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Association of Parathyroid hormone and vitamin D with vaginal *Lactobacillus* in First Trimester Pregnant in Hilla City

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«وَقُلِ اعْمَلُوا فَسَيَرَى اللَّهُ عَمَلَكُمْ وَرَسُولُهُ وَالْمُؤْمِنُونَ وَسَتُرَدُّونَ إِلَى عَالِمِ الْغَيْبِ وَالشَّهَادَةِ فَيُنَبِّئُكُمْ بِمَا كُنْتُمْ تَعْمَلُونَ»

صدق الله العلي العظيم

سورة التوبة (الآية ١٠٥)

Dedication

To

All my dreams my daughter Ruqaya.

My parents.

My brothers and sisters.

My love and support Hayder.

My second family.

My friend Reema.

Acknowledgment

First and foremost, my gratitude to Allah.

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Finally, I would like to thank all pregnant women who participated in this study.

Summary

A wide variety of pathogenic agents including bacteria, fungi, and virus can cause infections vaginitis especially during pregnancy. The present study is designed to investigate the isolation and identification of *Lactobacillus* spp. from women affected with bacterial vaginosis during first trimester of pregnancy and also determination of Vitamin D level, Parathyroid hormone, and some hematological parameters.

The number of pregnant women that included in the present study were ninety (90) women, (50) women were infected with bacterial vaginosis (BV) in first trimester of pregnancy, the remaining (40) women were used as a control group (non-BV) and they were also pregnant in first trimester. Age of the pregnant women were (16-45) years. All samples were collected during a period ranged from November (2021) to February (2022). Samples collection were done in Babylon province, Imam Ali general hospital and private clinics, sample collection done by Gynecologists physician , for each participant blood sample and high vaginal swab (HVS) were collected. All samples tested for serum level of Vitamin D and Parathyroid hormone (PTH) using the enzyme linked immunosorbent assay technique (ELISA) and also tested for complete blood count (CBC) by automated hematology autoanalyzer, and for HVS were cultured on De man, Rogosa and Sharpe (MRS) agar and broth for isolation of *Lactobacillus* spp. then identified using VITIK-2 device, and for antibiotic and antibacterial activity Muller-Hinton Agar (MHA) was used.

The data of this study showed that the body mass index (BMI) of BV group have higher BMI compared to those healthy women. The mean vitamin D levels in serum were significantly lower (P=0.001) in BV group (19.91±11.2) than control group (33.71±14.9) The lower the vitamin D, the greater the risk of developing BV. The mean levels of PTH were significantly higher (P=0.021) in BV group (32.7±13.6) than control group (26.5±10.9).

Moreover, the mean count of White blood cells (WBCs) were significantly higher (P=0.01) in BV group (7.77±2.7) than control group (6.47±1.6), and for platelet were also signficantly higher (P=0.009) in BV group (230.2±77.6) than control group (191.7±53.4), But for Packed cell

volume (PCV) there were no significant diffrence (P=0.230) between groups.

All 90 vaginal swabs were cultured on MRS agar and broth, were investigated on *Lactobacillus* spp. bacteria as a reverse indicator of bacterial vaginosis in first trimester pregnant and healthy women, 7 (14%) vaginal swab of BV group diagnosed as *Lactobacillus* whereas 43 (86%) vaginal swab showed no growth result, and 30 (75%) vaginal swab of control group diagnosed as *Lactobacillus* whereas 10 (25%) showed No growth result, then the identification was made with VITEK-2 device, and two types of *Lactobacillus* were detected: *Lactobacillus gasseri* and *Lactobacillus casei*.

The results showed that all *Lactobacillus* spp. isolates were from healthy women showed higher resistance to antibiotics used in this study cefixime, cefepime, Nitrofurantoin, Amoxicillin/clavulanic acid, and Amoxicillin with percentage of resistance (100%, 80%, 60%, 60%, 30% respectively) in non-BV group and (100%, 71%, 71%, 43%, 28% respectively) in BV group.

In this study, the cell free supernatant (CFS) extracted from *Lactobacillus* spp. from healthy women were more effective against the pathogenic bacteria isolates than the CFS from *Lactobacillus*. spp. from women with vaginosis.

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List of Abbreviations

Abbreviation	Meanings
1,25(OH)2D	1,25-Dihydroxy vitamin D
AMC	Amoxicillin/Clavulanic acid
AR	Antibiotic resistance
ATP	Adenosine triphosphate
AX	Amoxicillin
BMD	Bone mineralization density
BV	Bacterial vaginosis
CaSR	Calcium-sensing receptor
CBC	Complete blood count
CFM	Cefixime
CFS	Cell- free- supernatant
EDTA	Ethylenediaminetetra acetic
ER s	Endoplasmic reticulum
F	Nitrofurantoin
FBC	Full Blood count
FEP	Cefepime
GBS	Group B streptococci
GDM	Gestational diabetes mellitus
Hb	Hemoglobin
HGT	Horizontal gene transfer

HPV	Human papillomavirus
Hsv-2	Herps simplex virus type 2
HVS	High vaginal swab
LAB	Lactic acid bacteria
MCV	Mean corpuscular volume
MHA	Muller-Hinton Agar
MRS	Deman , Rogosa and Sharpe
NaPi2a	Type II sodium phosphate cotransporter
NaPi2c	Type IIc Sodium–Dependent Phosphate Transporter
non- BV	Non- bacterial vaginosis
PCV	Packed cell volume
PHPT	Hyperparathyroidism
PID	Pelvic Inflammatory Disease
PLT	Platelet
PTB	preterm birth
PTGs	Parathyroid glands
PTH	Parathyroid hormone
PTHrp	Parathyroid hormone-relater protein
PTHrP	PTH-related protein
QVS	quantitative ultrasound
RBCs	Red blood cells
RER	Rough endoplasmic reticulum
RP	Related peptide
RTI s	Reproductive tract infections
SHPT	Secondary hyperparathyroidism
STI	Sexually transmitted
UTI	Urinary Tract Infection
V-D3	Vitamin D3
VDD	Vitamin D deficiency
VDR	Vitamin D receptor
Vit A	Vitamin A
Vit D	Vitamin D
VVC	Vulvovaginal candidiasis
WBcs	White blood cells
WRA	Women of reproductive age

Chapter One

Introduction

1.1-Intoduction

Vaginal symptoms are one of the most prevalent causes for visits in clinical practice, and they arise in women throughout their reproductive lives, leading to numerous consultations with an obstetrician or gynecologist (Muvunyi *et al*.,2009). Vaginitis can take many different forms, including irritating, sexually transmitted illnesses, hormonal, foreign body, and infective vaginitis. There is a variety of things that can cause pain to the sick women (Kerry *et al*., 2018). *Trichomoniasis, Vulvovaginal candidiasis,* and bacterial vaginosis are the 3 most prevalent causes of vaginitis, in 40 to 50 % of cases with a recognized cause, bacterial vaginosis is to blame, following by *trichomoniasis* (20 to 25 % of cases) and *vulvovaginal candidiasis* (15 % to 20% of cases) Only 5 to 10 percent of vaginal infections are caused by atrophic, irritant, allergic, and inflammatory vaginitis (Workowski *et al*.,2015).

Bacteria, fungi, parasites, and viruses all have the infectious vaginitis is a possibility. Numerous bacteria which are characterized by proliferation and lacking Lactobacilli, which produce hydrogen peroxide, are associated with vaginitis. *Staphylococcus aureus*, *Klebsiella pneumoniae*, Group B *Streptococci* (GBS), *Escherichia coli, Listeria monocytogenes*, *Acinetobacter spp*., *Neisseria gonorrhoeae,* and bacteria species produce bacterial vaginosis are only a few of the bacteria that may induce vaginitis. The most critical component for *Lactobacillus spp*. to keep the vaginal flora in equilibrium. *Lactobacillus spp.* functions as a probiotics and preventive agent in the treatment and prevention of vaginal infection by producing antagonistic chemicals such as hydrogen peroxide, lactic acid, and bacteriocin (Jomehzadeh *et al*.,2020). ,

The probability of Preterm birth probability is doesn't decrease although the symptoms improve with treatments for vaginitis throughout gestation (Koss *et al*.,2012, Brocklehurst *et al*.,2013). Increased vaginal secretions, which often smell like fish due to the presence of amine compounds, and a white to

yellowish white color accompanied by a burning sensation when urinating and the appearance of Clue-cell in the secretions, which are torn cells of the vaginal epithelium accompanied by a raise in pH up to 4.5, which is known as Amsel Criteria, are common symptoms of the disease (DeCherney *et al*.,2013)

Probiotics are *Lactobacillus* spp. that are non-pathogenic and generate antibacterial compounds. Living microorganism which, if administered in the right concentrations, improve the host's health (World Health Organization, 2005).

