- To compare between the performance of the Rapid Plasma Reagin Test (RPR, Non-treponemal test) and the Rapid Syphilis Test (RST, Treponemal Test) in the diagnosis of syphilis.

- To determine risk factors associated with syphilis among pregnant women.

CHAPTER TWO

LITERATURE REVIEW

2. LITERATURE REVIEW:

2.1 History of syphilis:

Syphilis epidemic raged in Europe in the late 1400s, leading many historians to assume that the disease was introduced to Europe by Columbus sailors on their return from the West Indies (Hudson, 2010). An alternate theory (the pre-Colombian theory) holds that syphilis developed as a sexually transmitted variant of cutaneous treponemal infections originating in Central Africa, which spread rapidly through Europe during the many wars of the late 15th century (Rothschild, 2005).

2.2 Causative organisms:

Treponema pallidum is a member of the order Spirochaetales, family Spirochaetaceae, and genus *Treponema* *Treponema* pallidum is the causative organism for syphilis. It is a delicate, motile spirochete bacterium. Humans are its only natural source (Wright and Jones, 2003).

2.2.1 Characteristics of *Treponema pallidum*:

Treponema pallidum is a motile helical bacterium with a central protoplasmic cylinder, cytoplasmic membrane, peptidoglycan and outer membrane, which resembles a Gram-negative bacterium (Peeling and Hook, 2006). *Treponema pallidum* is typically thin, with six to 14 spirals and tapered ends (Miklossy, 2008).

The bacterium size ranges from a length of 6 μm to 20 μm and a width of 0.10 μm to 0.18 μm, which means light microscopy is inadequate for its visualization, however, it can be viewed using dark-field microscopy (Peeling and Hook, 2006; Woods, 2005). Some of the most characteristic features include the presence of periplasmic flagella and a paucity of immunogenic outer membrane spanning proteins, which have earned *T. pallidum* the name of "stealth pathogen" (Peeling and Hook, 2006; Salazar *et al.*, 2007). Furthermore, *T. pallidum* is typically deprived of a few common features, such as a lipopolysaccharide (LPS) and an iron acquisition mechanism (Peeling and Hook, 2006).

2.2.2 Culture characteristics:

Characteristic features of *T. pallidum* include a slow generation time of 30 to 33 hours and an inability to grow in vitro, which may limit research but also implies that an antibiotic with a long half-life is required for treatment (Peeling and Hook, 2006; Salazar *et al*., 2007; LaFond and Lukehart, 2006). Only a few generations of *T. pallidum* have been cultivated on rabbit epithelial cell monolayers when stored at 33°C to 35°C under micro-aerobic conditions (Peeling and Hook ,2006; Woods, 2005). Rabbit models have been used successfully for in vivo propagation of the spirochaete by inoculating the testis (rabbit infectivity test). A primary infection and progression of disease similar to that in humans has been most closely portrayed in rabbit models compared to other animal models (Peeling and Hook, 2006).

2.2.3 Metabolism:

T. pallidum is a chemoheterotroph which encodes few proteins; therefore, it has very limited metabolic capacity. T. pallidum is also microaerophilic, meaning that it requires a very low concentration of oxygen (Norris, 1993).

2.2.4 Genome:

Treponema pallidum's genome was sequenced for the first time in 1998, at which time a small circular genome comprised of 1 138 006 bp and 1 041 open reading frames (ORF) was identified (Fraser, *et al*., 1998; LaFond and Lukehart, 2006; Woods, 2005). The G+C content of the genome is 52%, which is higher than the 39% G+C content identified in Borrelia burgdorferi, a closely related species (Fraser *et.al*, 1998; Norris *et al*., 2001). The temperature-sensitive *T. pallidum* was noted to lack sigma-factor 32, which plays a crucial mediator role in the prokaryotic heat-shock response (Porcella and Schwan, 2001).

2.2.5 Periplasmic flagella and motility:

Motility is an important function associated with the haematogenous dissemination of pathogenic bacteria (Giacani *et al*., 2009). Corkscrew-like motility has been observed in *T. pallidum*, which is made possible by the presence of three to six periplasmic flagella attached at the subterminal ends of the cell (LaFond and Lukehart, 2006; Liu *et al*., 2010; Izard *et al*., 2010). The classical flagella appearance includes a basal body-motor complex, a hook and a filament (Liu *et al*.,

2010; Izard *et al*, 2010). The proteins involved in bacterial motility can be associated with the chemotaxis system or the basal body complex (LaFond and Lukehart, 2006). A type III secretion system is believed to be necessary for auto-assembly of the flagella and once the apparatus is completed, a chemotaxic influence determines the direction of motility (Rajagopala *et al*., 2007).

2.3 Transmission:

The transmission of *T. pallidum* relies on intimate contact with the human host, which can occur during sexual intercourse, transplacentally to the foetus (congenital syphilis), during a blood transfusion or during an organ transplant, the latter two are rare routes of infection (Giacani *et al*., 2009; LaFond and Lukehart, 2006). The most common sexual route of transmission appears to be limited to the primary and secondary stages of disease and relies on contact with the lesions formed at these stages. The required contact with lesions means there is a low risk of sexual transmission during the latent stage of disease despite the intermittent release of *T. pallidum* in to the bloodstream during that period (LaFond and Lukehart, 2006). The latent stage of disease may not present with any clinical manifestations but still poses a risk for congenital infection in untreated mothers as well as progression towards clinical diseases in immune compromised patients (Peeling and Hook, 2006).

