Sudan University of Science and Technology

College of Graduate Studies

Frequency of Group B *Streptococcus agalactiae* (GBS) Among Sudanese Pregnant Women with Previous Miscarriage in Khartoum State, 2017

تردد المكورات العقديه القاطعه للدر (مجموعه ب) بين النساء الحوامل اللاتي عانين من حالة اجهاض سابقة في ولاية الخرطوم .2017

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الآيـــــــــــــة

قال تعالى:

(رَبِّ قَدْ آتَيْتَنِي مِنَ الْمُلْكِ وَعَلَّمْتَنِي مِنْ تَأْوِيلِ الْأَحَادِيثِ فَاطِرَ السَّمَاوَاتِ وَالْأَرْضِ أَنْتَ وَلِيِّي فِي الدُّنْيَا وَالْآخِرَةِ تَوَفَّنِي مُسْلِمًا وَأَلْحِقْنِي بِالصَّالِحِينَ)

صدق الله العظيم

سورة يوسف الآيه (101)

Dedication

This work is dedicated

To

my family, friends and my honest Abdelsamed

Acknowledgment

Firstly all thanks to Allah

I would like to thank my family for their patience.Special regard, respect for my supervisor Prof. Yousif Fadlalla Hamedelnil.

Great thanks to teaching staff in Sudan University of Science and Technology.

I am grateful a lot to my teacher Sohair Ramadan for technical support and encouragement.

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Abstract

Streptococcus agalactiae (S.agalactiae) is one of the most important etiologic agent of several infection,cause abortion, and a present a major public health problem. This study aimed to detect the frequence of *S. agalactiae* among pregnant women with previous miscarriage by using PCR.Fifty samples of vaginal and high vaginal swabfrom pregnant women , were collected from different hospitals in Khartoum,25 from each vagina and high vagina.

Patients's data was collected by structured interview questionnaire and analyzed by statistical package for social sciences (SPSS), version 11.5.

DNA was extracted by boiling method and alcohol was used to concentrate the nucleic acid . PCRtechnique was used to amplify the DNA of Group B *Streptococci* (GBS), the result showed 4 (8%) of each vaginal and high vaginal swabs were positive GBS.

Thirty four (68%) from subjects were normal delivered, 8 (16%) were caesarean section delivery, and 8 (16%) were no previous delivery, Frequency of GBS according to age grouping (19-26), (27-34) and (35-42) were positive ± negative (0 ± 11 ; 5 ± 20 ; 3 ± 11) respectively . Mean and standard deviation of agepregnant women, Mean± SD (30.8±5.4) respectively, frequency of *Streptococcus agalacticae* among pregnant women with previous miscarriage, positive (16%) and (84%) negative, the mean of age according to

freqeunce of *Streptococcus agalacticae* positive (33%) and (30%) negative.

Finally found the frequency of *Streptococcus agalacticae* in pregnant women with previous miscarriage according to type of delivery 5(10%) normal delivery, 1(2%) caesarean section delivery and 2(4%) no previous delivery.

ملخص الأطروحه

تعتبرالمكورات العقديه القاطعه للدر من أكثر العوامل شيوعا المسببه لعدة أمراض , و تسبب الاجهاض من تمزغ الغشاء قبل الاسبوع 37 من الحمل.

تهدفهذه الدراسة لمعرفة تردد بكتريا المكورات العقديه القاطعه للدرالمسببه للاجهاض في النساء الحوامل اللاتي يعانين من حالة اجهاض سابقه باستخدام تفاعل البلمره المتعدد , شملت الدراسه 50 من النساء الحوامل أخذت مسحات من المهبل و عمق الرحم من مستشفيات مختلفه بولاية الخرطوم و كانت 25(50%) من العينات من مسحات من الرحم و 25(50%) ممسحات عمق الرحم.معلومات النساءجمعت بواسطة استبيان من خلال معاينة وحللت بواسطة الحزمة الإحصائية للعلوم الإجتماعية.تم التعرف على البكتريا بطريقه مباشرة عن طريق اختبار البلمرة المتعدد و فيها تم استخلاص الحمض النووي للبكتريا باستخدام الغليان ثم الكحول لتركيز الحمض النووي و تم الكشف عن وجودها عن طريق تفاعل البلمره المتعدد الذي يضاعف الحمض النووي للبكتريا .و كانت النتيجه ايجابيه بعدد 4 (8%) لكل من من عينات مسحات المهبل وعمق الرحم ،النسب حسب نوع الولاده كانت(68 %)ولادهطبيعيه , (8 %)ولاده قيصريه و(8%)اخرون لم يسبق لهم الولاده، أكثر حالة اجهاض كان في الفئه العمريه ما بين (27-34)، متوسط الاعمار والانحراف المعياري للنساء الحوامل يساوي (30.8±5.4)، تردد البكتريا وسط النساء الحوامل اللاتي عانين حالة اجهاض سابقة كانت ايجابية بنسبة (16%) وسلبية بنسبة(84%)، متوسط العمر للنساء الحوامل حسب تكرار البكتيريا كان ايجابي (33%) وسلبي (30%)،ايضا كانت نسبة انتشار البكتريا في النساء الحوامل اللاتي عانين من حالة اجهاض سابقه حسب نوعالولاده كالاتي 5(10%) ولاده طبيعيه , 1(2%) ولاده قيصريه و 2(4%) لم يسبق لهم الولاده.