Lactobacilli spp. are the most common type of bacteria in the vaginal tracts of healthy women . An imbalance in the local microbiota can make women more susceptible to diseases like bacterial vaginosis (BV) and *vulvovaginal candidiasis* (VVC). Despite the fact that antimicrobial therapy is often effective, repeated use of antimicrobials leads to a high rate of recurrence and a rise in microbial resistance. (Santos *et al*., 2016).

PTH (parathyroid hormone, also known as the peptide hormone parathormone (also known as parathyrin) is produced by the parathyroid glands and regulates serum calcium levels through acting on bones , kidneys, and the gut (Coetzee *et al*.,2004).

Vitamin D is a fat-soluble, which is essential for both children and adults' calcium metabolism. A sufficient intake of vitamin D is essential for maternal health, fetus skeleton development, and the best results for both the mother and the fetus is when calcium requirements rise in the 3rd trimester of pregnancy. The metabolites of this vitamin play an essential role in control of mineral metabolism by altering calcium absorption in tissue of intestinal and having additional effect on the bones and many other tissue of body (Mulligan *et al*., 2010).

Vitamin D and parathyroid hormone (PTH) are two hormones that control the body's serum calcium levels (Holick, 2013). The calcium absorption is improved when 1,25(OH)2D enters the bloodstream and goes to the small intestines. In the kidney, the most conspicuous endocrine effects of 1,25(OH)2D are tight controls of their own equilibrium by suppressing 1-hydroxylase and stimulating 24-hydroxylase at the same time. An unbroken 1,25(OH)2D-vitamin D receptors scheme is essential for both baseline and PTH-induced osteo-clastogenesis. Mature osteoclast remove phosphorus and Ca from the bones, preserving optimum blood level of the 2 elements (Dusso *et al*.,2005; Holick ,2007).

Aim of the study

The aim of the present study was employed to explore the interaction between vaginal *Lactobacillus* and vitamin D deficiency of pregnant women in first trimester

To carry out these aims the following parameters were followed :.

- Investigation on *Lactobacillus* spp. bacteria as a reverse indicator of bacterial vaginosis in first trimester pregnant and healthy women
- Diagnosis of BV in first trimester pregnancy.
- Isolation and identification of *Lactobacillus spp*. from BV and Non-BV pregnant women.
- Determination of the antibacterial activity of Cell- Free Supernatants (CFS) extracted from *Lactobacillus spp.*
- Estimation of vitamin D ,parathyroid hormone level, and Count Blood Cells (CBC) assay.
- Estimation of the correlation between Vitamin D level and parathyroid hormone (PTH) with BV in first trimester pregnancy .

Chapter Two

Literature Review

2.1: Vaginitis

Definition and Symptoms of Vaginitis

Vaginitis defined as vaginal symptoms such as itching, burning, irritation, odor, and vaginal discharge is among the most prevalent causes for women seeking medical treatment is vulvovaginal pain (Hainer and Gibson ,2011). The pH of the vaginal fluid has been demonstrated to have an important function in determining vaginal health. During the reproductive years, the vaginal pH should be less than 4.5, but it should be higher than 4.5 before menarche and after menopause. Vaginal epithelial cells grow in response to estrogen, their glycogen levels rise, and *lactobacillus* manufactures lactic acid from glycogen, resulting in an acidic pH (pH 2.8–4.2) that promotes vaginal health. A vaginal pH greater than 4.5 may indicate bacterial vaginosis, *Trichomonas vaginalis*, group B *streptococcus*, or other pathogenic organisms in the case of vaginitis. As a result of epithelial cells' enhanced proliferation rate, the thickness of epithelial cell layers has grown (Patton *et al.,* 2000).

Some symptoms related to vaginal are inflammation caused by *streptococcus*, *staphylococcus*, and *E. coli* as 'aerobic vaginitis.' Vaginal *Streptococcus agalactiae* (also known as Group B *streptococcus*) and *E. coli* have long been linked with invasive maternal and newborn infections (van de Wijgert *et al.,* 2014).

Clinicians consider vaginal discharge as a common presenting sign in a variety of conditions (primary care, gynecology, family planning, and department of genitourinary medicine). Vaginal discharge is likely to be either useful or harmful. Despite the fact that atypical vaginal discharge frequently leads to sexually transmitted infections (STI) testing, vaginal discharge is a poor predictor of STI presence. It is typical for bacterial vaginosis to return following treatment, and this may be made worse by personal hygiene routines like

vaginal douching that disturb the normal flora in the vagina. STIs and bacterial vaginosis are commonly linked (Mitchell, 2004).

Reproductive tract infections (RTI's) cover three types of infections: sexually transmitted infections (STIs), infections caused by overgrowth of organisms which are commonly found in the reproductive system, and infections connected with medical operations, such as abortion and the implantation of intrauterine devices are all covered by the term "reproductive tract infections" Female RTIs, such as vaginitis and cervicitis, frequently start in the lower genital tract and cause symptoms such abnormal vaginal discharge, genital pain, itching, and a burning sensation when urinating (Rabiu *et al.*, 2010).

Infectious vaginal discharge in pregnant women can lead to abortion, premature rupture of membranes, chorioamnionitis, preterm, low birth weight, and postpartum endometritis, among other issues (Passini *et al*., 2010). *Lactobacillus* species dominate the healthy human vagina, they perform an important role in preventing women from genital infection. Lactobacilli deficiency can disrupt the microbial equilibrium in the vaginal area, leading to bacterial vaginosis (BV) syndrome. (Aroutcheva *et al*., 2001), which is linked to a quantitative and qualitative shift from naturally occurring *lactobacilli* to a mixed microflora dominated by anaerobic bacteria (Forsum *et al* ,2005).

2.2 Bacterial Vaginosis

Bacterial vaginosis is a bacterial imbalance in the vaginal flora caused by an overabundance of anaerobic bacteria and a deficiency of Lactobacillary flora. Women may experience signs of a typical vaginal discharge, but they are frequently asymptomatic. (Brocklehurst *et al*., 2013)

Among women of reproductive age, (BV) is a common infection of the genital tract that's been related to neonatal deaths, earlier miscarriage, repeated

abortion, premature delivery, and post-abortal infection. Following delivery, infection that result in preterm labor as well as other pregnancy complications might still exist, putting the infant at significant life-threatening danger. The public knowledge of the function of BV in premature births (PTB) is limited and inconsistent since many infection are subclinical (Africa *et al*., 2014)

Clinical symptoms, such as exfoliated epithelial cell containing Gram-variable polymorphic rod-shaped bacteria clinging to their surface, and a fishy odor, are all indicators of bacterial vaginosis (Turovskiy *et al*., 2011). The most common anaerobic microorganisms detected in BV are *Prevotella* species, *Gardnerella vaginalis*, *Mycoplasma homins*, and *Mobiluncas* species (Rampersaud, 2012; Zarbo *et al*., 2013). BV is related to an increased risk of Pelvic Inflammatory Disease (PID), STIs, and prematurity (Fredrick *et al.*, 2011; Tomusiak *et al.,* 2013). Vaginal discharge is typically caused by infection with *Neisseria gonorrhoeae* and/or *Chlamydia trachomatis*, while cervical discharge is usually caused by infection with *Neisseria gonorrhoeae* and/or *Chlamydia trachomatis*. (Zemouri *et al*., 2016)

Non-infectious reasons of vaginal secretions include vaginal douching, utilizing scented soap for cleansing, certain laundry detergent, toilet paper, personal care products, as well as other feminine care items that may irritate and generate unusual discharge. Foreign body (like a stuck tampons, condoms, or toilet paper) can sometimes cause a foul-smelling or bloody discharge (Le Doare and Heath, 2013).

Lactobacillus colonization requires attachment to epithelial surfaces, and one of the proposed mechanisms by which they could defend the vagina against pathogen colonization is through competing for adhesion receptor sites (Monteagudo-Mera *et al*., 2019). Interactions between specific adhesins on the bacterial surface and receptors on host tissues are part of bacterial adherence to mucosal surfaces (An and Friedman, 2000). Low-birth-weight newborns,

preterm delivery, and pelvic inflammatory illness have all been linked to bacterial vaginosis. Human Papillomavirus (HPV), Herpes Simplex Virus type 2 (HSV-2), *Trichomonas vaginalis*, *Neisseria gonorrhoeae,* and HIV are all more likely in women with BV. Given the high prevalence and severity of related morbidity, it was vital to properly diagnose and treat BV-affected women, particularly pregnant women (Hemalatha *et al.,* 2013).