2.4 Epidemiology of Syphilis:

Syphilis is a multistage disease that is usually transmitted through contact with active lesions of a sexual partner or from an infected pregnant woman to her fetus (Stamm, 2002). Efforts to eliminate syphilis have met with only modest success (Hook and Peeling, 2004). Despite the availability of new diagnostic tests and antibiotic therapy, syphilis has reemerged in several developed countries. While the widespread epidemics of syphilis that occurred in Russia in the 1990s and more recently in China mostly involved heterosexuals, smaller outbreaks in the United States, Canada, and England predominately involved men who have sex with men (MSM)(Martin *et al*., 2009).

A major concern associated with increased rates of syphilis is that active, early syphilis (i.e., primary and secondary stages) enhances transmission of human immunodeficiency virus (HIV) by 2- to 5-fold, thus promoting the spread of HIV (Douglas, 2009).

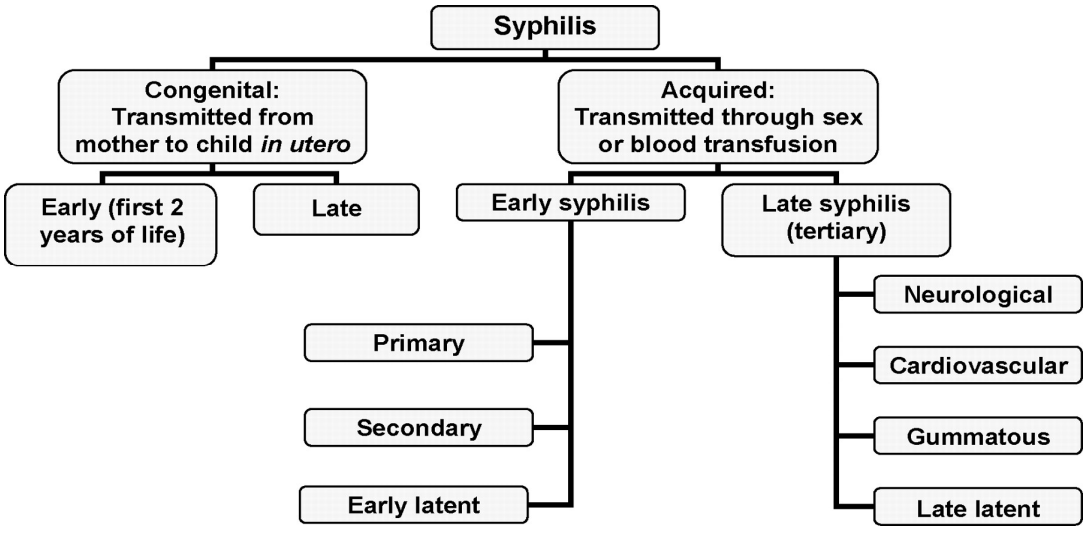
2.5 Pathogenesis of syphilis:

Dissemination of Treponemes to the immunologically privileged sites (e.g. brain, eyes, placenta, fetus, ovaries, testicles), where the immune response is reduced or absent, may help Treponemes to evade the immune system and promote chronic infections (Houston and Cameron, 2012).

T. pallidum initially enters from mucosal or cutaneous lesions of an infected person through skin or mucous membrane abraded during sexual activity. The organisms reach local lymph nodes within 30 minutes after skin inoculation. Viable treponemes attach to cells, after which polymorphonuclear leukocytes accumulate in the area of infection (Musher *et al.*, 1983). Some of the greatest concerns with regard to *T. pallidum* infections include the altered progression of the disease in HIV infected persons to a more malignant accelerated form and the increased risk of HIV and syphilis co-infections (Peeling and Hook, 2006; Giacani *et al.*, 2009). Furthermore, there is a risk of congenital infections, which may result in a myriad of clinical manifestations if the child is born or result in the untimely death of the child (Peeling and Hook, 2006).

2.6 Classification of Syphilis:

Syphilis is classified (WHO, 2003) as congenital or acquired as shown in Figure 1. There are four stages of syphilis: primary, secondary, latent, and late (tertiary).



(Fig 1) WHO clinical classification of syphilis (WHO, 2003).

2.6.1 Acquired Syphilis:

Divided into 4 stages— primary, secondary, and tertiary stages in which characteristic manifestations occur and a latent stage in which the patient is asymptomatic but seropositive. The latent stage occurs with the resolution of the secondary stage. Identifying the appropriate stage of disease is important because it affects duration of treatment (Peterman *et al*., 2005).

2.6.1.1 Primary syphilis:

Primary syphilis is acquired by direct contact with open lesions of an individual with syphilis. *T. pallidum* is transmitted through small abrasions in the skin. The initial manifestation of primary syphilis is called a chancre, a painless skin ulceration at the point of exposure (Singh and Romanowski, 1999). The average time from exposure to the appearance of the chancre is 3 weeks (Zetola *et al*., 2007). The chancre may persist for 4 to 6 weeks and usually heals spontaneously even without treatment. Inguinal lymphadenopathy can occur. A diagnosis of primary syphilis is often missed because the initial lesion goes undetected by the patient. This may be the result of an atypical location versus an atypical morphology of the initial lesion (Angus *et al*., 2006).

