بسم الله الرحمن الرحيم

Sudan University for Sciences and Technology

College of Graduate Studies

Serodetection of Syphilis among Recurrent Miscarriage and Non Miscarriage women in Wad Madani Teaching Hospital

الكشف المصلي لمرض الزهري لنساء الإجهاض المتكرر وغير المجهضات في مستشفى ود مدني التعليمي

A dissertation submitted for partial fulfillment for the requirement of MSc degree in Medical Laboratory Sciences (Microbiology)

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الآيـــــة

قال تعالى :

(نَرْفَعُ دَرَجَاتٍ مِّن نَّشَاء وَفَوْقَ كُلِّ ذِي علْم عَلِيمٌ)

سورة يوسف (76)

Dedication

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I dedicate this research to:

My mother

My father

The oasis of

Kindness and forgiveness

To my family

My friends

To my supervisor prof. Yousif Fadlalla

Who enlighten my way

As a torch of hope

And success

Acknowledgments

First of all my deep thank to ALLAH , and to my supervisor Prof. Yousif Fadlalla for his guidance , understanding and encouragement through out the study.

My appreciations to all working staff of Wad Madani Teaching Hospital in Al Gazira State for giving me the permission and their cooperation to do this study.

It would not have been possible to write this thesis without and support of the Kind people around me , to only some of Whom it is possible to give particular mention here.

Abstract

This study was conducted among recurrent miscarriage and non miscarriage women in Wad madani teaching hospital to determine the prevalence of syphilis among those with previous miscarriage and those who had no miscarriage before . A total of ninety samples (n=90) were collected, during the period from April to June 2018, to demonstrate the frequency of syphilis and to compare between different diagnostic methods used for detection of syphilis.

All samples were tested by using immunochromatography test (ICT), and ELISA.

50% of the participant had recurrent miscarriage women, and 50% as confirmatory test had no miscarriage before (normal delivery).

All samples were negative by ICT , and 7 were positive by using ELISA technique (specific treponemal antibodies were detected).

Six of positive samples were detected in non miscarriage women (control) and one positive was in women who had previous miscarriage (cases).

2 positive cases were among age group 15-25 years, 4 among 26-35 years, and 1 among 36-45 years.

ملخص الاطروحه

في هذه الدراسة تم جمع عدد تسعين عينه دم من مستشفى ود مدني التعليمي قسم النساء والتوليد بغرض معرفة الانتشار المصلي لمرض الزهري في النساء اللاتي تكرر عندهن الاجهاض واللاتي لم يحدث لهن اجهاض .تم اجراء هذا البحث في مستشفى ود مدني التعليمي وذلك في الفتره من ابريل وحتى يونيو 2018. كل العينات تم إختبارها باختبارين مختلفين ، اولها الكشف عن الاجسام المضاده بطريقة الكشف المناعيه السريع ICT وثانيها باستخدام فحص الانزيم المناعي المرتبط .

كل العينات اعطت نتائج سالبة باستعمال الفحص المناعي السريع 7 من العينات أعطت نتائج موجبة باستخدام فحص الانزيم المناعي المرتبط.

المشاركون في هذه الدراسه 50% نساء اجهضن بصوره متكرره و50% لم يجهضن.

6 حالات ايجابية في مجموعه النساء اللاتي لم يجهضن و حاله واحده في مجموعه النساء اللاتي اجهضن.

بحسب العمر كان توزيع الحالات الايجابيه كالاتي : حالتين في الفئة العمريه مابين 15-25 ، وأربعه حالات ايجابيه في الفئه العمريه مابين 26-35 ، وحاله واحده في الفئه العمريه 36-45 .

Table of contents

List of tables

Introduction

Syphilis is primarily a sexually transmitted disease (STD) caused by the bacteria *Treponema pallidum* (Genc and Ledger, 2000). *Treponema pallidum* has a characteristic helical shape, and is a member of the Spirochete family of bacteria. Sometimes Syphilis infection can be transmitted by blood transfusion or transmitted directly from the mother to a baby during pregnancy (Sanchez and Wendel, 1997).

(*T. pallidum*) causes acquired syphilis which transmitted congenitally or by sexual intercourse. Infectious syphilis includes patients with primary, secondary or early latent infection (Watson *et al*.,2002).

Vasculitis which results from infection with *T. pallidum* underpins the various manifestations of syphilis. At the site of inoculation a papule appears which rapidly ulcerates to form a chancre (Correa, 1994). The organisms multiply at the site of the ulcer and spread to the local lymph nodes. Subsequently, the Treponemes are disseminated haematogenously, most of the organisms are destroyed, with and, along with immune complexes, and it's responsible for the manifestations of secondary syphilis, only small foci remaining in the spleen and lymph nodes. These foci containing organisms are responsible for the persistence of serological markers of infection and these remaining treponemes after many years may lead to the manifestations of tertiary, neurological and cardiovascular syphilis (Mascola *et al*.,1984).

Congenitally infected baby stillbirth, miscarriage usually results from syphilis infectious during pregnancy (Saloojee *et al*.,2004). In early latent (asymptomatic) syphilis the risk of vertical transmission remains about 30% to 60 %. But risk of transmission diminishes as maternal syphilis advances (Buff *et al*., 2007).