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Abbreviation

1- GBS Group B Streptococci

2- PCR Polymerase chain reaction

3- V S Vaginal swab

4- HVS High vaginal swab

5- TE Tris EDTA

6-TBE Tris Borat EDTA

Chapter one

Introduction

Streptococci are Gram – positive bacteria which cause diverse human diseases (Parks *et al,* 2015). They are typically β-hemolytic and produce zones of hemolysis that are only slightly larger than the colonies (1–2 mm in diameter). The group B *streptococci* hydrolyze sodium hippurate and give a positive response in the so-called CAMP (Christie, Atkins, Munch – Peterson) test. Group B *streptococci* are part of the normal vaginal flora and lower gastrointestinal tract in 5–30% of women. Infection of Group B Streptococcal during the first month of life may present as fulminant sepsis, meningitis, or respiratory distress syndrome. Substantial reductions in the incidence of early–onset neonatal group B streptococcal infections have been observed after the 1996 recommendations for screening pregnant women at 35–37 weeks of pregnancy, this is done by using either broth – enriched culture or molecular methods on rectal and vaginal swabs obtained at the time of screening. Intravenous ampicillin given to mothers who are colonized with group B *streptococci* and are in labor prevents colonization of their infants and subsequent group B Streptococcal disease. Group B Streptococcal infections are increasing among non pregnant adults, two expanding populations, namely elderly adults and immunecompromised hosts, are most at risk for invasive disease. Predisposing factors include diabetes mellitus, cancer, advanced age, liver cirrhosis, corticosteroid therapy, HIV, and other immune compromised states. Bacteremia, skin and soft tissue infections, respiratory infections, and genitourinary infections in descending order of frequency are the major clinical manifestations (Mores *et al,* 2013).

Streptococcus agalactiae is a commensal organism, but it may cause infection in susceptible hosts including newborns as well as in pregnant or postpartum women . prevalence of group B *Streptococci* and pregnancy outcome in 405 Brazilian women with gestational age between 35 and 37 weeks (Rocchetti *et al*, 2011). Conventional diagnostic methods, such as culture, biochemical tests,and enzyme – linked immune sorbent assay (ELISA), are methods of diagnosis and then developed to be used of molecular detection of PCR assay to screen *Streptococcus agalactiae.*

Miscarriage is one of the most common yet under-studied adverse pregnancyoutcomes. In the majority of cases the effects of a miscarriage onwomen's health are not serious and may be unreported. However in themost serious cases symptoms can include pain, bleeding and a risk of haemorrhage (Engelhard *et al*, 2001). Most pregnant women who carry group B *streptococcus* (GBS) bacteria have healthy babies. However, there's a small risk that GBS can pass to the baby during child birth, sometimes GBS infection in newborn babies can cause serious complications that can be life threatening, but this is not common, extremely rarely, GBS infection during pregnancy can also cause miscarriage, early (premature) labour, or stillbirth.

GBS is one of many bacteria that can be present in our bodies. It doesn't usually cause any harm. When this happens it's called "carrying" GBS or being colonised with GB, it's estimated about one pregnant woman in five in the UK carries GBS in their digestive system or vagina, around the time of labour and birth, many babies come into contact with GBS and are colonised by the bacteria. Most are unaffected, but a small number can become infected.

1.2 Justification

The miscarriage one of the problems that exposed of pregnant women, miscarriage is defined as the spontaneous loss of a pregnancy during the first 24 weeks of gestation (Engelhard *et al*, 2001).

GBS is most infectious agent cause miscarriage among pregnant women with in 35 to 37 week in pregnancy .

Our study aimed to study frequency of *Streptococcus agalactiae* among pregnant women suffered from previous miscarriage in Sudanese pregnant women.

About my knowledge no previous study of GBS in pregnant women.

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1.3 Objectives

1.3.1 General Objective

To study the frequency of *Streptococcus agalactiae* among Sudanese Pregnant Women with miscarriage.

1.3.2 SpecificObjectives

1-To determine frequency of *Streptococcus agalactiae* among pregnant women with miscarriage by using polymerase chain reaction.

2-To investigate association between *Streptococcus agalactiae* and miscarriage.

3-To investigate the frequency of *Streptococcus agalactiae* according of vaginal and high vaginal swab.

4-To detect the of *Streptococcus agalactiae* according types of delivery.