2.6.1.2 Secondary Syphilis:

Secondary syphilis is the result of hematogenous dissemination of *T. pallidum*, which subsequently causes systemic symptoms. This stage typically occurs 6 to 8 weeks after the primary infection (Lautenschlager, 2006). Manifestations of secondary syphilis can mimic many other diseases, and cutaneous manifestations are common. Manifestations include a generalized rash, fever, malaise, myalgias, alopecia, and generalized lymphadenopathy.The initial rash of secondary syphilis begins as a faint exanthem with macular pink lesions that are asymptomatic and are often overlooked (Baughn and Musher, 2005; Lautenschlager, 2006).

2.6.1.3 Latent Syphilis:

Because transmission usually occurs during the primary and secondary stages when lesions are present, it is important to diagnose and treat syphilis early. Latent syphilis is defined as having serologic evidence of infection without signs or symptoms of disease.Latent syphilis is defined as having serologic evidence of infection without signs or symptoms of disease. Latent syphilis

can be classified as early or late depending on the time frame from initial infection (early, < 1 yr and late, ≥1 yr) (Workowski and Berman, 2006).

2.6.1.4 Tertiary Syphilis:

Tertiary (late) syphilis generally occurs 5 to 20 years after initial infection and can present with mucocutaneous, cardiac, ophthalmologic, neurologic, or osseous abnormalities (Hutto, 2001). Tertiary disease will develop in approximately one third of untreated individuals, manifesting as gummatous (benign) syphilis, cardiovascular syphilis, or neurosyphilis (Workowski and Berman, 2006). The incidence of late gummatous syphilis has declined dramatically since the introduction of antimicrobial agents (Pereira *et al*., 2007).

2.6.2 Congenital Syphilis:

In resource poor countries or in instances where prenatal screening is neglected, an untreated mother could pass the *T. pallidum* infection to her unborn child in utero or during delivery (Woods, 2005). Congenital infections can result in abortion, stillbirth, neonatal mortality or severe sequelae**.** The risk of vertical transmission can be as high as 95% during primary syphilis but rarely occurs during the tertiary stage of the disease (Stamm, 2002). The consequence of vertical transmission during the primary and secondary stage of syphilis is pre-term birth or foetal death. In the event of the infected foetus' survival and birth, the child could demonstrate signs of growth impairments (Woods, 2005). The pathogenesis of *T. pallidum* relies on the naturally suppressed immune response in the mother during pregnancy and the ability of the spirochaete to transverse the placenta (Peeling and Hook, 2006). Kasowit's law suggests that each successive pregnancy will have a greater chance of progressing normally due to less severe immune responses (Chakraborty and Luck, 2008). Almost all cases of congenital syphilis are easily prevented by antenatal screening for syphilis and treatment during pregnancy (Watson *et al*., 2002).

2.6.2.1 Early Congenital Syphilis:

The symptoms of early syphilis can be varied. ''Snuffles'' or persistent rhinitis is often the earliest presenting symptom, occurring in 4–22% of newborns (Chawla *et al*., 1998). Other common, but often nonspecific, symptoms of congenital syphilis include non-tender generalised

lymphadenopathy, conjugated hyperbilirubinaemia and hepatosplenomegaly (Sangtawesin *et al*., 2005). Glomerulonephritis resulting in nephritic syndrome may also occur. The rash in early syphilis is classically a vesiculobullous or maculopapular rash occurring on the palms and soles and may be associated with desquamation. Other rashes including erythema multiforme have been reported (Sangtawesin *et al*., 2005).

2.6.2.2 Late Congenital Syphilis:

Late congenital syphilis occurs in children over the age of 2, but most often presents in puberty (Walker, 2002). Late syphilis can affect many organ systems, although the sites most often involved include the bones, teeth and nervous system. A poor response to intensive treatment is often documented during the management of these late manifestations (Uchiyama *et al*., 2005).

2.7 Laboratory diagnosis:

The causative organism, the spirochete *Treponema pallidum*, cannot be easily cultured or identified under a standard microscope (Goh, 2005). The detection of *T. pallidum* can be broadly discussed under three topics, namely: microscopy, serology and molecular techniques. Due to the inability of the spirochaete to be cultured in vitro on routine bacteriological media or in rabbit epithelial cell monolayers for more than a few generations, detection has been mostly reliant on serological techniques and microscopy (Martin *et al*., 2009).

2.7.1 Microscopy:

Microscopy is an immediate and direct technique employed for the detection of the small *T.pallidum* bacterium utilising dark-field microscopy or immunofluorescence in exudates from primary chancres and mucous membranes, which is seldom performed in the clinical setting and relies on the suspicion of the clinician. Microscopy is limited by the difficulty in detecting the spirochaete in less reliable mucous membrane lesion specimens, which may contain other indistinguishable saprophytic spirochaetes (Emerson, 2009).