Maternal infection is, however, entirely treatable with penicillin which also prevents vertical transmission and can be diagnosed by serological methods (Mullick *et al*., 2005) during the first two years of infection. Transplacental transmission is usually occurs but it is rare after four years, although cases of transmission up to 10 years after acquisition of syphilis have been reported (Yang *et al*., 2009).

About a third of babies born to mothers with early syphilis are born without infection and a third with congenital syphilis; a third of pregnancies will result in stillbirth or early- trimester miscarriage . Between half a million and a million cases of congenital syphilis occur each year worldwide, and in some poor countries up to a fifth of neonatal mortality is directly attributable to syphilis (Ferguson and Varnado, 2004). Congenital syphilis is classified as either early or late congenital syphilis depending on whether it presents before or after 2 years of age, the prognosis is particularly poor if symptoms of syphilis are present in the first few weeks after birth (Grros *et al*., 2013). Congenital syphilis can be easily prevented by antenatal screening for syphilis and treatment during pregnancy (Ray, 1995).

Penicillin is effective in the treatment of all stages of syphilis *T. Pallidum* grows very slowly, which requires that the penicillin be present in bactericidal concentration for weeks . If the patient is allergic to penicillin, doxycycline can be used but must be given for prolonged periods to effect a cure (Katz and Klausner,2008).

1.1 .1 Rationale

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 was 9% reported by Abdelbagi *et al* (2008) and 2.4% according to WHO(1999).

It is important to screen for syphilis among pregnant women in first trimester, because primary syphilis may be asymptomatic. 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.

Screening and treating mothers for syphilis during pregnancy can prevent adverse pregnancy outcomes associated with maternal infection.

This study sought to determine the prevalence of syphilis among the miscarriage and non miscarriage women in Al Gazira state.

1.1.2 Objective:

1.2.1 General objective:

To determine sero-detection of syphilis among spontaneous recurrent miscarriage and non miscarriage women in Gezira state.

1.2.2 Specific objective:

To detect treponemal antibody by using ICT.

To confirm result by using ELISA.

To compare between ICT and ELIZA.

CHAPTER TWO
LITERATUE REVIEW

Literature Review

2.1 History of syphilis:

In 1831 Ricord has designed a larger study on syphilis and gonorrhea and succeeded to show that the last occurs only after contact with gonorrohea patients, whilst the former only after contact with syphilis patients (Tampa *et al;* 2014).

It was not earlier than 1905 that Schaudinn (1871-1906) and Hoffman (1868-1959) have discovered the etiologic agent of syphilis, whom they have named *Spirochaeta pallid*, on various syphilis lesions, proving its existence in both fresh and Giemsa coloured specimens. It was them who changed the name of the bacterium subsequently to *Treponema pallidum*.(Tampa *et al;* 2014)

In 1906 Landsteiner introduced the use of the dark-field microscopy method for the detection of the *spirochete* of syphilis.In 1910 the German bacteriologist August Wasserman(1866-1925) came with the first serologic test for syphilis and in 1949 Nelson and Mayer have conceived *Treponema pallidum* immobilization test (TPI)the first specific test for *T.pallidum* (Tampa *et al;* 2014).

2.2 Previous Study in Sudan and other different countries:

Globally, about 1 million pregnancies are affected each year by syphilis due to maternal infection, and because of these about half of the pregnancies result in stillbirth or neonatal death (Walker and Walker, 2002). In many developing countries syphilis remains a major cause of adverse pregnancy outcome (Schmid, 2004). There are several pregnancy outcomes associated to maternal syphilis, which are spontaneous abortion, stillbirth, low birth weight (LBW), premature delivery and congenital syphilis (Radolf, *et al.,* 1998). Antenatal syphilis screening is inexpensive and effective towards the reduction of syphilis impact on pregnancy, and yet syphilis continues to be a threat to pregnant women in low resource settings (Walker and Walker, 2002). A study in Mozambique has

shown that about a third of babies born to mothers with early syphilis are born without infection, a third are born with congenital syphilis, and a third of pregnancies will result in miscarriage or stillbirth (Gloyd *et al.,* 2007). Prevalence of antenatal syphilis in Africa ranges from 3% to 17% (Temmerman, *et al.,* 2000). In resource-poor countries syphilis is responsible for up to a fifth of neonatal deaths (Watson-Jones, *et al.,* 2002).

The 1993 World Development Report (WDR) cites antenatal syphilis screening as one of the most cost effective ways to improve children's health (World Bank Development Report, 1993). Despite this, only an estimated 38% of women attending for antenatal care in Africa receive syphilis screening (Gloyd, *et al.,* 2001).

Congenital syphilis, in particular, is estimated to inflict over 1.5 million pregnant women in Sub-Saharan Africa with approximately 60% of the acute cases leading to foetal death. This amounts to nearly 500,000 infant deaths from syphilis in sub-Saharan Africa alone, rivalling those due to HIV (Schmid, 2004). A study in Tanzania found that maternal syphilis was responsible for some 50% of all stillbirths (Watson-Jones, *et al.,* 2002). Different studies on maternal syphilis in African countries showed high rates of seropositivity for example, 4% in Kenya and Malawi, (Bique, *et al.,* 2000) 6 to 15% in South Africa, 8% in Zambia, 14% in Zimbabwe, and 5–15% in Mozambique (Bique, *et al.,* 2000). Meanwhile 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).