Although bacterial vaginosis (BV) is associated with an increased risk of premature birth during pregnancy, treatment with metronidazole was not effective and, in fact, hazardous to the pregnancy. Although broader spectrum antibiotics, such as clindamycin, and combination antibiotherapy have demonstrated superior benefits, not all research agree (Gilbert *et al*., 2017). BV-associated bacteria toxin (lipopolysaccharide) can cross the placenta and injure fetuses' brains. Toxins have the potential to induce irreversible neurological brain damage like cerebral palsy, an increased risk of Parkinson's disease, and/ or schizophrenia (Hashemi *et al*., 2000). BV has also been related to an increased possibility of HIV infection (Urakubo *et al.,* 2001; Sha *et al.,* 2005), as well as herpes simplex virus type 2 infection (Sha *et al*., 2005; Eriksson *et al.,* 2005). Antibiotics, such as clindamycin or metronidazole, are commonly used to treat BV, and can be either orally or administered topically Antibiotics, on the other hand, do not eliminate all vaginal harmful microorganisms. About 60% of BV patients respond to this therapy, and there is a significant recurrent rate of 30% to 40% (Ling *et al.,* 2004; Larsson and Forsum, 2005). Furthermore, antibiotics disrupt the healthy vaginal microbiome, and the risk of acquiring antimicrobial drug resistance rises rapidly as the overall usage of anti- microbial (feminine hygiene and treatments) preparation rises. Metronidazole and Clindamycin inhibit *Lactobacillus spp*. in vitro at concentration lesser than those used topically to treat the bacteria (Aroutcheva *et al*., 2001; Macklaim *et al.,* 2015).

2.3 *Lactobacillus*

Lactobacilli are the most common bacterial genus found in the healthy vaginal tract of women of reproductive age (Witkin *et al.*, 2007). By regulating the environments as well as other bacterial via the formation of lactic acid, bacteriocins, and competitive exclusion, are believed to improve vaginal hygiene (Zhou *et al*., 2004;Fredricks *et al*., (2005), stated that the two main species present are *L. crispatus* and *L. iners*, and *L. crispatus* or *L. gasseri*.

The other organisms *L. jensenii*, *L. gallinarum*, or *L. vaginalis* may be presented in many instances as well (Pavlova *et al*., 2002). However a modified microbiota, like the lack of *lactobacilli*, may accompany vaginosis, the existence of lactobacilli also isn't required for vaginosis prevention (Witkin *et al*., 2007). *Lactobacillus* is a phylogenetically diverse genus that currently has over 125 species (Dellaglio *et al*., 2005). Probiotics are *Lactobacillus spp*. that are non-pathogenic and generate antibacterial compounds. This is described as a living microorganism which, when administered at the right concentration, boost the host's health (World Health Organization, 2005). As a result, they can be discovered in human body places where dietary substrates are easily available 126 (O'Callaghan and Toole, 2011).

The mechanisms by which *Lactobacillus* controls the colonizations of other bacterial species in the vagina decide whether or not pathogenetic or etiological treatments (Li *et al.*, 2005; Kovachev and VatchevaDobrevska, 2014).

2.4 Characteristics of *Lactobacillus* SPP.

Lactobacillus spp. are Gram-positive rods that are catalase negative, nonsporing, and facultative anaerobic, though they prefer to grow in microaerophilic conditions ,their Gram stain morphology can range from short, plump rods to long slender rods, in chain or palisades, and their colony

morphology can range from small to medium gray colonies on MRS that usually exhibit alpha hemolysis on blood agar (Dover *et al.,* 2008; Razzak *et al.,* 2011).

The pathogenicity of commensal *Lactobacillus* is undetermined, although the ability to create acids and the ability to grow and survive in an acidic environment are the most known predictors of cariogenicity of *lactobacilli* (Edelman, 2005; Badet and Thebaud, 2008). Other chemicals released by *Lactobacillus* are vital for maintaining vaginal microbiota balance (Shopova 2003; Stoyancheva *et al.,* 2014). Another defense component generated from Lactobacillus vaginalis is H2O2 (Vallor et al. 2001; Kovachev, 2018). *Lactobacilli* have a strong oxi-reduction potential in the vaginal environments, which prevents anaerobic bacteria from colonizing (Zarate and Nader-Macias, 2006; Gil *et al.,* 2010). *Lactobacillus* adheres strongly to the stratified, nonkeratinizing vaginal epithelium, causing pathogenic bacteria like Candida albican, Gonococcus vaginalis, E. coli, Strep. agalactiae, and Strep. aureus to be excluded and rejected (Parolin *et al*., 2015).

It is most likely the same method, *Lactobacillus* adheres to the vaginal wall predominantly by adhesins, which are produced as fine fimbriae on the cell surfaces and bind extracellular matrix ingredients such as laminin, collagen, and fibrokinetin that surround the host epithelium (Antikainen *et al.,* 2002; Younes *et al*., 2016). The varying affinity of adhesion for certain receptor, or their relative locations in the case of steric hindrance, are blamed for changes in vaginal epithelial cell adhesions (Zarate and Nader-Macias, 2006). After engaging and activating plasminogen system receptors, *Lactobacillus* is incapable to keep the produced cells plasmin activity (Lahteenmaki *et al*., 2001; Lorca *et al.,* 2002). Physiological antiproteases, unlike pathogenic microorganisms, are thought to be unable to combat the plasminogen proteolysis mechanism (Styriak *et al*., 2003; Buck *et al.,* 2005*).*

Lactobacillus contributes to vaginal health by producing chemicals (mostly organic acid) that acidify the environments and hinder the growth of infections (Tachedjian *et al*., 2017). To defend their ecological niche against competing organisms, *Lactobacillus* spp. as well as other bacteria produce antimicrobial peptides (bacteriocins) that are applied topically (Sablone *et al.*, 2000; Diep and Nes, 2002). The transmembrane potential and pH gradients are depleted as a result of their antimicrobial action, which is often brought on by the cellular membranes being permeable, which results in an efflux of ATR amino acids and ion. (Oscáriz and Pisabarro, 2001)

2.5 Bacteriocin as Antibacterial

Bacteriocins are multifunctional proteinaceous compounds generated by ribosomes that have strong antibacterial activity at specific quantities. They are created by bacteria and archaea to prevent the growth of bacteria that are similar or closely related. Antimicrobial action against pathogenic and degrading microorganisms is demonstrated by these compounds, indicating their biotechnological potential. (Negash and Tsehai, 2020). As previously mentioned, bacteriocin production was tested using both an agar spot test and a well-diffusion experiment (Delgado *et al*., 2007).

Bacteriocins have antibacterial properties against Gram-positive and Gram-negative bacteria, as well as fungus (Stoyancheva *et al.*, 2014). Bacteriocin kill pathogen by compromising cell wall integrity and inhibiting DNA and synthesis of proteins (Dover *et al.*, 2008). This results in a lake of adenosine-triphosphate (ATP). Bacteriocins have inhibitory effects similar to antibiotics, however they differ in terms of production, toxicity, method of action, and resistance mechanism (Kaur *et al.*, 2013).

Bacteriocin resistance is not commonly found in antibiotic-resistant bacteria, nor it is genetically determined. Furthermore, bacteriocin is more

active when the vaginal pH is between 4.0 and 7.0, and their effectiveness diminishes rapidly outside this range. According to toxicology studies, vaginal Lactobacillus spp. produces antimicrobial peptides that are non-haemolytic, don't really irritate the vaginal endothelium, and therefore do not affect the viability of the vaginal *Lactobacillus* spp. (Li *et al.*, 2005). This suggests that bacteriocins and the Lactobac from which they originate are safe (Stoyancheva *et al*., 2014).

2.6 Antibiotic and *Lactobacillus Spp.*

Due to the extensive use of antibiotics, pathogenic and non-pathogenic microorganisms linked to people, animal, and the environmental have developed and transmitted antibiotic resistance (AR) (Hernando-Amado *et al.,* 2019). The rapid spread of AR among bacteria, along with a dearth of new antibiotics, is a huge public health problem around the world (WHO, 2014). The selection pressure imposed by antibiotic in the environments has aided the spread of AR through horizontal gene transfer (HGT) (von Wintersdorff *et al.*,2016).

Antibiotic that is used to treat infection in people is frequently the same as that utilized in veterinary medicine, leading to the rapid spread of AR gene among bacteria in the food chain (EFSA, 2016). *Lactobacillus* spp. Are found in the native microbiota of food, as well as human and animal mucosa. Some Lactobacillus strains are utilized as starter cultures and supplement cultures to manage food fermentation, while others are used as probiotic to prevent and treat intestinal disorders (Sáez-Lara *et al.*, 2015).