Immunofluorescence is more sensitive this uses the indirect fluorescent technique with killed *T.pallidum* as antigen. The organisms are fixed on a slide to which serum is added. The antibody in the serum unites with treponemes and is made visible with fluorescent stain (Wright and Jone, 2003).

2.7.2 Serological tests:

2.7.2.1 Non-treponemal tests:

Include the Venereal Disease Research Laboratory (VDRL) and Rapid Plasma Reagin (RPR) tests. These are sensitive tests that are easily analyzed, inexpensive and reliable. Specificity is variously reported as 93-98% with sensitivity varying with stage of disease (Peelin and Ye, 2004). These tests detect the cross-reaction of antibody (IgG and IgM) to syphilis with cardiolipin. False positive non-treponemal tests may occur in patients who are pregnant, i.v. drug users, those with systemic inflammatory diseases such as systemic lupus erythematosus, or after a recent viral infection (Edwards, 2000). The traditional algorithm used for the screening of syphilis makes use of a non-treponemal assay typically followed by treponemal assays for confirmation (Zanto, 2010).

2.7.2.2 Treponemal tests:

These tests specifically detect antibodies against *T. Pallidum* and are positive for life in the vast majority of infected patients regardless of stage or treatment history (Edwards, 2000). Treponemal assays remain positive regardless of treatment and become reactive sooner thus successfully detecting infection in the primary stage earlier Examples of treponemal tests include: treponemal enzyme immunoassay (EIA), T. pallidum haemagglutination assay (TPHA), *T. pallidum* particle agglutination (TPPA), fluorescent treponemal antibody absorption test (FTA-abs) and *T. pallidum* recombinant antigen line immunoassay, and rapid point of care (POC) syphilis tests based on RPR and immunochromotographic based strips(ICT) (Emerson, 2009). TPHA ,TPPA these are very valuable and simple tests using an indirect haemagglutination method with red cells or by gelatine particles. Together with VDRL, it is probably the best combination for routine use.False positive reactions occur in up to 2% (Wright and Jones, 2003).

In general, both non-treponemal and treponemal tests can be interpreted in the usual manner for patients co-infected with syphilis and HIV, although false-positive and -negative results have been described (Ballard *et al*., 2007).

2.7.2.3 Immunochromotographic based strips (ICT):

The ICT has a sensitivity range of 93.7% to 100% and specificity ranging from 94.1% to 100% (Emerson, 2009). Immunochromotographic strips (ICSs) in which 1 or multiple *T.pallidum* recombinant antigens are applied to nitrocellulose strips as capture reagents (Zarakolu *et al*., 2002). The World Health Organization compared the performance of 8 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).

2.7.3 Molecular methods:

Molecular methods are not commonly used in the detection of *T. pallidum* in a clinical setting but can be considered a complimentary technique to be used in combination with conventional dark-field microscopy or serology (Peelin and Ye, 2004; LaFond and Lukehart, 2006). Some of these molecular methods used for detection of the pathogen include the use of PCR and Real-time PCR assays (Morshed *et al*., 2009). The application of PCR in the detection of *T. pallidum* DNA has the advantage of being a diagnostic method with the ability to characterise strains susceptible to macrolide antibiotics. The sensitivity of PCR detection assays has been found to vary depending on the specimen types and the stage of disease (Martin, 2009).

2.8 Treatment:

Adequate treatment of maternal infection is effective for preventing maternal transmission to the fetus and for treating fetal infection (Alexander *et al*., 1999). Penicillin G, administered parenterally, is the preferred drug for treating of syphilis. The effectiveness of penicillin was established through clinical experience and randomized controlled clinical trials. It provides weeks of treponemicidal levels of penicillin in the blood, but it does not efficiently cross the blood brain barrier. Aqueous crystalline penicillin G is the drug of choice for neurosyphilis treatment (Workowski and Berman, 2010). Treatment failure was described in few cas reports, particularly in patients with HIV infection, but there is no documented penicillin resistance in *T. pallidum* (Stamm, 2010). CDC recommends that pregnant women should be treated with the penicillin regimen appropriate for their stage of infection (Workowski and Berman, 2010). In primary, secondary, and early latent syphilis, benzathine penicillin G 2.4 million units IM in a single dose is recommended (Workowski and Berman, 2010). In late latent syphilis or latent

syphilis of unknown duration, benzathine penicillin G 7.2 million units total should be administered, as 3 doses of 2.4 million units IM each at 1 week intervals. In case of neurosyphilis, aqueous crystalline penicillin G 18–24 million units per day, administered as 3–4 million units IV every 4 hours or continuous infusion, for 10–14 days represents the suggested treatment (Workowski and Berman, 2010). Pregnant women who have a history of penicillin allergy should be desensitized and treated with penicillin. In case of HIV positive patients, placental inflammation from congenital infection might increase the risk for perinatal transmission of the virus. No sufficient data are available to recommend a specific regimen for HIV infected pregnant women (Workowski and Berman, 2010).

2.8.1 Jarisch-Herxheimer reaction:

The Jarisch-Herxheimer reaction can occur in some patients 2 to12 hours after receiving therapy for active syphilis. It is characterized by fever, headache, myalgia and malaise, and it is caused by the release of treponemal endotoxin-like compounds during penicillin-mediated lysis (Murray, 1996). The Jarisch-Herxheimer reaction can increase the risk of premature labor and/or fetal distress during the second half of pregnancy (Myles *et al*., 1998).