Antenatal syphilis screening is a written national policy in nearly all African Ministries of Health; however, screening is performed sporadically at best. In 1997 survey completed by 22 sub-Saharan Africa countries produced estimates

that fewer than 38% of women already attending antenatal care were likely to have been screened. Failure to screen for syphilis in pregnancy was estimated to have resulted in at least 1 million missed opportunities annually to have identified and treated pregnant women with active syphilis (Gloyd, *et al.,* 2007). While prevalence of maternal syphilis reported in some sites of southern Sudan was to range from 12% to 21% in 2007 (Surveillance report, 2007).

2.3 causative organism:

Syphilis is caused by *Treponema pallidum* sub species pallidum. It is delicate motile organism (Rothschild, 2005).

2.3.1 Characteristics of *Treponema Pallidum:*

T.pallidum has not been grown in bacteriologic media or in cell culture . It grows very slowly . The medical importance of that fact is that antibiotics must be present at an effective level for several weeks to kill the organism and cure the disease . It belongs to family Spirochetes . The Spirochetes are Gram negative bacteria, long, thin, helical and highly motile, contain flagella between peptidoglycan and outer membrane (Wright and Jones,2003).

The bacterium size ranges from a length of 6 mm to 20 mm and a width of 0.10 mm to 0.18 mm, which means light microscopy is inadequate for its visualization, however, it can be viewed by using dark field microscopy (Peeling and Hook,2006;Woods,2005).

Treponema Pallidum cannot be cultured and have slow generation time of 30 to 33 hours, which may limit research work but also implies that an antibiotic with long half- life require for treatment. Only a few generation of *T.Pallidum* have been cultivated on rabbit epithelial cell monolayers when stored at 33^0 C to 35^0C under micro –aerobic condition. Rabbit models been used successfully for in vivo

propagation of the spirochetes by inoculation the testis (rabbit infectivity test). 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) . Also one of the most important characteristic is drying kill the spirochetes rapidly and also heating, trivalent arsenical, mercury and bismuth.

2.3.2 Metabolism

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

2.3.3 Genome :

The *T. pallidum* genome is a circular chromosome of a pproximately 1,138,000 base pairs,which is small for bacteria. Most pathogenic bacteria have transposable elements, but *T. pallidum* does not ,which suggests that the genome is highly conserved and may explain its continued susceptibility to penicillin. There are few genes involved in energy production and synthesis of nutrients, indicating that *T. pallidum* obtains these from the host (Carrol *et al;* 2016).

2.3.4 periplasmic flagella and motility :

Spirochetes contain axial filaments known as end flagella which found between peptidoglycan layer and outer membrane, also contains an outer sheath of glycosaminoglycan . 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 sub terminal end of the cell . The classical flagella appearance includes a basal body –motorcomplex , hook and filament (Liu *et al* ., 2010*)*. The protenins involved in bacterial motility can be associated with the chemotaxis system or the basal complex . A type **III** secretion system is believed to be necessary for auto-

assembly of the flagella and once the apparatus is completed , a chemotaxis influence determines the direction of motility (Rajagopala *et al* .,2007).

2.4 Transmisstion:

Venereal syphilis is a worldwide disease of only humans; there is no animal reservoir **(**LaFond and Lukehart, 2006). Syphilis is acquired by direct contact, usually sexual, with active primary or secondary lesions. Studies have shown that 16 to 30% of individuals who have had sexual contact with a syphilis-infected person in the preceding 30 days become infected (Peeling *et al.,* 2004). Actual transmission rates may be much higher. Infection also occurs when organisms cross the placenta to infect the foetus in a pregnant woman causing congenital syphilis and this happens particularly during the first two years of infection **(**LaFond and Lukehart, 2006). *T.pallidum* may also occasionally be transmitted as a blood-borne infection (Peeling and Hook, 2006).

2.5 Epidemiology of syphilis:

Syphilis occurs world wide, and its incidence is increasing .It is one of the leading notifiable disease in the United States .Many cases are believed to go unreported , which limits public health efforts . There has been a marked increase in incidence of the disease in homosexual men in recent years.(Levinson, 2014). The wide spread epidemics of syphilis that occurred in Russia in 1990 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).

2.6 Clinical Manifestation for Syphilis

Syphilis is a chronic illness which, without treatment, may proceed through the primary, secondary, and tertiary stages over a period of many years (Peeling and Hook, 2006).

The primary stage is typically marked by the appearance of a single painless lesion (the hard chancre) at the site of inoculation this appears on average about 21 days post-infection. In a few cases, there may be multiple primary lesions. Primary lesions most often occur on the genitalia and may be accompanied by regional lymphadenopathy. Even without treatment, primary lesions typically resolve spontaneously due to the painless chancre and occurrence at invisible sites (Peeling and Hook, 2006). While the lesions of primary syphilis are localized to sites of initial inoculation, the pathogen is thought to invade intercellular junctions of the endothelium, resulting in haematogenous dissemination of the organism during the primary stage, seeding the central nervous system and remainder of the body. Resolution of primary lesions is followed on average (6–8 weeks later) by the secondary stage, at which time manifestations of dissemination may occur at virtually any location or organ but most commonly at other cutaneous and mucosal locations (French, 2007). Secondary syphilis may lead to a broad range of syndromes such as hepatitis, iritis, nephritis, and neurological problems (early meningovascular syphilis) with headache and involvement of the cranial nerves, particularly the (auditory) nerve. These complications of secondary syphilis are relatively uncommon, occurring in less than 10% of individuals (Rompalo, *et al*., 2001).