Lactobacillus is found in the native microbiota of food, as well as human and animal mucosa. Some Lactobacillus strains are utilized as starter cultures and supplement cultures to manage food fermentation, while others are used as probiotics to prevent and treat intestinal disorders (Sáez-Lara *et al.*, 2015;

EFSA, 2018). In reality, most Lactobacillus species have been found to be resistant to these antibiotics (Abriouel *et al.*, 2015). Lactobacilli susceptibility to antibiotics must be determined, and the lack of transferrable AR genes in selected strains must be ensured (Salvetti and O'Toole, 2017). Over the last 15 years, studies have been published that show bifidobacteria and lactobacilli are typically sensitive to tetracycline, erythromycin, chloramphenicol, ampicillin, penicillin, quinupristin-dalfopristin, clindamycin, linezolid, and clarithromycin (Abriouel *et al.*, 2015). Resistance to aminoglycosides can develop through a variety of pathways, (Doi *et al.*, 2016) including

I. Aminoglycoside acetyltransferases, nucleotidyltransferases, or phosphotransferases which are responsible for the enzyme modifications and deactivation of the antibiotic.

II. A higher efflux

III. Reduced permeability

IV. Modification to the 30S ribosomal subunit that prevent this family of antibiotic from adhering.

Some phylogroups of *Lactobacillus* spp. as well as other LAB species, including *Leuconostoc* spp., have a built-in strong resistance to vancomycin (Goldstein *et al*.,2015).

2.7 Probiotic

Probiotic are described as living microorganism ,if these probiotic were given in the right amounts, help the host's health (Reid, 2008).

Probiotics have been suggested as an alternative therapy for BV and may be applied locally or orally to the vaginal system in the form of pantyliners, suppositories, or tampons. Oral probiotics may enhance the long-term outcomes of BV therapy with vaginal suppositories including *L. acidophilus* by

harmonizing the vaginal and gastrointestinal flora (Antonio and Hillier, 2003; Delia *et al.*, 2006). By colonizing the vaginal system, the newly imported *Lactobacillus* spp. would beat off vaginal infections and stop the introduction of new illnesses. Despite the fact that the majority of trials were small and sometimes lacked control group, several studies on the treatments of BV with probiotic have shown mainly favorable outcomes (Reid *et al.*, 2001; Uehara *et al.*, 2006)

In vitro properties such as generation of antimicrobial agents (H_2O_2, bacteriocins), ability to adhere to vaginal epithelial cells, and antimicrobial effectiveness against pathogens have all played a role in the selection of *Lactobacillus* spp.-based probiotics. Other desirable criteria for probiotics include their ability to strongly acidify the vaginal environment with lactic acid in order to have beneficial effects on the host, such as immunomodulatory activities, the ability to survive and outcompete other bacteria, coaggregation, and antibiotic susceptibility other than those used to treat BV. (MacPhee *et al.*, 2010; Santos *et al.*, 2016).

Probiotics are becoming more widely accepted as an alternative to antibiotic therapy for controlling some infections and reestablishing the human bacterial ecology. *Lactobacillus* spp. protect the vaginal epithelium from pathogen colonization through two main mechanisms: blocking pathogen attachment and producing substances that inhibit pathogen multiplication. Various pathogens may respond differently to the same *Lactobacillus* strain in terms of blocking and inhibitory activities. This emphasizes the necessity of recognizing *Lactobacillus* spp. strains prior administering them humans as probiotics. The main indications for probiotic *Lactobacillus* use in urogenital tract infection include bacterial vaginosis, repeated vaginal candidiasis, post-infection prophylactic by re-establishing the vaginal flora to prevent any future

episode, and repeated Urinary tract infection (UTI) in healthy pre-menopausal and post-menopausal women. (Andreu, 2004).

2.8 Vagina Anatomy and Sex hormones

The vagina (meaning sheath in Latin) is an important part of the female reproductive tract or reproductive organs located in the pelvis. It is a thin-walled fibromuscular tube (approximately 8-10 cm in length) connecting the uterine cervix internally and the vestibule externally. A stratified, squamous and nonkeratinized epithelium is placed on a dense connective tissue that contains a vascular venous plexus, and act as an erectile tissue (Sato *et al*.,2018)

Bacterial communities in the human vagina are thought to have a critical role in protecting the host against infectious disease. In reproductive age women, it is thought they do so through the production of lactic acid resulting in a low pH environment that restricts the growth of pathogens and other opportunistic organisms (O'Hanlon *et al*., 2011)

During pregnancy, the placenta is the primary endocrine organ for maintaining pregnancy and fetal growth. The placenta releases hormones, including androgens, estrogens, and progestogens, and these hormones occur at extremely high concentrations in the maternal circulation. These hormones are important paracrine and autocrine regulators affect the growth and differentiation of the placental trophoblast, growth and maturation of the placental vascular tree, and uterine endovascular invasion by the extravillous cytotrophoblast (Armaly *et al*.,2018). Progesterone and estrogens also function as modulators of uterine vessels in that they decrease the resistance of the spiral uterine arteries and modulate the synthesis and release of angiogenic factors by placental cells (Berkane *et al*.,2017, Kumar *et al*.,2018)

Sex hormones contribute to the development and activity of the immune system, accounting for differences in gender-related immune responses. Both innate and

adaptive immune systems bear receptors for sex hormones and respond to hormonal cues (Moulton ,2018). It is well known that estrogen suppresses T and B cell lymphopoiesis and activates B cell function. ERs are found on thymocytes as well as thymic epithelial cells (Savino *et al*.,2016) Estrogen influences T cell development and lymphopoiesis, its effects on the thymus are complex (Zoller and Kersh,2006)

2.9 Complete Blood Count (CBC):-

2.9.1 Blood

In humans, blood is a biological fluid in circulatory systems that moves transporting metabolic waste products out from cells while providing or supplying crucial nutrients oxygen and nutrients. The produced constituents include RBCs, WBCs (neutrophil, monocyte, lymphocyte, eosinophil, and basophil), and platelets. Plasma is mostly water, with 7% ,the remainder is made up of protein (globulin, albumin, and fibrinogen) and 2% other substances (electrolyte, nutrient, breathing gases, waste product, and regulatory compound) (Thakare, 2021). Plasma volume increases in the first weeks of pregnancy, with the steepest increase occurring during the second trimester, after which it continues to increase further in the third trimester (De Haas *et al*.,2017) . During pregnancy, 25(OH)D level may be influenced by physiological hemodilution. Also, blood cell count (RBC), hemoglobin (Hb), and hematocrit could be the indicators of hemodilution. Thus, examined the 25(OH)D level as well as the red blood cell count (RBC), hemoglobin (Hb), and hematocrit across all the three trimesters among pregnant women (Takaoka *et al*.,2020)

Red blood cells, white blood cells, and platelets are all produced in the human body constituents of blood. The majority of cells in the blood (erythrocytes) are RBCs. Their primary function is to transport O_2 from the lung to the tissue and return CO_2 to the lungs. This is because haemoglobin, a

protein that binds to oxygen quickly and reversibly, is present. Under specific conditions, haemoglobin's affinity for oxygen varies, allowing for higher oxygen offloading at the respiratory tissues. The body's defense against pathogens is made up of white blood cells (leucocytes) ,Granulocytes and agranulocytes are two types of cells that kill infections via separate ways (Glenn and Armstrong, 2019).

Despite this diversity of responsibilities, the available platelet function tests focus on changes that are directly related to the hemostatic process (Harrison, 2009) .The typical platelet count in a mm3 of blood is between 150,000 and 300,000. (Des, 2020)

The complete blood count (CBC) is a widely ordered test that is routinely conducted on large, automated hematology analyzers in central laboratories. The results of the CBC are used to make critical and clinical choices like whether to transfuse, provide chemotherapy, or give antibiotics (Anthony, 2015)

The RBCs, WBCs (eosinophil, lymphocyte, neutrophil, monocyte, and basophil), and platelet make up the formed constituents. Plasma is mostly made up of water, with 7% of protein (globulin, albumin, and fibrinogen) and 2% of miscellaneous chemicals (electrolyte, nutrient, respiratory gases, waste product, and regulatory substance) (Thakare *et al.*, 2021). Erythrocytes have a role in cardiovascular system modulation through mechanisms that include their interaction with the endothelium. Bacteria, viruses, parasites, poisons, and tumors are all examples of invading microorganisms, and leukocytes operate to eliminate dead cells and detritus from the body. WBCs are divided into five types, each of them is classified as granulocytes or agranulocytes based on the presence or lack of granules and the staining properties of their cytoplasm (Des, J. T. R, 2020).

When a blood cell passes through a small hole surrounded by electrodes, the equipment generates an electrical pulse. As a result, the electronic counter not only counts the total number of cells, but also assesses the average of cell volume and cell size fluctuation. These metrics are known as the mean corpuscular volume (MCV) and the RBC distribution width, respectively, in the context of RBCs. Modern electronic counters may also analyze cell size and content in multiple ways, giving you more information on the different types of WBCs like neutrophils, lymphocytes, monocytes, eosinophils, and basophils (Tefferi *et al.,* 2005).