Chapter Two

Literature Review

2.1Definition

Group B *Streptococcus* (GBS) is the main etiological agent of maternal and fetal infections, sepsis and meningitis in the term newborns (Bassir *et al*, 2016). Group B *Streptococcus* are encapsulated Gram-positive cocci belonging to Lancefield group B, that frequently colonizes in human genital and gastrointestinal tracts. It is an important cause of illness in three categories of population: infants, pregnant women, and adults with underlying medical conditions. In pregnant women and postpartum women, GBS is a frequent cause of asymptomatic bacteriuria, urinary tract infection, upper genital tract infection (i.e. intraamniotic infection or chorioamnionitis), postpartum endometritis (8%), pneumonia (2%), puerperal sepsis (2%), and bacteremia without a focal site (31%). It also can cause focal infections such as pneumonia, meningitis, and endocarditis, albeit rarely. Invasive maternal infection with GBS is associated with pregnancy loss and preterm delivery (Tevdorashvili *et al*,2015).

2.2Etiology

Group B *streptococci* (GBS) are the leading cause of life-threatening neonatal bacterial infections in developed countries. In the newborn the bacteria initially colonised during passage through the birth canal. Maternal vaginal carriage is usually asymptomatic. GBS is also a commensal that colonizes in the gastrointestinal and genitourinary tracts, resulting in significant maternal and perinatal morbidity, and can be transmitted from a GBS-colonized mother to her newborn via the ascending route during labor and delivery, causing neonatal severe invasive diseases such as an early-onset

GBS (EOGBS) disease occurring within the first week and a late-onset GBS (LOGBS) disease occurring between 1 week and 3 months of life (Borchardt *et al*, 2006; Huber *et al*, 2011; Stoll *et al*, 2011; Okike *et al*, 2014).

2.3Pathogenesis

S. agalactiae is also an important cause of disease in parturient women, and in recent years it has emerged as a significant cause of serious disease in adults with underlying conditions such as diabetes and malignancy(Farley, 2001).

Rupture of membranes before labour onset and increased interval between membrane rupture and delivery are considered to be major risk factors. Other predisposing factors include premature delivery, low birth weight, dense vaginorectal colonization, and intrapartum fever (Edwards and Baker, 2001).

2.4 Epidemiology

Group B *Streptococcus* (GBS) is the leading cause of neonatal sepsis in the developed world. Little is known about its epidemiology in the developing world, where the majority of deaths from neonatal infections occur. Maternal carriage of GBS is a prerequisite for the development of early onset GBS neonatal sepsis but there is a paucity of carriage data published from the developing world, in particular South East Asia (Turner *et al*, 2012).In Australia miscarriage occurred in 25% of the women inthe study when the women were 31–36 years old (Hure *et al*, 2012).Since the early 1970s, Group B (*ß-haemolytic) Streptococcus* (GBS) (*Streptococcus agalactiae*) has been the leading pathogen causing serious perinatal infection in the USA as well as most developed countries. Control of this infection has therefore become a major priority in pediatrics. Significant advances have been achieved in the areas of

diagnosis and management leading to a reduction in mortality from 35-50% in the 1970s to less than half of this initial rate.

The incidence of invasive early-onset GBS disease decreased by more than 80% from 1.8 cases/1000 live births in the early 1990s to 0.26 cases/1000 live births in 2010; from 1994 to 2010 it is estimated that over 70,000 cases of early onset GBS invasive disease were prevented in the United States (Schrag and Verani, 2013).

2.5 Clinical manifestation

In pregnant women, GBS causes cystitis, amnionitis, endometritis, and stillbirth; occasionally, GBS bacteremia leads to endocarditis or meningitis.

In postpartum women, GBS can cause urinary tract infections (UTIs) and pelvic abscesse, in newborns, early-onset GBS infection occurs before age 7 days (mean age at presentation is age 12 hours) and primarily manifests as nonfocal sepsis, pneumonia, or meningitis.Late-onset disease in neonates occurs at age 7-89 days (mean age, 36 days), and nonfocal bacteremia and meningitis are the most common presentations (Smith and Smith, 2016).

2.6 Methods of diagnosis

2.6.1 Culture

Group B *Streptococcus* (GBS) can be cultured by aselective enrichment method using anorectal /vaginal swabs in LIM (Todd-Hewitt broth supplemented with selective antibiotics) or Trans Vag broth, followed by subculture on blood agar plates (BAP) (Carvalho *et al*, 2009). In recent years,alternative methods have become available that have improved sensitivity, decreased test turnaround time, and increased test efficiency and that are easier to perform in the laboratory (Block *et al*, 2008).Such methods include