2.9 Prevention and management:

The risk factors associated with the spread of syphilis is poverty, drug use, limited education, multiple sexual partners, a positive HIV status, single status and a history of abortion and other sexually transmitted infections (STIs) (Peeling and Hook, 2006; Woods, 2005). Another contributing factor towards the increased incidence of syphilis in some developing countries is the lack of access or poor healthcare, which includes poor antenatal care (Peeling and Hook, 2006). The prevention and management of *T. pallidum* infections is a multifaceted approach, which currently relies on the efficient detection and treatment of infected individuals, especially since no vaccine is available. The lack of a vaccine means that the preventative approach taken to reduce risk taking behaviour includes education of the population regarding the disease and the advertising of sexual protection. Antenatal screening is of particular importance in the prevention of congenital syphilis (French, 2007)**.**

CHAPTER THREE

MATERIALS AND METHODS

3 Materials 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 Omdurman Maternity Hospital and Almotakaml Medical Center in Khartoum.

3.3 Study area:

The study was conducted in Khartoum State.

3.4 Sample size:

A total of 100 samples were collected, 50 samples from Omdurman Maternity Hospital and 50 samples from Almotakaml Medical Center.

3.5 Study duration:

The study was conducted during the period from April to June 2014.

3.6 Data collection:

Clinical data were collected using a questionnaire with informed consent. The data included the age residence, gestational period history of previous abortion and genital ulcer.

3.7 Specimen collection, preparation and storage:

Venous bloods were collected as follows:

-The forearm the site of collection was disinfected using alcohol.

-The vein located (sometimes tourniquet) was used to locate the vein.

-Using sterile syringe 3 mls of blood were withdrawn, transferred into a sterile plain container, and allowed to clot naturally at room temperature.

-The clotted samples were centrifuged at 1500 rpm, for 5 minutes.

-The serum was collected in another plain sterile container with the corresponding laboratory number.

-The samples were transported in an ice bag, from the site of collection to the lab, where they were stored, for analysis.

Storage:

The samples were stored at -20^{o}C until the time of analysis (no more than 3month).

3.8 Laboratory Examination:

All serum samples were tested for syphilis using two screening serological tests, namely, Rapid Plasma Reagin Test (RPR) and Rapid Syphilis Test (RST). Reactive specimens with one or both of the screening tests were further assayed with enzyme linked immunosorbent assay (ELISA).

3.8.1 Rapid Plasma Reagin Test (RPR):

The kit used were manufactured by Spinreact (Spain).

3.8.1.1 Principle of the method:

The RPR-carbon is a 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 patient affected by syphilis.

3.8.1.2 Reagents:

RPR-carbon:

Carbon particles coated with a lipid complex, cardiolipin, lecithin and cholesterol in phosphate buffer 20mmol/L

Control positive:

Artificial serum with regain titer more or equal ¼.

Control negative:

Animal serum preservative

3.8.1.3 Procedure:

1. The reagents and samples were allowed to reach room temperature.
2. 50 micro liters of the sample and one drop of each positive and negative controls were placed into separate circles on the slide test.
3. The RPR-carbon reagent were swirlled gently before using, and the dropper was assembled and pressed gently to remove air bubbles from the micropipette.
4. One drop of the reagent was added next to the samples to be tested.
5. The drops was mixed with a stirrer, spreaded over the entire surface of the circle using different stirrers for each sample.
6. The slide was placed on a mechanical rotator at 80-100 r.p.m for 8 min.
7. The presence or absence of visible agglutination was examind macroscopically immediately after removing the slide test from the rotator it was considered positive if it showed slight to marked visible clumps of carbon particles.

3.8.2 Syphilis Antibody Rapid Test:

The onsite Syphilis Antibody Rapid test is a lateral flow chromatographic immunoassay. Permits the measurement of antibodies (IgM, IgG, and IgA) to recombinant antigens of *Treponema pallidum* in blood rapidly and reliably without instrumentation.

When an adequate volume of test specimen is dispensed into the sample pad of the strip, the specimen migrates by capillary action across the strip. Anti- *Treponema pallidum* antibody, if present in the specimen will bind to the *Treponema pallidum* conjugates. The immunocomplex is then captured on the membrane by the pre-coated *Treponema pallidum* antigen, forming a burgundy colored T band indicating a *Treponema pallidum* antibody positive test result.

3.8.2.1 Procedure

The specimen and test components were brought to room temperature and then were collected at least 150-200 micro liters or 3-4 drops of serum or plasma in a sample container.

The strip was then dipped into the specimen for at least 10 seconds.

The strip was removed from the specimen, and placed on a flat, dry surface.

The test result was read in 5-10 minutes. Positive result could be visible as short as 1 minute.

3.8.2.2 Interpretation of assay result

Negative result:

If only control band is developed

Positive result:

If both C and T bands were developed.

Invalid:

If no C band is developed

3.8.3 Enzyme Linked Immunosorbent assay ELISA:

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

3.8.3.1 Principle of the test:

Syphilis EIAII96 test kits use three recombinant antigens in a sandiwish test.

The antigen will detect *T.pallidum*-specific IgG, IgM and IgA; enabling the test to detect antibodies during all stages of infection.