2.9.2 Leucocytes

In medical practice, alterations in peripheral leucocyte count can be very useful, and the cells line implicated can be particular to certain conditions. Neutrophilia is a type of bacterial infection that is usually detected in individuals. The most serious infections are linked to increased neutrophilia and, in some cases, a degree of myeloid left shift (the existence of immature myeloid cell in the peripheral circulation) as well as 'toxic' neutrophil granulation. Non-infectious illnesses can also cause neutrophilia. It's a common side effect of steroid therapy, strenuous activity, and surgery or splenectomy, but it can also happen with systemic vasculitis, tissue necrosis/burn, and as a result of some cancers. Lympopenia is a typical side effect of steroid and other immunosuppressive medications, such as azathioprine. It is related with rheumatoid arthritis, systemic lupus, and sarcoidosis, and it can be a presenting characteristic in patient with Hodgkin lymphoma. Mild lymphopenia is a fairly typical finding in a routine FBC and should not prompt further inquiry in the absence of any additional symptoms(Yedgar *et al*., 2002; Poz *et al*., 2018).

In experience, investigating moderate isolated lymphopenia rarely yields positive results. Lympocytosis is a typical side effect of viral infection, it's often accompanied by a modest, self-limiting neutropenia. Stress lymphocytosis is

brought on by acutely occurring disorders such heart attack, severe trauma, and epileptic seizures, which is a typical occurrence in hospital patients. The lymphocytosis arises suddenly and lasts only a few days after the trauma. Mild lymphocytosis can occur after a splenectomy and can also be caused by smoking (Leach, 2014).

2.9.3 Hemoglobin: -

A 50 % reduction in anemia in women of reproductive age (WRA) is a World Health Assembly Global Nutrition Target for 2025, and accurate hemoglobin measurement is a global priority (WHO, 2020). The use of hemoglobin measurements as a screening approach for donor eligibility in the blood banking context is a must. Anemia is a disorder caused by a lack of circulating RBC, which limits their ability to transport O_2 throughout the body (McLean *et al.,* 2009).

Hb is a protein found in red blood cells that transports oxygen to the tissues. Hb concentrations below a certain threshold (70–130 g/L dependent on age, gender, pregnancy status, and degree of severity) are considered anemia (WHO, 2018). Anemia affects roughly 1.93 billion people, or 27% of the global populations, which is a serious public health issue, with WRA having the greatest frequency in low-income and middle-income nations and preschoolers (Kassebaum, 2016).

Hemoglobin levels in men range from 13.5 to 17.5, whereas women's levels range from 12 to 16 (Turgeon, 2005, Mairabäurl and Weber, 2011). Both normal hemostasis and pathological bleeding and thrombosis need the involvement of human platelets. These cells play an important role in tumor growth and metastasis, as well as vascular constriction and repair (George, 2000) Platelets also have a role in inflammation, associated diseases, and the advancement of atherosclerosis when they collaborate with other cells such as

white, endothelial, or smooth muscle cells. (Katz *et al.,* 2011; Badimon and Vilahur, 2014; Katz *et al.,* 2011).

2.9.4 Platelets

Megakaryocytes, which are found largely in the bone marrow, create circulating platelets. Megakaryocytes, like all other blood cells, are produced by a master stem cell (Michelson, 2019). In individuals with ongoing chronic infection, inflammation, or cancer, thrombocytosis is usually encountered as a reactive phenomena. The longer these disease processes go on, the more possible it is that thrombocytosis will develop. Other inflammatory indicators are frequently elevated in these patients, and the blood film tend to show small homogeneous platelet with minimal differences in size. Thrombocytosis is commonly connected with chronic bleeding and iron deficiency anemia, and it will go away after the bleeding source and iron shortage are addressed. The majority of instances observed in general medical practice are reactive thrombo-cytosis and thrombocytosis following splenectomy. Thrombo- cytosis is a symptom of a number of myelo-proliferative illnesses, and it is frequently associated with hemoglobin or platelet count abnormalities. Inflammatory indicators will not be elevated in these circumstances, and the blood film will often reveal big platelets with a wide range of individual sizes (Leach, 2014).

2.10 Parathyroid glands

PTGs (parathyroid glands) are nodular structures that are commonly seen along the thyroid's posterior wall. They produce parathyroid hormone (PTH), which is critical for maintaining calcium homeostasis in the body. Primary hyperparathyroidism (PHPT) is caused by an adenomatous, hyperplastic, or occasionally carcinomatous gland overproducing PTH, while secondary hyperparathyroidism is caused by hypocalcemia (SHPT). Chronic renal disease or malabsorption can cause hypocalcemia. (Apetrii, 2017).

The parathyroid gland is one of the extrarenal structures that produces calcitriol, it plays a important role in Ca and phosphate homeostasis via Ca, phosphate, and vitamin D feedback loops. Segersten *et al*. were the first to discover that human parathyroid glands produce 1OHase protein and mRNA, the enzyme is more abundantly expressed in hyperplastic glands from patients with primary and secondary hyperparathyroidism than in normal glands (Segersten *et al*., 2002). There are three forms of hyperparathyroidism: primary, secondary, and tertiary. In the absence of a known stimulus, primary hyperparathyroidism is defined by excessive secretion of the parathyroid hormone (PTH) from larger parathyroid glands, resulting in hypercalcemia. (DeLellis *et al*., 2008; Carlson ,2010).

The parathyroid gland is made up of four tiny glands that are implanted in the back of the thyroid gland. Its primary role is to produce and secrete parathyroid hormone (PTH), a polypeptide hormone it keeps serum calcium levels in check. PTH and level of serum calcium are inversely proportional. PTH, in concert with vitamin D, mobilizes calcium reserves and rises calcium absorption and re- absorption in the body when serum calcium levels are low. The parathyroid gland have a negative feedback from vitamin D and Calcium, which binds to a receptor in the gland when levels increase, inhibiting PTH production and release. (Schwartz *et al*., 2019; Demir *et al.,* 2020; Ilahi *et al*., 2021). Chief cells and oxyphil cells are the two main cell types found in the parathyroid glands. (Hakami and Khan, 2019; Khan *et al*., 2021).

PTH, an 84-amino-acid straight-chain polypeptide, is synthesized and secreted by the parathyroid glands' main cells. A bigger precursor is generated in RER ribosome and transported to the Golgi apparatus via the ER's cisternal channels, where it is membranes-bound glandular or storage granule after being enzymatically converted into a prohormone precursor. The amino-terminals end of the 90-amino-acid pro- hormone is stripped of a hexapeptide to create the

physiologically active hormone. During production, the granule and cellular membranes fusion releases PTH into the interstitium, in which it spreads into blood capillaries. PTH along with calcitonin and calcitriol, is involved in calcium homeostasis regulation (the active form of vit. D) (Mense and Rosol, 2018). Oxyphil cells are substantially larger (12–20 mm) than chief cells (6–8 mm) and feature an eosinophilic cytoplasm due to their high mitochondrial contents. Oxyphil cells are generated from transitional cells, which are small like chief cells but eosinophilic similar chief cells. The finding that glial cells do not express the 2 gene and that transitory and oxyphil cells both produce parathyroid hormone (PTH) strengthens the case for a chief-to-oxyphil cells trans- differentiation (Ritter *et al.,* 2012).

2.11 Parathyroid hormone

The parathyroid gland produces parathyroid hormone, which is a polypeptide that is produced and cleaved into an active form. A pre-pro-PTH, a 115-amino-acid polypeptide, is synthesized first, then cleaved to generate pro-PTH, which is 90-amino-acids long. It is subsequently cleaved a second time, this time at the amino-terminal end, to yield 84 amino acid active parathyroid hormone. This is the most important hormone in the body, since it is stored, secreted, and operated. Synthesis, cleavage, and storage are all expected to take less than an hour. When low serum calcium is observed, active PTH production can occur in as little as a few seconds. The method of secretion is exocytosis, in which the hormones are secreted through membranes vesicles carried to the cellular membranes and discharged after the vesicles merge with the outside membranes. Activated PTH has a short serum half-life and is swiftly eliminated from the bloodstream by the kidneys and liver. (Hans and Levine, 2021; Goyal *et al*., 2021).

Hypoparathyroidism is defined by a decrease in the actions of parathyroid hormone (PTH), which can be caused by either decreased secretion

or resistance to PTH. Studies in genetic anomalies of hypoparathyroidism patients have substantially increased understanding of the etiology of hypoparathyroidism. A new hypoparathyroidism classification based on these genetic anomalies is proposed, as well as a diagnostic method for hypoparathyroidism patients. Endo et al. (Endo *et al.,* 2007).