the use of modified media, such asGranada medium (GM) (De La Rosa *et al*, 1983, De La Rosa *et al*,1992), which utilizes the unique ability of beta-hemolytic strains of *S. agalactiae* to produce a red orange pigment, thereby allowing detection and identification of GBS in a single step. Enrichment broth modified from the original Granada medium has been developed and investigated more recently (Block *et al*, 2008; Church *et al*, 2008; Heelan *et al*, 2005; Martinho *et al*, 2008). These broth media have shown agood diagnostic performance compared to LIM broth with respect to sensitivity and specificity and have added the advantage of decreasing turnaround time for positive cultures by 24 h. The formulations of both solid and liquid media that enhance pigment production have changed considerably over the past few years, and comparisons between these new formulations are few. Each GBS culture was inoculated on a BAP and grown over night. Tubes of LIM, Strep B carrot broth (SBCB), Granada instant liquid biphasic (IGLB), and Northeast Laboratory GBS screening medium (NEL-GBS) broth were eachinoculated with 1.0 ml of each of the three concentrations, and tests were repeated three to four times over a 4-month period. LIM broths were examined for growth as judged by the increasein turbidity of the broth after 24 and 48 h. If the broth showed no visible growth after 24 h of incubation, the broth was subcultured to BAP and these plates were examined for growth after overnight incubation at 35°C. The SBCB, IGLB, and NEL-GBS broths were examined for pigment productionat 24 and 48 h. If a peach, orange, reddish-orange, or red colorwas evident, positive pigment production was recorded. If nocolor change was detected after 24 h of incubation, the tubeswere subcultured to BAP and incubated overnight. If growth ofGBS on BAP was evident, the culture was recorded as positive for growth (Carvalho *et al*, 2009). On Blood Agar Most strains of *S. agalactiae* produce grey mucoid colonies about 2 mm indiameter, surrounded by a small zone of *beta* hemolysis(clear area with decolorization ofhaemoglobin). About

5% of strains are non haemolytic.Placing discs of penicillin and Gentamicin on the plate can help to identifythese strains (penicillin sensitive, Gentamicinresistant), on MacConkey agar Most strains grow on thismedium, Neomycin blood agar a useful selective medium for isolating *S. agalactiae* from urogenital specimens, Orange pigment produced by *S. agalactiae* when cultured on serum starch agar anaerobically.

2.6.2 Biochemical reaction

2.6.2.1 CAMP (Christie, Atkins, Munch, Peterson)

This test to identifypresumptively *S. agalactiae* , it requires the use of a beta-lysin producing strain of *S. aureus*(NCTC 1803 or ATCC 25923) to detect the CAMPfactor, i.e. extracellular diffusible protein produced by*S. agalactiae*. This protein interacts with the staphylococcal beta-lysin on sheep (or ox) blood agar producing enhanced hemolysis (Davies *et al*, 2008).

The test is performed by streaking a knownbeta-lytic*Staphylococcus* strain across a 10% blood agar plate and theninoculating the test organism at right angles to it. The testorganism must not touch the staphylococcal inoculum. An*Enterococcus* species is also inoculated as a negative control.The test organism is presumed to be *S. agalactiae*, if after overnight incubation at 35–37 ºC, there is an arrow head shaped area of hemolysis where the staphylococcal organismmeets the test organism (Davies *et al*, 2008).

2.6.2.2 Bile aesculin stope

S. agalactiae does not hydrolysis aesculin.It is able to grow on bile agar. Group A*Streptococcus pyogenes* gives a variable aesculin hydrolysis reaction anddoes not grow on bile agar. Group D streptococci hydrolysis aesculin and can grow on bile agar(Davies *et al*, 2008).

2.6.2.3 Hippurate hydrolysis test

S. agalactiae hydrolyzes hippurate. The test is inexpensive and rapid performed using a saline suspension of thetest organism and a Rosco Diagnostic a hippurate hydrolysis. (Davies *et al*, 2008).

2.6.3 Immunological reaction

Lancefield grouping

*S. agalactiae*belongs to Lancefield Group B.Serological identification of the organism can bemade by testing the beta-haemolytic colonies froma blood agar culture (confirmed as catalasenegative, Gram positive cocci) for B antigen using Group B antiserum reagent. The technique issimilar to that described for grouping *Streptococcus* Group A(Davies *et al*, 2008).

2.6.4 Molecular detection

2.6.4.1 DNA extraction

It is generally accepted that the solvent of choice for the extraction of biologically active components of propolis (mainly phenolics, including different types of flavonoids) is 70% ethanol, with most commercial products extracted using this solvent system (Park andIkegaki, 1998 andCunha*et al*, 2004). The traditional method (maceration), however, is time consuming, requiring timeframes from 2 to 10 days (Cunha*et al*, 2004andWoisky andSalatino, 1998). Recently, modern extraction methods have been developed for the fast and efficient extraction of organic compounds from solid matrices, with microwave assisted extraction (MAE) and ultrasonic extraction (UE) among the most promising for the extraction of natural products (Huie, 2002 andKaufmann and Christen, 2002). MAE is the process of using microwave

energy to heat solvents in contact with a sample in order to partition some chemical components from the matrix into the solvent. The benefits of UE are thought to be due mainly to the mechanic effects of acoustic cavitation (Liu and Wang, 2004)

2.6.4.2 PCR amplification

ItisamoleculartechnologyaimtoamplifyasingleorfewcopiesoftheDNAtothous andsormillionsofcopies.

Types of PCR Conventional (Qualitative) PCR, Multiplex PCR, Nested PCR, RTPCR and qRT PCR, Quantitative PCR, Hotstart PCR.Touchdown PCR.Assembly PCR, Colony PCR.Methylation specific PCR and LAMP assay.