Sexual transmission of syphilis occur following lesion contact and thus it is effectively limited to persons with primary and secondary manifestations of

infection. Again, even without treatment, both primary and secondary lesions resolve and the infection enters a 'latent' stage in which clinical manifestations are absent. Despite the absence of clinical manifestations, during the latent stage of untreated disease, the infection can still be passed to children born of untreated infected mothers. Many years later, few people with latent syphilis may progress to late (tertiary) manifestations including neurosyphilis, cardiovascular disease, and lesions of the skin, bones or viscera (gummata) (French, 2007).

In the pre-antibiotic era showed that 15–40% of untreated infected individuals develop recognizable late complications (Lindstrand, *et al.,* 1993). Clinical manifestations in congenital syphilis are classified as either early or late congenital syphilis depending on whether it presents before or after 2 years of age. The prognosis is particularly poor if symptoms of syphilis are present in the first few weeks after birth (French, 2007).

2.7 Congenital Syphilis:

Congenital syphilis is a rare disease in most developed countries; but it remains a severe pregnancy outcome in developing countries (Walker and Walker, 2002). *T. pallidum* can be transmitted from the bloodstream of the infected woman to her developing fetus at any time during pregnancy, although risk of fetal infection is much higher during early maternal syphilis (the first year of infection) than during later stages (Sheffield, *et al.,* 2002).

Transmission of syphilis to a fetus depends largely on the duration of the disease in the mother. A long interval between infection and pregnancy results in a benign outcome in the infant (Lindstrand *et al.,* 1993). The risk of transmission is 70%–100% in women with primary or secondary syphilis, 40% with early latent syphilis

and 10% in late latent cases. About 40% of pregnancies in women with infectious syphilis result in the death of the fetus (Finelli, *et al.*, 1998).

Antibiotic treatment of the mother during the first two trimesters is usually sufficient to prevent negative outcomes, but later treatment or lack of treatment may result in foetal death, foetal damage, or birth of an infected infant (Mascola, *et al.*, 1985). Destructive effects are thought to depend upon the immune response of the foetus and include spontaneous abortion, stillbirth, and premature delivery. Affected infants typically have low weight at birth, and infants with congenital syphilis may be underweight even relative to other infants of the same gestational age (Mascola, *et al.*, 1985). Pulmonary haemorrhage, secondary bacterial infection, and severe hepatitis cause death of approximately 4% of *T. pallidum*-infected neonates soon after delivery (LaFond and Lukehart, 2006).

2.8 Laboratory diagnosis:

In laboratory diagnosis always there are three important approaches. Specimens can be pus or tissue fluid from lesions to look for spirochetes and blood serum for serological tests. (Emerson, 2009).

2.8.1 Direct diagnostic methods include:

Darkground microscopy is the traditional method for direct detection of *T.pallidum* in lesion exudates, It can provide rapid results, with identification of the organism by its characteristic morphology and motility. Immunofluorescence using fixed smears of lesion material, or tissue specimens, has several advantages and is of comparable sensitivity ,neither technique differentiates between the pathogenic treponemes, the resluts were non-specific.More recently described monoclonal antibodies are more specific (Gillesspie and Hawkey, 2006).

2.8.2 Indirect diagnosis:

Is based on serological tests for the detection of antibodies.

2.8.3 Serological tests:

Fall into two categories: non Treponemal tests for screening, and Treponemal tests for confirmation (Fears and Pope, 2001).

2.8.3.1 Non-treponemal tests:

These tests involved the use of non treponemal antigens . Extracts of normal mammalian tissue (e.g cardiolipin from beef heart) react with antibodies in serum samples from patients with syphilis. These antibodies, which are a mixture of IgG and IgM, are called regain antibodies . These tests are positive in most cases of primary syphilis and are almost always positive in secondary syphilis.

The titer of this nonspecific antibodies decrease with effective treatment, in contrast to the specific antibodies , which are positive for life.

False positive reaction occur in infections such as leprosy, hepatitis B, and Infectious mononucleosis and in various autoimmune disease . (Edwards, 2000).

2.8.3.2 Treponemal tests :

These tests involve the use of treponemal antigens and therefore are more specific than those described earlier. In these tests, *Treponema Pallidum* reacts in immunofluorescence or hem agglutination assays (TPHA) with specific treponemal antibodies in the patient serum .

These antibodies arise within 2 to 3 weeks of infection; therefore , the test results are positive in most patients with primary syphilis .The tests remain positive for life after effective treatment and cannot be used to determine the response to treatment or reinfection .They are more expensive and more difficult to perform than the nonspecific tests and therefore are not used as screening procedure .

Treponema Pallidum Particle Agglutination (TPPA) these are very valuable and simple tests using an indirect haemagglutination method with red cells or by gelatin particle together with Venereal Disease Research Laboratory(VDRL) , it is probably the best combination for routine use.