PTH receptors from a variety of other species have been cloned. Due to the diverse action of PTH in a number of different body tissues and the in vitro studies that have shown for numerous second messengers of hormones discharge from research findings with cell membrane shards improved in the PTH receptor, it was initially hypothesized that many various receptors would've been found to facilitate some of the pleotropic actions of this peptide hormones. Because of this, it came as a bit of a surprise when early cloning efforts in a variety of species only produced one G proteins-coupled receptors, now recognized as the common PTH/PTHrP receptors. This receptor is involved in the majority of PTH's classical functions in mineral ion homeostasis, as well as its effects on bones and kidneys. There are other receptor for both PTHrP and PTH, which are beyond the focus of this review; however, they have a variety of biological functions ascribed to them, most of which include contact with parts of either PTHrP and PTH beyond the amino terminal 34 residue (Jüppner *et al*., 2000). There are numerous ways to assess PTH in the blood: intact PTH, N-terminal PTH, mid-molecule PTH, and C-terminal PTH, and different assays are employed in different clinical settings. The typical PTH concentration is 8–51 pg/mL. (Longo *et al.*,2012).

PTH production is closely controlled on a transcriptional of post-transcriptional and levels by extracellular calcium concentrations. Hypocalcemia, glucocorticoids, and estrogen all stimulate PTH gene transcription. PTH intracellular breakdown can also be accelerated by hypercalcemia. Hypocalcemia, dopamine, adrenergic agonists, and

prostaglandin E2 all stimulate PTH secretion. The CaSR, which is found in the cell membrane of parathyroid cells, detects changes in serum calcium. The receptor allows the parathyroid gland to detect changes in plasma calcium concentration, resulting in desired changes in PTH release (Goodman and Puarles, 2008).

PTH reduces the quantity of NaPit2c and NaPit2a, in the renal proximal tubule brush border membranes, resulting in reduced phosphate reabsorption and phosphaturia. The sodium phosphate cotransporter responses to PTH involves a number of kinases, include proteins kinases A and C, mitogen-activated proteins kinase extracellular signal-regulated kinase 1/2, and a myosin motors (myosin VI) (Lederer *et al.,* 2003; Blaine *et al.,* 2009).

The rise in osteoclastic activity induced by PTH results osteopenia and osteoporosis are caused by localized bone weakening and a broad reduction in bone mineral density (BMD). Deficit calcium phosphorus intake results in a skeletal mineralization deficiency. (Holick ,2007).

This disorder, known as rickets, causes a variety of skeletal abnormalities in young children who have insufficient mineral in their skeleton. Muscle weakness is another symptom of VDD with affected youngsters having trouble standing and walking (Holick,2006, Gordon *et al*.,2008), and the elderly experiencing increased sway and more frequent falls. As a result, the risk of fracture increases. Vitamin D Deficiency was linked to a nearly 3-fold greater incidence of Bacterial Vaginosis in pregnant women, based on the National Health and Nutrition Examination Survey data BV (Hensel *et al*.,2011).

The diagnosis of parathyroid dysfunction has gotten a lot easier since the emergence of reliable and precise PTH testing (Marx, 2000). Several investigations, however, have shown that PTH assays are not interchangeable (Cantort *et al*., 2006; Souberbielle *et al*., 2006). Furthermore, preanalytic

factors and method variations can have a negative impact on clinical decision-making. (Torres,2006; Cantort *et al*.,2006; Joly *et al*.,2008).Variability can be influenced by a number of factors, including the test employed, the population studied, vitamin D status, and a number of pre-analytical circumstances (Joly *et al.*,2008; Parent *et al*.,2008).

PTH also modulates fetoplacental calcium homeostasis and skeletal development in pregnant women (prentice, 2000;Kovacs and Fuleihan, 2006). As a result, it's critical to comprehend the factors that influence PTH levels during pregnancy. 25(OH)D, Dietary calcium and blood calcium have all been examined in relation to PTH (Green *et al*.,2008;Kramer *et al*.,2016). However, the available information regarding the effects of calcium supplementation on PTH is debatable, because of the lack of agreement on the recommended calcium intake during pregnancy (Hemmingway *et al*.,2018; Cormick *et al*.,2019). Other factors such as socioeconomic and lifestyle characteristics have previously been suggested to PTH levels can be predicted in non-pregnant adults, but data on pregnant women is few (Jääskeläinen, *et al*.,2013;Hysaj *et al*.,2021).

Furthermore, despite the fact that quantitative ultrasound (QUS) is a valuable, safe, and inexpensive technology, data on bone quality in the research on pregnant women and PTH is limited (Brandao *et al.*,2013; Hellmeyer *et al.*,2015).

2.12 Vitamin D :-

Vitamin D a lipid-soluble substance that promotes bone metabolism and helps maintain calcium and phosphorus balance. The role of vitamin D in extracellular health has received a lot of attention. When it comes to metabolism during pregnancy, there is a lot of research and disagreement about the impact of vitamin D levels on fetuses and neonates (Salle, 2000; Wagner, 2012).

Vitamin D deficiency is common in pregnant women all over the world (Wagner ,2012).

Vitamin D is important for bones well-being and plays a key role in the mineralization of bones. Vitamin D3 is essential to the maintenance of a constant relationship between calcium and phosphate ions, which are key factors in strengthening and protecting teeth. (Brito *et al.,* 2004). In human skin, solar rays facilitate the formation of vitamin D3 from 7-dehydrocholesterol. Vitamin D3 undergoes two-step hydroxylation to form 25-hydroxy vitamin D3 (25-OHD3) and biologically active 1,2-dihydroxyvitamin D3 (1,25-(OH)2D3) ((Suda *et al.,* 2003; Bikle, 2014).

Vitamin D insufficiency in pregnancy causes newborn vitamin D deficit, which increases the risk of rickets since newborn vitamin D reserves rely on maternal vitamin D levels (Dawodu *et al*., 2005). Vitamin D regulates calcium and phosphate equilibrium, as well as bone mineralization (Holick, 2003). Serum 25(OH)D levels of 80 nmol/L are now widely accepted as a marker of vitamin D insufficiency (Dawson-Hughesb *et al*.,2005). Vitamin D production in the skin is seasonal; vitamin D shortage is frequent in winter.1,25(OH)2D increases the development of antimicrobial peptides in respiratory epithelial cells, protecting the lungs against infection. It has been claimed that vitamin D insufficiency is a "seasonal stimulant," explaining pandemic influenza's exceptional seasonality (Cannell *et al.,* 2006; Bischoff-Ferrari *et al.,* 2006). The ideal vitamin D content throughout pregnancy and in newborn is still up for debate (Marshall *et al.,* 2013).

A serum level of 11 ng/mL is assumed to be enough to prevent rickets in babies, while a serum level of >30 ng/mL is needed to control secondary rickets. Hyper- parathyroidism and increased calcium absorption deficient values are less than 20 ng/mL, and the optimal concentration is >30 ng/mL (Holick *et al*., 2011). Vitamin D insufficiency was linked to issues with

embryonic bone formation and growth. (Cavalier *et al*., 2008). It may impair newborn skeletal development, bones growth, and insufficient bone mass development, and also cause osteomalacia during gestation, skeletal abnormalities, and fetus minerals bones acquisitions in infant (Dawodu & Akinbi, 2013; Viljakainen *et al.*, 2010).

Extraskeletal effects of vitamin D insufficiency in pregnancy includes an increased risk of gestational diabetes, hypertension, and maternal bacterial vaginosis, but not caesarean section delivery. Infants are more likely to experience intrauterine development retardation, low birth weight, and early-onset sepsis as a result of it. Furthermore, adequate vitamin D levels during pregnancy are thought to benefit both the mother and the child's immune systems. (Pooraziz, 2015; Aghajafari *et al.,* 2013).

Early-onset infection in term neonate can be prevented with enough vitamin D treatment during pregnancy (Cetinkaya *et al.*, 2014). Inadequate vitamin consumption, a lack of fortified foods, lifestyle, seasonal change, color of skin, low supplement complain, premature delivery, body covering, obesity, and living at high latitudes are all causes of vitamin D deficiency (Maghboli *et al.,* 2006; Hagenau *et al*., 2009).

In several countries, vitamin D insufficiency was documented in large proportions among pregnant women and babies. According to a large study, the occurrence of vitamin D insufficiency differs between 18 and 84 percent in different countries, depending on ethnicity, geography, culture, and customs. (Kovacs *et al.,* 2001). Measuring vitamin D insufficiency in Iran, and varied figures for pregnant women and neonate have been reported (Maghbooli *et al.*, 2007). Vitamin D insufficiency is rather common in healthy pregnant mothers and newborns. Vitamin D is involved in calcium and phosphorus metabolism, which is important for bones health, as well as the physiopathology of various

autoimmune diseases, therefore finding techniques to minimize vitamin D deficiency looks to be key (Hashemipour *et al.*, 2004).