2.7.Treatment

Group B *streptococci* are uniformly sensitive to penicillin and ampicillin. Although resistance to penicillin or ampicillin has not be documented, some isolated have shown minimum inhibitory concentrations (MICs) approaching the upper limits of susceptibility for some of the beta-lactam agents.Group B streptococci have never been as exquisitely sensitive to penicillin as group A beta-hemolytic streptococci; therefore, the initial therapy for group B streptococcal infection has always been high-dose parenteral penicillin or ampicillin.

Penicillin or ampicillin plus an aminoglycoside has demonstrated synergy but has not been shown to provide a better clinical outcome than penicillin or ampicillin alone. Testing for aminoglycoside sensitivity is important because synergy is not observed if the organism is not sensitive to aminoglycosides.

Keep in mind that given group B streptococcal isolate can be resistant to one aminoglycoside and sensitive to another.

While clindamycin and erythromycin were at one time uniformly active against group B streptococci, resistance has been increasing. One large study that examined the susceptibility patterns of over 4800 group B streptococcal isolates found that 32% were resistant to erythromycin, 15% were resistant to clindamycin, and 99% of clindamycin-resistant strains were also resistant to erythromycin.As a result, sensitivity testing is important before these agents are used. Oral clindamycin remains an excellent agent to follow a course of parenteral therapy for bone, soft-tissue, and lung infections if the isolate is susceptible.

Because of possible resistance with clindamycin, vancomycin remains the initial treatment of choice for group B streptococcal infection in patients who are allergic to penicillin. Penicillin, ampicillin, or vancomycin remains the treatment of choice for endocarditis. While vancomycin may be adequate in group B streptococcal meningitis in patient who are allergic to penicillin, skin testing and desensitization for penicillin therapy might be considered. Penicillin has not been demonstrated to be superior to vancomycin for group B streptococcal endocarditis.

While fluoroquinolones appear to have efficacy against isolates of group B streptococci, resistance to fluoroquinolones has recently been reported.

Similarly, linezolid, a new antibiotic with efficacy for aerobic gram-positive cocci, should have activity against group B streptococci. It is available in parenteral or oral form. However, no clinical studies have evaluated linezolid in group B streptococcal infections.(Christian*et al*,2017).

2.8. Prevention

The continuing magnitude and severity of group B streptococcal disease and its attendant mortality and morbidity underscore the desirability of prevention methods. Two approaches have been proposed: chemoprophylaxis and immunoprophylaxis(Dennis*et al*, 1991).

Chapter Three

Material and Methods

3.1.Type of study

Cross sectionalstudy.

3.2. Study area

Data was collected from Alsudi Maternal Hospital (Northern Omdurman near Aieslamia University) and Omdurman Maternal Hospital (Aldayat) (Almourada).

3.3.Working area

Sudan University of Science and Technology, Research lab.

3.4.Study population

The swabs were collected from pregnant women suffering from history of miscarriage.

3.5.Study duration

This study was carried out during 9 months between January and October, 2017.

3.6.Data collection

The data was collected by structured questionnaire.

3.7.Ethical consideration

All patients were informed about the purpose of the study before collection of the samples (orally) and the ethical approval was obtained from Sudan University of Science & Technology Research Board, and from the hospitals authorities.

3.8.Sample size

Fifty swab samples were collected from vaginal and high vaginal pregnant women in third trimrster. Each sample was immersed in a plastic swab tubes (using cotton swabs) containing 5ml of Tris HCL buffer (pH8.0).Pellets from these samples were obtained by centrifugation (10min at 3,000xg), then re-suspended in 2ml Tris HCL buffer (PH 8.0) and stored at -20˚C until used

(Jacobs*et al*,1995).

3.9.Extraction of DNA

The cell pellets in Tris HCL(PH8.0), were centrifuged for 10min at 3,000xg, the the cell pellets were re-suspended in 50µl tris EDTA buffer(TE(PH8.0)),then were centrifuged as before ,then re-suspended in other TE buffer(100 µl) bufferd (Mansour, 2010). The thermal lysis was performed (10 min at 100°C), to lyse bacterial cell wall(de-Paris *et al*, 2011).To concentrate the nucleic acid obtained from the boiling method, 400 µL of ice-cold ethanol was added into the micro tube containing nucleic acid, it was mixed gently and left in a deep freezer (-18 to -20°C) for 10-30 minutes; then, it was centrifuged for 10 minutes at the maximum speed rpm. The supernatant was decanted.Finally, 50 µL of sterile 1x TE [Tris-EDTA buffer] buffer was added to the micro tube and the pellet was gently dissolved. The nucleic acid was used for PCR or was preserved at -20°C until further use (Mashouf*et al*, 2014).