False positive reaction occur in up to 2% (Wright and Jones,2003)

2.8.3.3 Immunochromatographic based strips (ICT):

The most Simple rapid tests for treponemal antibody commercially available. An evaluation of these assays was recently published by the WHO sexually Transmitted Diseases Diagnostics Initiative, The rapid assays are potentially suitable for non laboratory use in the developing world, and they may also have a role as point of care tests elsewhere (Gillesspie and Hawkey 2006).

2.8.4 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 . Some of these molecular methods used for detection of the pathogen include the use of PCR and Real time PCR assays the application of PCR in the detection of *T.pallidum* DNA has the advantage of being a diagnostic method with the ability to characterize strains susceptible to macrolide antibiotics. The sensitivity of PCR detection assay has been found depending on the specimen types and the stages of the disease (Morshed *et al.*, 2007).

2.9 Treatment:

Penicillin is effective in the treatment of all stages of syphilis . A single injection of benzathine penicillin G (2.4 million units) can eradicate *T.pallidum* and cure early (primary and secondary) syphilis .Benzathine penicillin is used because the penicillin is released very slowly ,from this depot preparation . *T.pallidum* grows

very slowly , which required that the penicillin be present in bactericidal concentration .If the patient is allergic to penicillin , doxycycline can be used but Must be given for prolonged periods to effect a cure . In neurosyphilis , high doses of aqueous penicillin G are administered because benzathine penicillin penetrates poorly into the central nervous system .No resistance to penicillin has been observed .However ,strains resistant to azithromycin have emerged.

Pregnant women with syphilis should be treated promptly with the type of penicillin used for the stage of their disease. (Workokowski and Berman, 2010)

2.10 Prevention

To prevent congenital syphilis, it is imperative to screen for syphilis early in pregnancy; but this remains a challenge. A study found that pregnant women who came for a first antenatal care visit had a median gestational period of 20 weeks (Saloojee, *et al.*, 2004). Promotion of earlier attendance at antenatal care is a simple prevention strategy which should be advocated (Romoren and Rahman, 2006). Another study also determined that antenatal screening and treating women during pregnancy can prevent maternal syphilis (French, 2007). Interventional program launched in Nairobi by the Nairobi City Council department of public health in early 1990s, has been subjected to monitoring and evaluation; and shows that the effort lead to the downward trend of syphilis during pregnancy from prevalence of 7.2% in 1993 to 3.4% in 1998, and also changes in health seeking behaviour and improved health care (Temmerman, *et al.*, 2000). Likewise in Shenzhen (China) a screening and interventional program led to high increase in coverage for antennal care clinics; which lead to a cost effective decrease in incidence of congenital cases by half from 43.3 per 100,000 to 22.0 per 100,000 pregnant woman screened in 2002 and 2003 (Cheng, *et al.*, 2007).

CHAPTER THREE

MATERIALS AND METHODS

Materials and methods

3.1 Study design:

This is a case control study conducted in Gezira State (Wad Madani)

3.2 Study area:

This study was conducted in Gezira State (Wad Madani teaching hospital Department of Obstetrics and Gynecology).

3.3 Study population:

Pregnant women with and without history of miscarriage were included.

3.4 Study duration:

Study was carried out during April to July, 2018.

3.5 Sample size:

A total of 90 plasma samples were collected .

3.6 Inclusion criteria:

Pregnant women of more than 18 and less than 44 years old.

3.7 Exclusion criteria:

Pregnant women of less than 18 and a bove than 44 years old.

3.8 Data collection:

Data were collected by using a questionnaire and filled by the investigator during each time when blood samples were collected.

3.9 Ethical consideration:

Permission to carry out the study was obtained from the College of Graduate Studies Sudan University of Science and Technology, verbal consent was taken from each patient after informing the her with the objective of the study.

3.10 Specimens collection , preparation and storage:

A volume of 5 ml blood were collected from each patient using venipunctures technique then displaced into Ethylene diamine tetra acetic acid (EDTA) container.

Each blood specimen was centrifuged at 3000 g for 5 minutes to obtain the plasma. The later was gently collected into plain container and stored at -20 °C until the serological analysis.

3.11 Laboratory Examination:

All specimens were tested for antibodies against syphilis by using screening serological test, Immunochromatographic test (**ICT**) . All these specimens were confirmed by sandwich ELISA.

3.11.1 Immunochromatographic based strip test (ICT):

3.11.1.1 principle

The SD BIOLINE Syphilis 3.0 contain a membrane strip, which is pre-coated with recombinant *Treponema pallidum* antigens (17,15 KDa) on test band region. The recombinant *Treponema pallidum* antigens-colloid gold conjugate (17,15 KDa) , patient sample and sample diluents moves along the membrane chromatographically to the test region (T) and forms a visible line as the antigen – antibody-antigens gold particle complex forms. Therefore, the formation of a visible line in the test region (T) indicates a positive result for the detection of *Treponema pallidum* specific antibodies (IgG, IgA, IgM) . when the *Treponema Pallidum* specific antibodies (IgG, IgA, IgM) are absent in the sample , no visible color band in the test region (T).

3.11.1.2 Procedure:

The test components and the specimen were brought to room temperature and then processed.

About 10 µL of plasma were placed on the sample well (s) used micropipette.

4 drops of diluent were added into sample well (s).

The results were interpreted within 5-20 minutes.