The plastic wells are coated with a mixture of the 15Kd, 17Kd, and 47Kd recombinant antigens of *T.pallidum*. Specific antibodies in the serum or plasma specimens combine with these antigens and with the same antigens conjugated to horsedish peroxidase , when conjugate is added to well in which the specimen has been incubated . After unreacted materials have been removed by

washing, the presence of bound enzyme indicating the presence in the specimens of specific antibodies is revealed by a colour change in the substrate/chromogen mixture. The intensity is compared to that in control wells to determine the presence or absence of specific antibody.

3.8.3.2 Procedure:

Reagents and samples were allowed to reach room temperature

50µl of samples were added, negative control(x4) positive control (x2) to each well.

Then 50µl of conjugate was added to each well and mixed by tapping the plate gently and Incubated at 37^{o}C for 30 minutes.

Washed 5 xs with working strength wash buffer. Short soak time of about 30 seconds is recommended between each wash cycle.

50 µl of the chromogen/substrate were added to each well. Then the plate was incubated for 30 minutes at room temperature.

50 µl of the provided stopping solution were added to each well.

Carefully wipe the plate bottom, measuring the absorbance using plate reader within 30 minutes of stopping the reaction using sulphric acid, 450nm wavelength filter was used.

3.8.3.3 results:

For the assay to be valid, the mean optical density (OD) value of positive control must be greater than or equal to 1.000 and the mean OD value of negative control must be less than or equal to 0.080.

Cut off value:

Calculate as the mean of the negative control values plus 0.10

i.e. $\frac{NC1+NC2+NC3}{3} + 0.1$

OD value of the sample < cut off value, the specimen was considered negative

OD value of the sample ≥ cut off value, the specimen was considered positive

CHAPTER FOUR

RESULTS

4. RESULTS:

In this study a total of 100 blood samples were collected from pregnant women in different gestational period, 50 samples from Omdurman Maternity Hospital and 50 samples from Almotakaml Medical Center, to detect seroprevalence of syphilis among pregnant women. The participant's ages ranged from 18 to 42 years, with mean age 27.69 years (Figure 2).

All specimens were tested for syphilis by the two serological tests, 4% were positive using RPR and 2% using RST, and then 4 reactive specimens (2 by RPR only and 2 by both serological tests) when tested by ELISA 2 specimen were positive (Figure 3).

The results exhibited in table 1 showed that the two positive cases was among the age group 23-27 year and among age group 28-32 year. No genital ulcers where showed in positive cases one in each respectivety.

The two positive cases was one the third and fourth gestational period.

Among the two positive cases one showed history of previous abortion while the other one had no previous abortion.