The American Academy of Pediatrics recommends that all breastfed infants born to mother with vitamin D deficiency receive 400 IU/day of oral vitamin D supplementation, which should be continued until they reach the age of six. The dose must be 600 IU/day in situations of dark skin (Wagner & Greer, 2008). A healthy lifestyle should be encouraged, with outdoor activities and sun exposure, as well as the use of fortified foods (Dovnik *et al.*, 2014). In 90 percent of newborns, Shakiba found that a four-month gap in vitamin D sufficiency had clinical effects (Shakiba *et al.*, 2014).

Vitamin D is necessary for bones health it plays an important role in bone mineralization (Suda *et al.*,2006), Vitamin D deficiency in the mother causes vitamin D deficit in the baby, increasing the risk of rickets (Dawodu A *et al.*, 2005). A more recent evidence reveals that deficiency of vitamin D is linked to non-skeletal health effects in addition to impaired skeleton mineralisation (Holick, 2004; Pasco *et al*., 2008). Vitamin D is stored in the form of 25-hydroxyvitamin D (25(OH)D) and circulating plasma concentration of 25(OH)D is a good predictor of vitamin D level. There is no consensus on what constitutes appropriate vitamin D level, therefore, many cut-off points have been employed to determine degrees of deficiency: either a severe deficit of 12•5 nmol/l, an inadequacy of 50 nmol/l, or an inadequacy of 80 nmol/l (Lips,2004; Hollis, 2007; De Ronne and De Schepper, 2013).

The definitions and importance of vit D deficiency are still up for debate. 40 percent of European are vitamin D insufficient, with 13 percent having severe deficiencies, according to new big observed data (Cashman *et al*., 2016) It has been questioned if this widespread shortage is significant and whether supplement is necessary (Zhang *et al*., 2019)

This was also evident in the topic's meta-analyses, which was conducted with inadequate methodological standards (Amrein *et al.,* 2017). Low vitamin D levels are becoming more frequent around the world, and various research ranging from basic science to clinical application have found a strong link between chronic diseases and acute situations. Furthermore, pathological connections of vitamin D with energy balance and immunological and endocrine system modulation are accompanied by the huge amount of observational data presently available (Bouillon *et al.,* 2013). Vitamin D supplementation appears to ameliorate three poor pregnancy outcomes: 40 percent fewer preterm births, 50 percent fewer cases of gestational diabetic, and 60 percent fewer cases of preeclampsia (Rostami *et al.,* 2018).

These findings are in line with past research on the subject (Wagner *et al.,* 2009). Furthermore, similar to the genomic and epigenetic consequences of vitamin D supplementations, vitamin D insufficiency during pregnancy appears to be able to trigger certain genomic pathway linked to autoimmune illness in infancy and later life (Hossein-nezhad *et al.,* 2015; Novakovic *et al.,* 2009). Because The placenta, is like the kidneys, may convert 25(OH)D to the active form 1,25(OH) 2D, further basic study on the particular vitamin D metabolism in pregnancy is needed in the future (Hossein-nezhad *et al.,* 2012).

In 2016, conducted a comprehensive review of the relationship between vitamin D deficit during pregnancy and PTB, finding that pregnant women with vitamin D deficiency had a higher risk of PTB According to an update in 2017 update on this issue's comprehensive study (Qin *et al*., 2016; Zhou *et al*.,2017). Vitamin D supplementation alone during pregnancy can minimize the risk of PTB, whereas circulatory 25-OH D insufficiency in pregnant women can raise the risk of PTB. Despite systematic reviews examining the link between vitamin D deficit and PTB during pregnancy, no research has found a relationship between vitamin D insufficiency and PTB during different pregnancies.

Furthermore, high-quality meta-analysis is becoming more widely recognized as one of the most important strategies for obtaining evidences (Yao *et al*.,2016; Tian *et al.*,2017).

When exposed to UV radiation, the skin produces vitamin D3 (cholecalciferol), a fat-soluble steroid derived from 7-dehydrocholestrol. The hepatic enzyme 25-hydroxylase catalyzes the hydroxylation of vitamin D at position 25 to generate 25-hydroxyvitamin D, also known as calcidiol. Before reaching the kidneys, 25-hydroxyvitamin D is produced in the liver and enters the circulation, where it is bound to vitamin D binding protein. Two enzymes 1-hydroxylase and 24-hydroxylase found in tubular cells of the kidney can further hydroxylate calcidiol, resulting in 1,25(OH)2D, the most active form of vitamin D, or 24,25-dihydroxyvitamin D, an inactive metabolite (Dusso et al.,2005). As a result, the vitamin D hormonal system can take many different forms, ranging from cutaneous precursors through dietary components to the most active metabolite, 1,25(OH)2D, which regulates calcium homeostasis and bone health by acting on target organ receptors. The serum concentration of 25(OH)D, the precursor form of physiologically active vitamin D, is the most accurate indicator of total vitamin D storage or status (Holick ,2007). It has been related to an increased risk of preeclampsia, gestational diabetes, low birth weight, and PTB in both mothers and babies (Agarwal *et al.*, 2018).

Vitamin D Deficiency was found to reduce the link between smoking and BV in non-pregnant women. Disorders of the pelvic floor as people get older, illnesses of the pelvic floor, such as urine and fecal incontinence, are becoming more common. With a 30% failure rate, pelvic floor issues continue to be among the greatest prevalent indications for gynecologic surgeries. They were associated to osteoporosis and low BMD. Low levels of 25(OH)D are public in women, and lower level has been associated to an increased risk of pelvic floor disorders (Badalian and Rosenbaum, 2010) .

Complications of Vitamin D Deficiency Calcium, bone metabolism and phosphorus, are disrupted as a result of VDD. The absorption of dietary calcium and phosphorus is reduced by VDD, leading in an increase in PTH levels (Lips *et al*.,2006; Heaney, 2004).

Chapter Three

Materials and Methods

3. Material and Methods

3.1 Material

3.1.1 Laboratory Equipment and Instrument

The laboratory equipment and instrument used in the present study have been recorded in the table (3-1) below.

Table (3-1): Equipment and Instruments

No	Laboratory Equipment and Instrument	Manufacturer/Origin
1	Anaerobic jar	Fisher(UK)
2	Autoclave	Hirayama (Japan)
3	Benze burner	Amal (Turkish)
4	Centrifuge	Hettich (Germany)
5	Compound light microscope	Olympus(Japan)
6	Densichek Turbidity meter	Biomerieux (France)
7	Disposable and glassware	Afco(Jordan)
8	Distillator	Deltalab(Spain)
9	Electric sensitive balance	Sarteorius ag (Germany)
10	ELISA	BioTeck (USA)
11	Hematology Auto analyzer	Dymind (China)
12	Hot plate with magnetic stirrer	IKA (Germany)
13	Incubator	Memmert (Germany)
14	Inoculation loop	Himedia(India)
15	Micropipette	Dragon (china)
16	Refrigerator	Concord (Lebanon)
17	Safety hood	EuroClone (Italy)
18	VITEK 2	Biomerieux (France)

3.1.2 Chemical Materials Utilized in the Study

The biological and chemical materials that were used in the study have been itemized in the table below.

Table (3-2) Biological and Chemicals Material

No	Biological and Chemical Materials	Manufacturer
1	Glycerol ($C_3H_8O_3$)	Merck(England)
2	Gram stain	Spectrum (Germany)

3.1.3 Commercial kit

The commercial kits that were used in this study were illustrated in the table (2-3).

Table (3-3): Commercial kit

No	Type of Kit	Origin
1	Vitamin D Elisa	Monobind (USA)
2	PTH Elisa	Monobind (USA)
3	VITIK 2 ANC Card	Biomerieux (France)

3.1.4 Culture Media

Table(3-4) contains a list of the cultural media employed in this study.

Table(3-4): Culture Media and the Purpose of Use

No	Culture Media	Company(Origin)	Purpose of use
1	**De man, Rogosa and Sharpe (MRS) agar & MRS broth**	Himedia(India)	For *Lactobacillus* spp isolation
2	**Muller-Hinton Agar (MHA)**	Himedia(India)	Antibiotic susceptibility

3.1.5 Antibiotic Discs

Table(3-5) provides a list of the antibiotics utilized in this study.

Table (3-5): Antibiotic Discs, Symbols and Potency

No	Anti- biotic disc	Symbols	µg/disk	Manufacture /Origin
1	Cefixime	CFM	5	Bioanalyse (Turkey)
2	Cefepime	FEP	10	
3	Nitrofuranation	F	100	
4	Amoxicillin/ Clavulanic acid	AMC	30	
5	Amoxicillin	AX	25	

3.2 Study Design

Study design shown in figure (3-1).