3.10. Polymerase chain reaction

The sequences of the primerforward5'-CGC TGA GGT TTG GTG TTT ACA-3', and reverse 5'- CAC TCC TAC CAA CGT TCT TC - 3'(405bp) . PCR was carried out by adding the template DNA (DNA extracted)from vaginal and high vaginal swab, 100pmol/µL of GBS primer and Distilled water(water for injection) to Maxim PCR Pre Mix Kit (i-Taq) for rxn:
Template DNA3µ/L,Primer (F: 100Pmol/µL) 1µ/L , Primer (R: 100Pmol/µL) 1µ/L Distilled water15µ/LTotal reaction volume20µ/L added to pre master mix. The amplification process was consisted of initial denaturation at 94˚C for 3 min followed by 30 cycles each consist of 94˚C for 1 min ,1 min at 51˚C forprimer annealing,1 min at 72˚Cfor elongation and a final period of extension at 72˚C for 5 min.Amplification was done using forward and reverse and primer.

3.11. Agarose gel electrophoresis

The PCR products were electrophoresed on 2% agarose gel containing 1.5ethidiumbromide and analyzed under UV light. 5µL of the PCR product were loaded on the gel along with 4µL of 100bpdeoxyribonucleic acid (DNA) ladder was applied with each batch of patients samples.The gel was run at 1X TBE(Tris borate EDTA) buffer (10X TBE: Tris base , boric acid and Na EDTA).

3.12.Visualization of PCR products

The gel tray was flooded by 10X TBE buffer near the gel cover surface, then 5µl of PCR products of each sample was injected into each well.The first well of casting trayinjected 4µl of DNA ladder (100bp). The electrophoresis apparatus was connected to the power supply. It was done

at 400v for 20min, after that the gel was removed by gel holder and visualized by U.V trans illuminater. The results were photographed using gel documentation system.

3.13. Statistical analysis

Data was analyzed by statistical package for social sciences (SPSS), version11.5. Qualitative data was represented as frequency and Percentage. Quantitative data was presented as mean ± SD.

Chapter Four

Results

A total of 50 Sudanese pregnant women in third trimester were enrolled in this study,25 samples were vaginal swab and 25were high vaginal swab. The mean of age of pregnantwomenwas 31 years.(Table4.1).

Table4.1:Mean and standard deviation of age.

Variables	Mean	SD
Age	31	5.4

Thirty four (68%) from subjects were normal delivered ,8 (16%) were caesarean delivery ,and 8 (16%) were of no previous delivery (Figure 2).

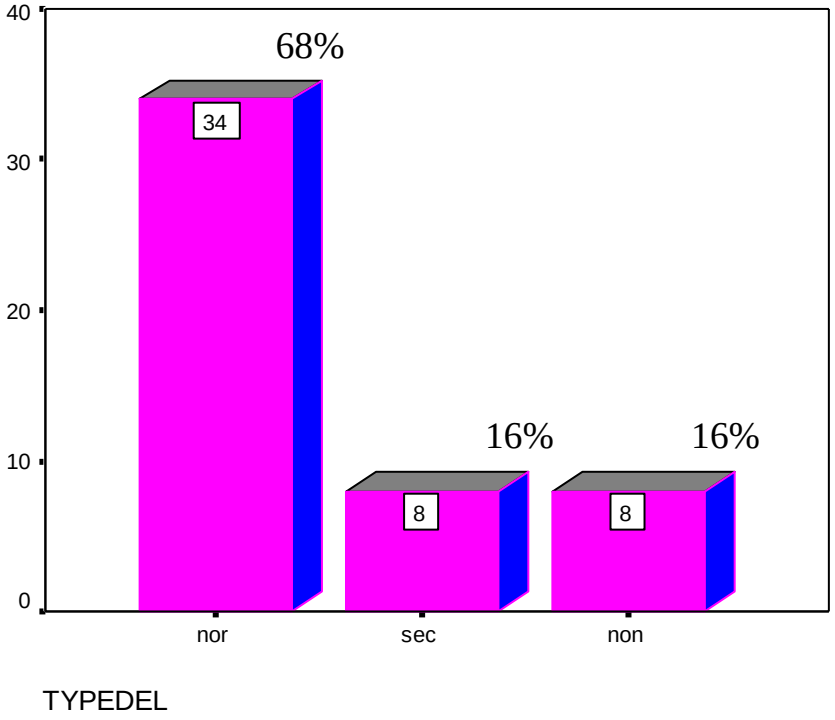


Figure (1): The frequencies of delivery types.

Frequency ofGBS according to age grouping (19-26), (27-34) and (35-42) were positive ± negative (0 ± 11 ; 5 ± 20 ; 3 ± 11) respectively(Table 4.2)

Table 4.2:Frequency of GBS according to age grouping.

Age grouping	**Result**	
	Positive	**Negative**
19-26	0	11
27-34	5	20
35-42	3	11

Frequency of GBS among pregnant women with previous miscarriage, (16%) positive and (84%) negative (Table4.3).

Table4.3:Frequency of GBS among pregnant women with previous miscarriage.