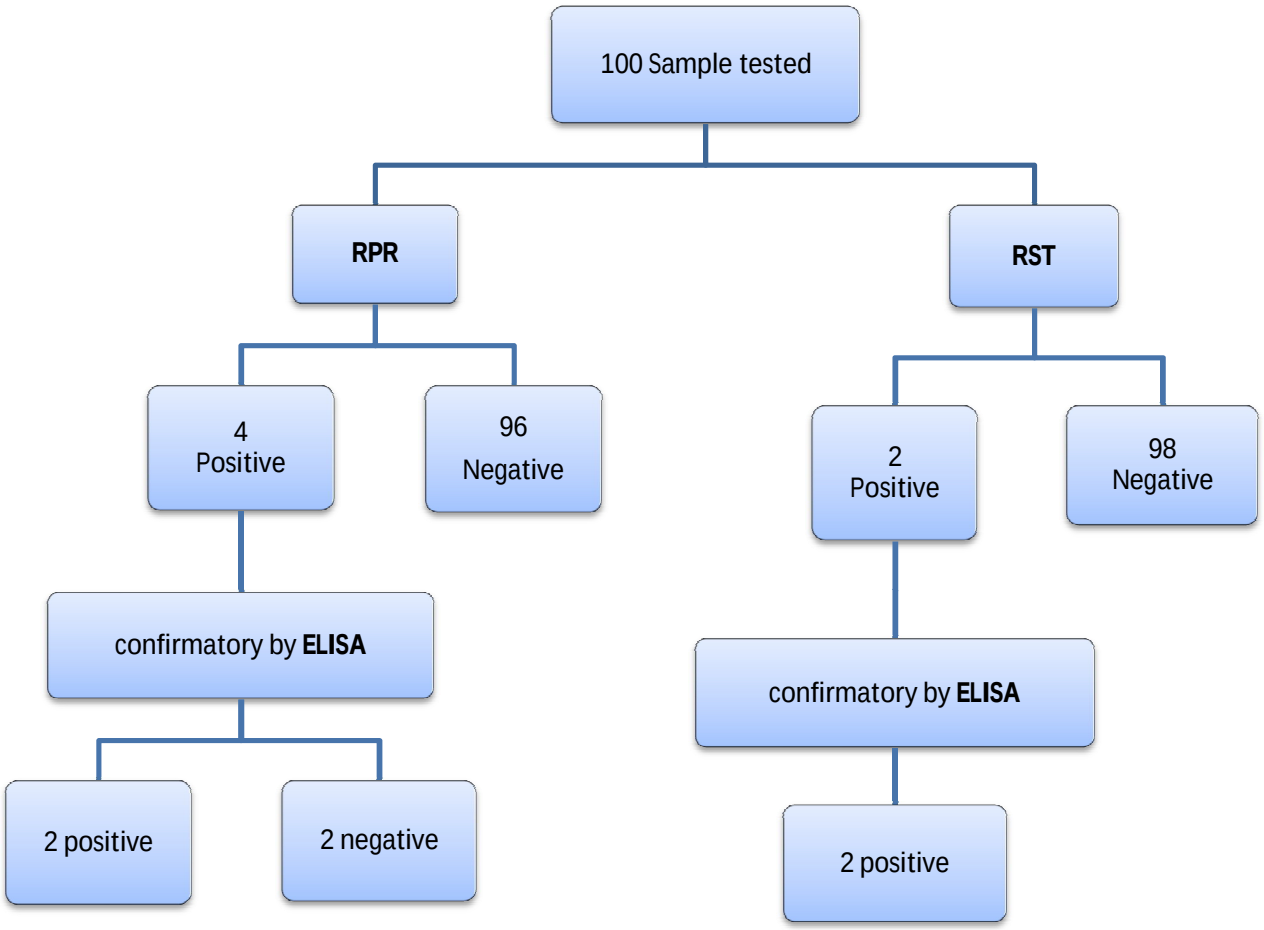


Fig 2. The results of three serological tests (RPR, RST and ELISA)

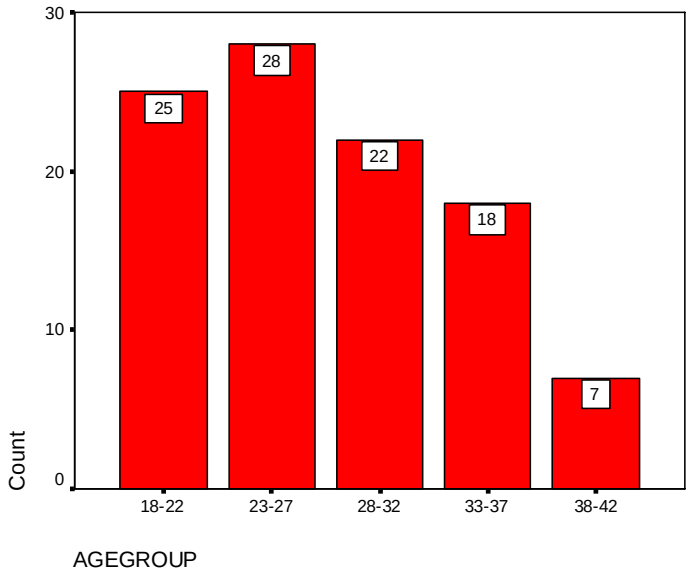


Fig.3: Distribution of the population according to the age group (year)

Table 1: Distribution of syphilis among different age groups

Age group	Total cases tested	Positive cases
18-22 year	25	0
23-27 year	28	1
28-32 year	22	1
33-37 year	18	0
38-42 year	7	0
Total	100	2

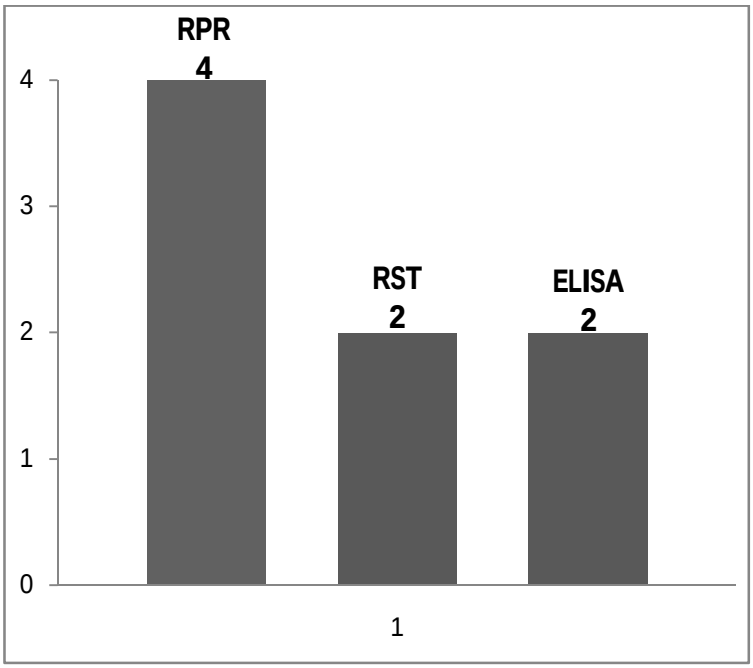


Fig4. Percentage of positive cases by the three Serological techniques.

Table 2. Relation between tests results and previous history of abortion.

Subject	Positive cases	negative cases	total
With history of abortion	1	8	9
With no history of abortion	1	90	91
Total	2	98	100

CHAPTER FIVE

DISCUSSION

Chapter five

5.1 DISCUSSION:

Syphilis is a multistage disease that is usually transmitted through contact with active lesions of a sexual partner or from an infected pregnant woman to her fetus (Stamm, 2002). Efforts to eliminate syphilis have met with only modest success (Hook and peeling, 2004). Despite the availability of new diagnostic tests and antibiotic therapy, syphilis has reemerged in several developed countries.