3.11.1.3 Interpretation of assay result:

- **Positive result:**

  If both control and test bands were developed.

- **Negative result:**

  If only control band is developed.

- **Invalid**

  If no control band is developed.

3.11.2 Enzyme Linked Immunosorbent assay:

The reagents were supplied by Fortress Diagnostic-England . Fortress syphilis Elisa is an invitro diagnostic kit for the detection of antibodies to *Treponema pallidum* in human serum or plasma.

3.11.2.1 Principle of the assay:

The detection of anti-TP antibodies is a chieved by antigen sandwich enzyme linked – immunosorbent assay. Where the micro wells are coated with recombinant *Treponema pallidum* antigens expressed in *E.coli* . The samples are incubated in the micro wells together with recombinant TP antigens conjugated to Horse Radish Peroxidase (HRP). The pre-coated antigens express the same epitopes as the (HRP) conjugate antigens , but are expressed in different hosts . In case of presence of anti-TP antibodies 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 immunocomplex is captured on the solid phase.

After washing to remove sample and unbound conjugates , chromogenic solution containing TMB and urea peroxidase are added in to the wells . In the presence of the antigen - antibody sandwich complex , the colorlesschromogenic is hydrolysesby the bound HRP conjugate to a blue colored product , which turn yellow upon addition of the stop solution . This color is then read photometric ally and is directly proportional to the amount of the antibody in the sample .Wells containing samples negative for anti – TP remain colorless.

3.11.2.2 Storage and stability:

Kits: Components stored at 2-8°C.

Specimens: Plasma stable for 72 hours at 2-8°C if delay occur stored frozen at -20°Cor less. Multiple freeze – thaw cycles should be avoided. Samples should be free from contamination and haemolysis.

3.11.2.3 Procedure:

Reagent preparation: The reagents and samples allowed to reach room temperature (18-30°C) for at least 15 – 30 minutes. The wash buffer concentrate was checked for the presence of salt crystals. If crystals have formed in the solution, resolubilize by warming at 37° C until crystals dissolve. The stock wash Buffer was diluted 1 to 20 with distilled or deionized water. Useing clean vessels to dilute the buffer.

Numbering wells: The strips needed was set in strip holder and numbered sufficient number of wells including three negative control, two positive control and one Blank.

Adding HRP conjugate: 100µl HRP conjugate were added in to each well except the Blank well.

Adding sample: 20 µl of positive control, negative control, and specimen were added into their respective wells. Upon addition of the sample the HRP conjugate sample mixture will appear blue. A separate disposable tip was used for each specimen.

Incubating: The plate was mixed by tapping gently and covered over the plate with the plate cover and incubated for 60 minutes at 37°C.

Washing: At the end of the incubation, the plate cover was removed and discarded. Each well was washed 6 times with diluted wash buffer. Each time, the microwells allowed to soak for 30-60 seconds. After the final washing cycle, the plate was turned down onto blotting paper or clean towel, and tapped to remove any remaining solution.

Colouring: 50 µl of chromogen A and 50 µl chromogen B solution were dispensed into each well , and mixed by tapping the plate gently. The plate was incubated at 37°C for 15 minutes avoiding light. The enzymatic reaction between the chromogen solutions and the HRP conjugate produced blue color in positive control and anti – TP positive sample wells.

Stopping reaction: 50 µl stop solution were added into each well and mixed gently. Intense yellow color developed in positive control and anti -TP positive sample wells.

Measuring the absorbance: The reference wavelength was set at **630** nm. The cut-off value were calculated and the results evaluated (the absorbance was read within 5 minutes after stopping the reaction.

3.11.2.4 Interpretation of Result:

According to manufacture instructions as follow:

The results were calculated by relating each samples optical density (OD) value to the cut – off value (C.O) of the plate.

Calculation of cut-off value: Cut-off value (C.O)= *Nc + 0.18

*Nc = the mean absorbance value for three negative controls.

=0.049+0.058+0.037=0.144/3= 0.048

0.048 +0.18=0.228

C.O = 0.228

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

Positive Results (S/C.O. > or =1): Samples giving an absorbance greater than or equal to the cut-off value were considered initially reactive, which indicates that anti treponemal 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 1.1 are considered. Repeatedly positive samples can be considered positive for anti –TP antibodies.

3.11.2.5 Quality control

The test results are valid if the quality control criteria are verified. The OD value of the positive control must be equal to or greater than 0.800 at 450/630nm.

The OD value of the negative control must be less than 0.100 at 450/630nm.

3.12 Data analysis

Statistical Package of Social Sciences (SPSS version 16). Computer software was used for data analysis using Chi-Square test.

CHAPTER FOUR

Result

4.Results:

All specimens were negative by using ICT rapid test . Only 7 of the samples were positive when tested by Enzyme Linked Immunosorbant Assay (ELISA)(table 1).

Distribution of syphilis among miscarriage women and non miscarriage women according to age group (result given by ELISA)

The result exhibited in table 2 showed that seven positive cases , 2 positive cases in age group {15-25} , 4 positive cases in age group {26-35} and 1 positive cases in age group {36-45}.