Variable	Result	
	Positive	**Negative**
Miscarriage	8(16%)	42(84%)

There was no deferent between frequency*Streptococcus agalacticae* in vaginal and high vaginal swab (Table 4.4).

Table 4.4:Comparisonthe frequency of*Streptococcus agalacticae*between vaginal and high vaginal swab.

Samples	Result		*P.value*
	Positive	**Negative**	
V.S	4(8%)	21(42%)	1.0
H.VS	4(8%)	21(42%)	

No statistically significance value frequency of *Streptococcus agalacticae*and the types of delivery, (*p.value*0.742) (Table4.5).

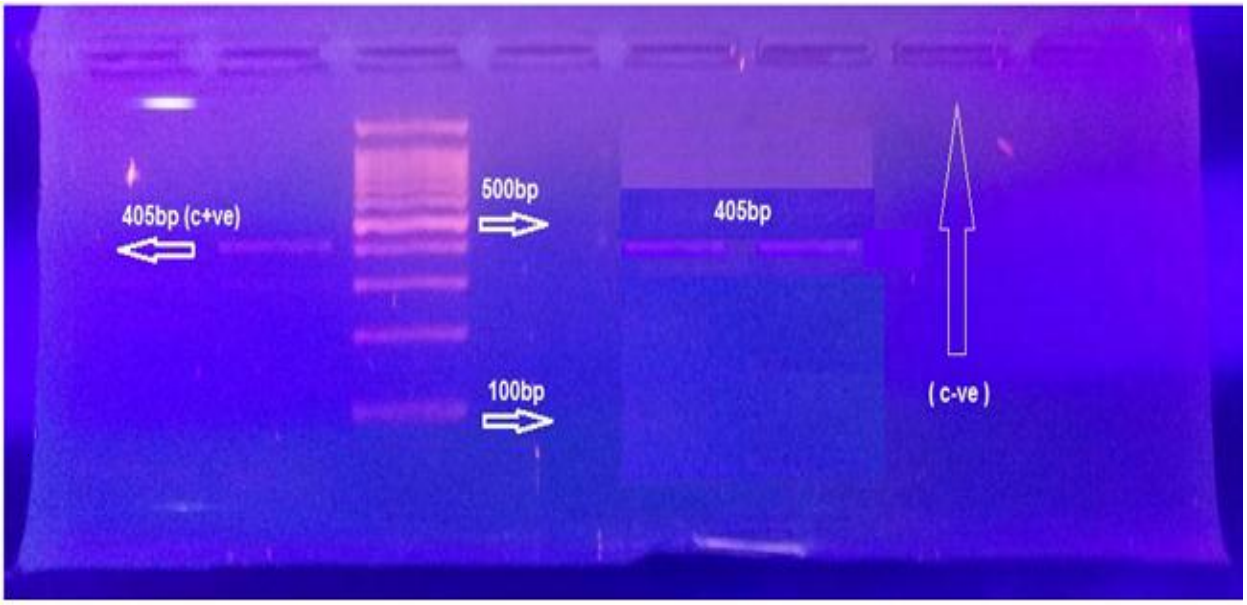
Table4.5: Frequency of *Streptococcus agalacticae*accordingto the types of delivery.

Types of delivery	**Result**		***p.value***
	Positive	**Negative**	
Normal	5 (10%)	29(58%)	0.742
Caesarean section	1(2%)	7(14%)	
No delivery	2 (4%)	6(12%)	

The mean of age according to occurrence of *Streptococcus agalacticae*positive (33.3)and negative (30.3) (Table4.6).

Table4.6: Mean of age according to occurrence *Streptococcus agalacticae.*

Variable	Result	
	Positive	**Negative**
Age	33.3	30.3



Figure(2) : PCR product

Lane 1: Negative sample, Lane 2: Control positive (405 bp),

Lane 3: 100 bpDNA molecular marker (Ladder),

Lane 4: Negative sample, Lane 5& 6 : Positive sample(405 bp),

Lane 7: Control negative.

Chapter Five

Discussion

This study aimed to investigate the association of prevalence GBS among Sudanese pregnant women with miscarriage, by using molecular methods. In the present study out of 50 samples 4 (8%) positive from vaginal swabs, 4 (8%) positive from high vaginal swabs while 42(84%) were negative from vaginal and high vaginal swabs, There was no statistical significance association between GBS and miscarriage in pregnant women (*P.value* 0.103).

Our study was dissimilar to their obtained by Giakoumelou*et al* (2016), who reported 25.4% of investigated women were positive for *Streptococcus agalactiae* , the difference might be attributed togeographical region, the result was also not agree with Romanik*et al* (2014), who reported*Streptococcus agalactiae* colonization rate was 28.7%, the difference might be attributed to geographical region , Al-Sweih *et al* (2004) in Kuwait, showed positive for GBS in 18 (16.4%) the difference might be attributed togeographical region and method of detection , Xie*et al* (2016) in Northwest China, found 6.4 % of vaginal swab were positive the difference might be attributed togeographical region and method of detection , Bidgani *et al* (2016) in China, showed (27.7%) were positive by culture and (43.8%) samples were positive by PCR , the difference might be attributed togeographical region and method of detection.GBS is present in24–25% of women of reproductive age (Ralph *et al* 1999; Wilson*et al* 2002),the difference might be attributed togeographical region and type of study design .