The prevalence of syphilis obtained in this study was found to be 2 %, it is relatively, similar to previous reports of WHO (WHO,1999). However, other study conducted in pregnant women in Ethiopia showed seropositivity of syphilis was 2.9% (Kebede and Chamiso, 2000). This results disagree with that obtained by Abdelbagi *et al* (2008) dispite the same location.

Reports from South Sudan revealed high prevalence of syphilis among pregnant women, this reported that Of the 231 pregnant women participants, 51 (22.1%) were positive for syphilis with the rapid plasma reagin test. The prevalence of syphilis was high in pregnant women with a gestation period of 18-24 weeks which is the second trimester of pregnancy. This finding could be attributed to a possible common behavior or practice where pregnant women tend to utilize antenatal care clinics from their second trimester of pregnancy (Emmanuel *et al*., 2010). In North-west Ethiopia the majority of pregnant women with syphilis were in their third trimester of pregnancy (Azeze *et al*.,1995).

Prevalence of syphilis among pregnant women was 1.03% in South Africa (Mullick *et al*., 2005). But it was 0.02% in Saudi Arabia (Sharifa and Al-Sibiani, 2008).

Among the two positive cases one showed history of previous abortion while the other one had no previous abortion and the past history of genital ulcers in pregnant women in this study was not found to be associated with positive cases of syphilis.

Due to the limited number of seropositive subjects (only two) of this study was difficult to find association between the age of the patient, history of abortion with sero-positivity to syphilis.

There were two RPR positive cases which were not confirmed by ELISA, This might be due to the fact that RPR was not a specific test, false positive results have been reported in some diseases and pregnancy and, therefore, must be confirmed by treponemal tests. RPR was highly sensitive compared to RST. RST is more specific than RPR, is used to detect specific antibodies against the spirochetal antigens (West *et al.*, 2002).

5.2 Conclusion:

Seroprevalence of syphilis in this study was 2% which was relatively similar to WHO (2.4%)

This study showed that RPR test was more sensitive compared to RST, which was more specific.

There is a relationship between the age and disease (the two positive cases was among the age group 23-27 year and among age group 28-32 year).

5.3 Recommendations:

1- Further indepth researches including large sample size are recommended.

2- All pregnant women should be screened serologically for syphilis at the first prenatal visit and at delivery.

3- Universal screening in pregnancy, will reduce vertical transmission and all the adverse effects of congenital syphilis.

4- Regular health education for pregnant women in antenatal clinics, to inform them about their health and the risk of syphilis to both born and the un-born child are required.

5-Use of advanced technigue e.g PCR is required.

6- For diagnosis of syphilis all results must be confirmed by more specific method.

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Appendix (1)

Date:……..

Sample number:……

Name:……

Age:…….....

Resident:…….....

Gestational Period:……

Tel.…….

Did you have history of previous abortion?

A) Yes

B) NO

Did you have history of previous genital ulcer?

A) Yes

B) NO

Did you administrate any treatment?

A) Yes

B) No

Signature…….

Appendix (2)

1 Equipments:

Centrifuge

Automatic micropipette

Mechanical rotator

Alarm clock

Microwell reader

Microwell washes system

Incubater

2 other materials:

alcohol.

Tourniquet

cotton

sterile syringe 5 mls

plain containers

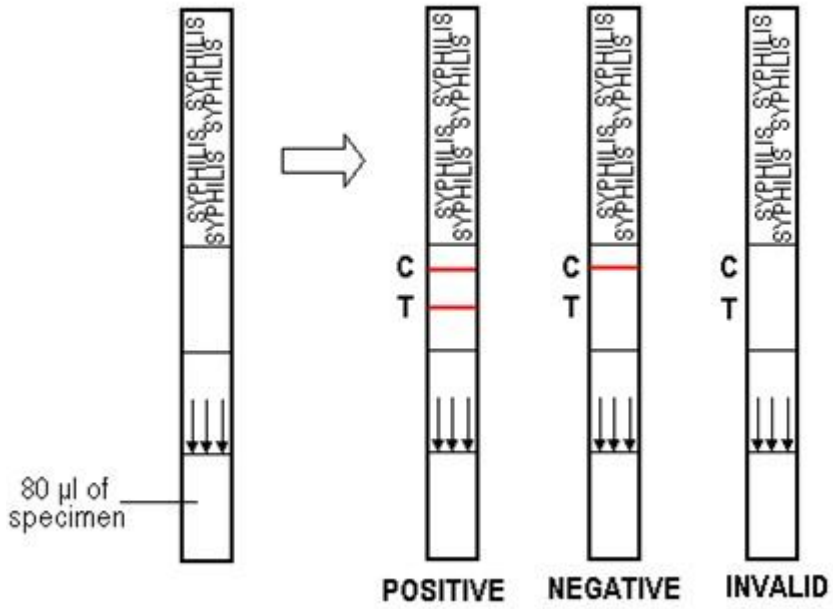
Disposable gloves

Safety box

Disposable yellow tips

Test tube and racks

Appendix (3)



Syphilis Antibody Rapid StripTest