Table 1: The distribution of positive and negative results using ICT and ELISA

Result	**ICT**	**ELISA**
Positive (+)	0	7
Negative (-)	90	83
Total	90	90

P>0.5

Table 2: Distribution of positive cases among different age groups

Age group	**Total cases Tested**	**Positive cases**	**%**
15 - 25	32	2	2.22
26 - 35	43	4	4.44
36 - 45	15	1	1.11

P>0.5

CHAPTER FIVE

DISCUSSTION

Discussion

Syphilis is a chronic infectious disease caused by the spirochaete *T. pallidum.* It has significant long-term morbidity for mothers, and can cause severe complications in pregnancy, which may result in spontaneous abortion, stillbirth and other negative outcomes including congenital syphilis. Congenital syphilis results in serious sequelae in live born infected children (Genc and Ledger, 2000).

The aim of this study was to detect sero-prevalence of syphilis disease among spontaneous recurrent miscarriage and non miscarriage women were enrolled in AL Gezira State.

Blood samples were collected from 45 recurrent miscarriage women and 45 non miscarriage women of different ages.

50% Non miscarriage women (control) and 50% miscarriage women (cases)

All serum samples were examined by using screening nonspecific serological tests (ICT) which gave negative result for all samples , and then confirmed the result by ELISA test. 7 samples showed positive result.

The positive result were found in the range 15-25 (2), 26-35(4), and the last one in 36-45. Six positive result were found in control group and one positive result found in case group .

This result is similar to the study of (Jabbar, 2015) which was carried out on 400 unscreened for syphilis pregnant women admitted in labour or because of abortion to the department of obstetrics and Gynecology of Abu-grab Hospital in Baghdad, Iraq during March 2012 to January 2013, from 400 patients admitted during the period of this study, 270 patients were in labour 90 of those were primiparous women, 180 were multiparous the rest (130) were cases of abortion. The results of serological test showed out of 400 patients only 12 (3%) were

positive, three (25%) primiparous women,7 (58.33%) multiparous and 2 (16.66%) aborted.

syphilis reemergence as a major problem in suburban area with high risk of pregnancy loss, most of the patients infected with syphilis were multiparous women (Jabbar, 2015) , this could be due to that consecutive pregnancy and labour lead to increased susceptibility to infection as a result to depressed the immune system and increase exposure to contaminants medical material infected with *Treponema pallidum* (Liu *et al*., 2011). Result of current study demonstrated that the age of patients rang of (19-30) years, which is in agreement with other previous study who found increase incidence of syphilis among reproductive age group (Arnold and Ford 2000; Lan and Nathali, 2008).

Syphilis prevalence data from the rest of Africa has been reported to range from a low of 2.5% in Burkina Faso, 13.7% in North West Ethiopia (Azize *et al.,* 1995), 17.4% in Cameroon (WHO, 1999) and a high of 42% in Mozambique (Folgosa *et al.,* 1996). It is nearly similar to study done in Khartoum the capital of Sudan by Abdel-bagi *et al.,* (2008), reported maternal syphilis to be 9% among the antenatal care attendees.

5.2 Conclution:

The result of this study conclude that:

The serodetection of syphilis obtained in this study among recurrent miscarriage women and non miscarriage women was 7.8 %.

Infection with syphilis is higher in the age group 26 – 35 years.

5.3 Recommendation:

There is a need for regular health education for pregnant women in antenatal clinics to inform them about their health, avoidance of risky behaviors and the risk of syphilis to both born and the un-born child.

1- More studies are needed with large sample size to support the result which was obtained in this work.

2-ICT can be used just for screening but to confirm the diagnosis for the patient, it is better to use other more advanced techniques like ELISA or more better Polymerase chain reaction (PCR).

3-For the diagnosis of syphilis all results must be confirmed by more specific and sensitive methods like PCR .

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Appendixes

Appendix(1)

Sudan University of Science and Technology

College of Graduate Studies

Microbiology Department

Questionnaire

General information:

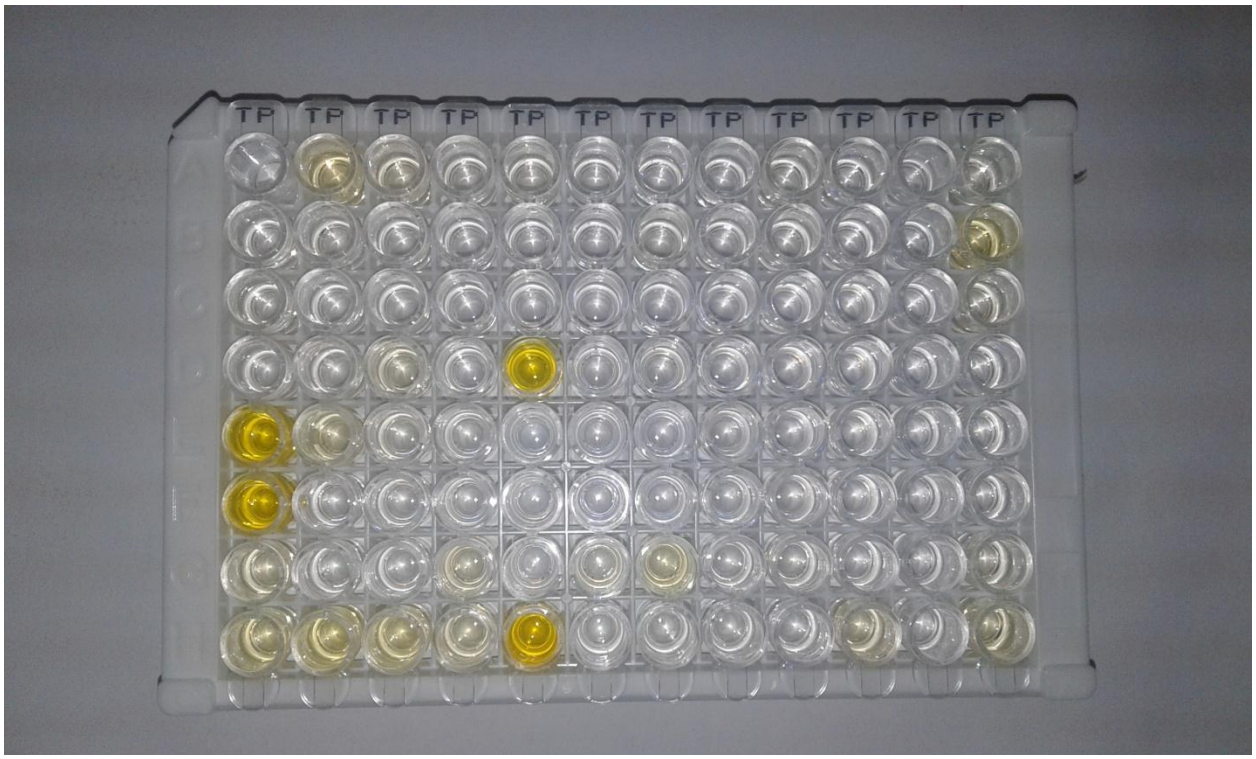
Date: …/…/…… Time: _____ (am/pm) NO ☐

Name: _____

Gender: …….. Age: ……years

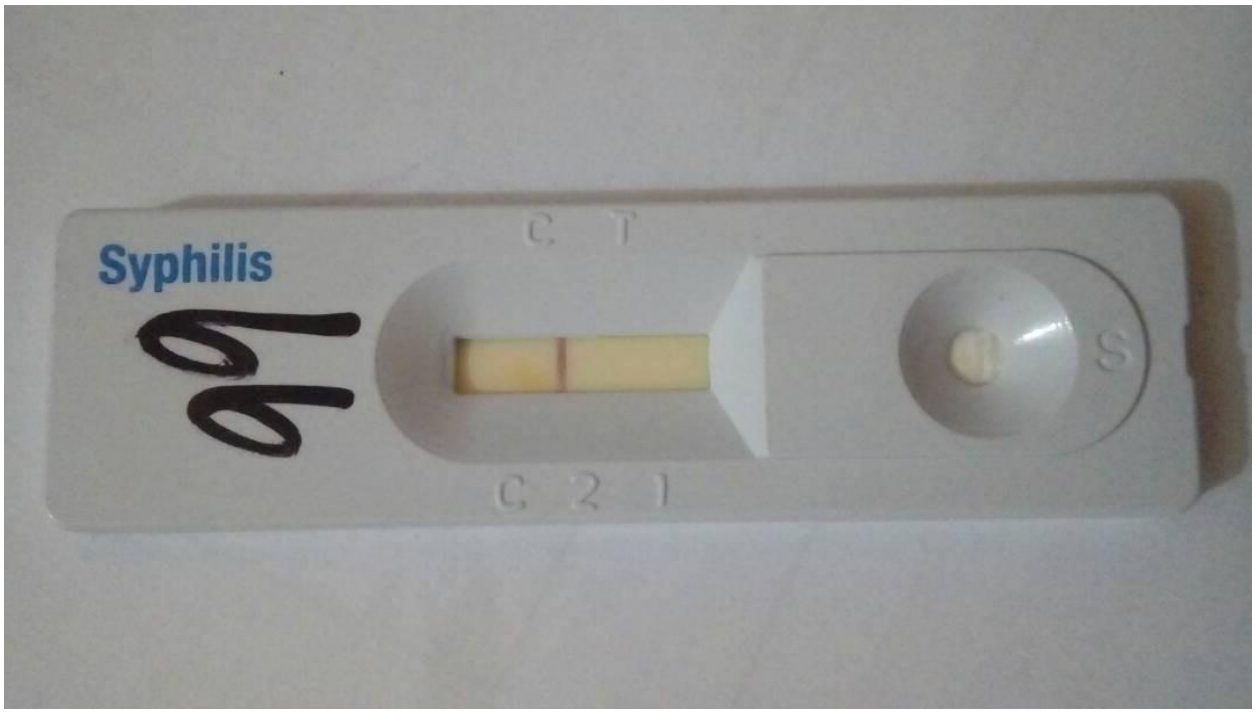
Clinical information:

- Have you ever been pregnant before?
- Yes……..……No…….
- Did you have miscarriage?
- Yes……..……No…….
- Did you have miscarriage before that?
- Yes…….No ……
- If present how many time?
- One ◯ Two ◯ Three ◯ More ◯
- At what stage occur:
- 1st◯ 2nd ◯ 3rd◯



ELISA microtiter plate yellow wells indicate positive results the tow lower left was control positive.

ELISA kits and reagens



Immunochromatographic base strip (ICT) negative test