Munir *et al* (2016) in Lahore, who observe 14%.of pregnant women was positive GBS, the difference might be attributed togeographical region and method of

detection.Also Marconi*et al* (2010) inBrazil,who reported significant relationship between GBS and previous history of miscarriage in pregnant women. the difference might be attributed togeographical region, method of detection and type of study design.

The different frequency rates between several studies may be associated withgeographical region, technique in sample collectiondifferences in the elapsed time after sampling for transporting clinical specimens to be processed, as well as using different type of PCR, also the difference in sample size may be attributed these difference.

In my knowledge not published data about this study

Chapter Six

6.1 Conclusion

From this study we concluded that, Group B *Streptococci* present in Sudanese pregnant women with miscarriage , and in 31 years mean of age.

Also we found the equal percentage in both samples vaginal and high vaginal swab .

The type of delivery is not affected by GBS.

6.2 Recommendations

Further study with large sample size and involving all part of the Sudan recommended to get conclusive results on the situation of Group B *Streptococci* among pregnant women .

We recommended to use rectal swab beside the vaginal swabfor identification the GBS.

Also recommended using conventional technique with PCR.

Molecular technique other than PCR like sequencing , restriction fragment length polymorphism (RFLP)and genotyping should be used to confirm the GBS.

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Appendix

AppendixI: reagents

Preparation of Tris EDTA(PH 8.0)

1 M Tris – HCL PH 8.0 .

0.5 M EDTA PH 8.0.

DW.

Preparation of TrisHCL(PH 8.0)

6.0 M Tris HCL .

Tris base.

0.5 NNaOH.

Preparation of primers (forward and reverse)

The stock solution we prepared by adding 300µl from DW then were pellet at 14000r For 5 min and placed at 4^0C over night . the working solutions were prepared by adding 10µl from stock solutions of each primer was dissolved in 90µl of DW mixed and placed at -20^0C .

Preparation of 10X TE buffer

Amount 108 grams of Tris base were added to 55 g of boric acid and 40ml of 0.5 EDTA , then in to liter de ionized water PH 8.0.

Preparation of 1X TE buffer

10 ml of 10X was added to 90 ml of deionized water and heated until completely dissolved.

Preparation of ethidium bromide

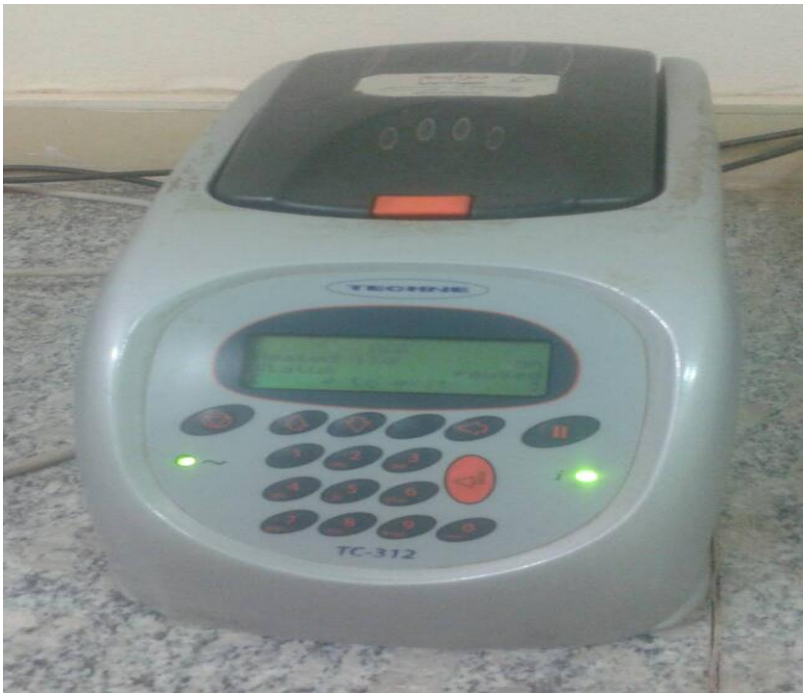
5 milligrams of ethidium bromide was dissolved in 500 µl DW and kept in brown bottle.

Preparation of Agarose

Two gram of agarose powder was dissolved in 100ml 1X TE buffer and heated in microwave for 90 minute . Then the mixture was cooled ,Then 1.5µl of ethidium bromide were added , mixed well and poured into a casting tray that was taped up appropriately and was equipped with suitable comb to form well in place . Any bubbles were removed and the gel was allowed to set at room temperature. After solidification the comb was gently removed and the spacer from the opened slides was removed.

Appendix II:

1: PCR Machine



2 Sensitive balance

3: Centerifug

4: Gel electrophoresis machine

5: Microwave

6: U V light machine