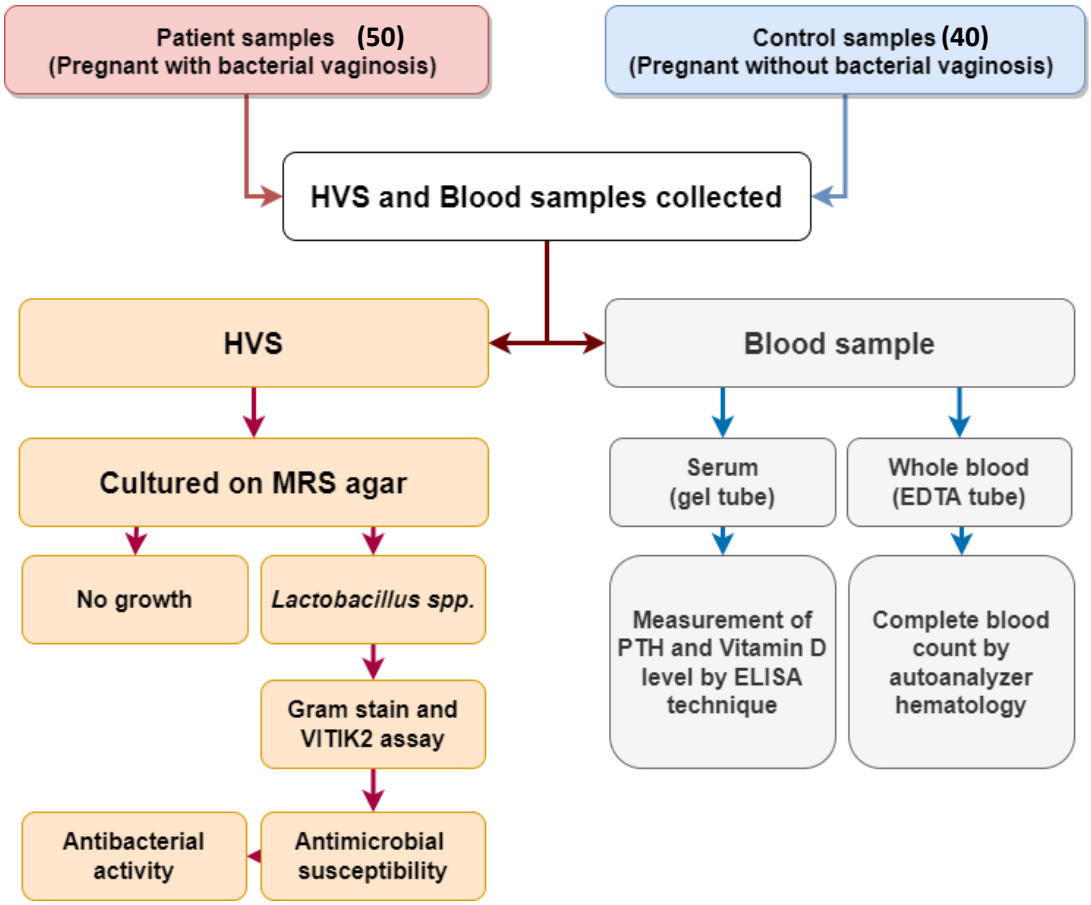

Figure (3-1): Study design

3.3 Methods

3.3.1 Specimens Collection

Ninety High vaginal swab (HVS) and blood sample were collected during four months from November 2021 until February 2022, they were collected from admitted patients to Imam Ali general hospital and private clinics, HVS swab collected using sterile cotton swab without media, and 4 ml of blood were collected and separated into two tubes, 2 ml for each tube, one tube with anticoagulant (Ethylene diamine tetra acetic acid (EDTA)) were used to get whole blood sample and the other was gel tube to get serum after centrifugation.

Samples were distributed as (50) sample from first trimester pregnant women suffering from bacterial vaginosis, and (40) from healthy first trimester pregnant women as control group, based on the Amesl criteria (Thin, gray/white vaginal discharge, Malodorous “fishy” discharge upon adding 10% potassium hydroxide, High vaginal pH (>4.5), Identification of vaginal epithelial cells heavily coated with bacteria (clue cells)) (DeCherney *et al*., 2013).

3.3.2 Culture Media Preparation and Sterilization Technique

3.3.2.1 De man, Rogosa and Sharpe (MRS) agar

MRS agar was used to isolation of lactic acid bacteria and it was prepared as in the following procedure:

To completely dissolve the medium, 65.13 grams had been mixed with 1000 ml of distilled water and heated to boiling. After that, it was autoclaved at 15 lbs of pressure (121° C) for fifteen minutes before being permitted to cooling to 45–50° C. Poured onto sterilized Petri plates after thoroughly mixing.

3.3.2.2 De man, Rogosa and Sharpe (MRS) broth

MRS broth was used for activation of lactic acid bacteria and it was prepared as in the following procedure:

54.3 grams were suspended in 1000 ml of distilled water, heated until completely dissolved and autoclaved at 121ºC for 15 minutes. Mixed well and poured into sterile test tubes.

3.3.2.3 Muller-Hinton Agar (MHA)

MHA were used for antibiotic susceptibility and antibacterial activity and prepared in the following procedure:

To completely dissolve the medium, 38.0 grams had been placed in 1000 ml of distilled water and then heated to boiling. After that, it was autoclaved at 15 lbs of pressure (121° C) for fifteen minutes before being left to cool to 45–50° C. Poured onto sterilized Petri plates after thoroughly mixing.

3.3.3 Preparation of Solution

3.3.3.1 Normal Saline Solution

Suitable to use sterile for all tests and investigations that required it, normal saline was utilized.

3.3.4 Preparation and method Gram stain

Gram stain was provided from spectrum company ready to use. The solution was used to study Gram stain reaction and their arrangement (Forbes *et al.*, 2007). Gram stain of isolates done using the following procedure:(Smith and Hussey, 2005)

- A cells smears that had been air-dried, heat-fixed, and flooded for 1 minutes with crystals violet staining solution.
- Slides cleaned for two seconds in a light, indirect stream of water.
- Slides covered with Gram's iodine, a mordant. one minutes of wait
- The slide washed for two seconds in a light, indirect spray of water from the faucet.
- Decolorizing agent drenched the slide. fifteen second wait
- Slides covered with safranin and counterstain. waiting between 30 and 60 secs. Slides should then be gently and indirectly washed with tap water, then dried with absorbent paper.
- A microscope used to see the outcomes of the staining technique while submerged in oil.

3.3.5 Laboratory Diagnosis

3.3.5.1 Isolation and Identification of Bacterial Isolates

All vaginal swabs were cultured on MRS agar and MRS broth under anaerobic conditions at 37 °C for 24- 48 hours. If a colony appeared, it was recognized based on it's own morphological (colony form, shape, colour, boundaries, type of pigment, elevations, and textures), and after being stained with Gram's stains, it was inspected using a light microscope, Isolates with growth of *Lactobacillus spp*. Stored in glycerol 40%. (Rogosa and Sharpe,1963; Winn *et al*. , 2006).

3.3.5.2: VITEK 2 assay

Isolated *lactobacillus spp*. was identified using VITEK2 ANC card for further identification by adding isolated colonies from a plate to 3 ml saline in clear plastic test tube to reach density equivalent to a McFarland No. 2.70 to 3.30 (according to manufacture instructions) metered by DensiCHEK Plus, to run the assay in VITEK2, final result appeared after approximately 6 hours.

3.3.5.3 Maintenance and preservation of isolated microorganism

This procedure was done according to (Harely and Prescott, 2002) as follows:

3.3.5.3.1 Short-term preservations

Isolate had been subcultured on the surfaces of suitable agar media after being kept for a few weeks. Parafilm was used to firmly wrap the plates, and they were kept at 4 °C.

3.3.5.3.2 Long-term preservations

By adding 0.4 ml of sterile glycerol to 2 ml of an exponentially bacterial growth in a sterile Micro- centrifuge tube and storing the mixture at -20 °C, isolate have been able to be kept for an extended amount of time in MRS broth media, which contains 20 percent glycerol.

3.3.6 Preparations of Bacterial Inoculum

MRS broth was used to activate the isolate for 18 hours at 37°C, and the proliferation were regulated to 0.5 McFarland using Densicheck device.

3.3.6.1 Antibiotic susceptibility test

In accordance with guidelines from the Clinical and Laboratory Standards Institute, the in vitro susceptibility of lactobacillus isolated to five antimicrobial drugs often prescribed by gynecologists to pregnant women was assessed using the disc - diffusion technique (CLSI, 2017). Using a clean cotton swab, bacterial inoculum was applied to Muller Hinton agar (MHA). In order to fully touch the agar inoculated with bacteria, antibiotic disks were put over MHA and carefully pushed down to achieve thorough contact. Following 24 hours. of incubation at 37°C, the diameter of the inhibition area in millimeters (mm). Results were interpreted as either sensitive or resistant in accordance with (CLSI, 2017).

3.3.6.2 Cell-Free Supernatants (CFS) preparations

Cell-free *lactobacilli* supernatant had been grown in MRS medium for 24 hours at 37 C. After that, the culture had been centrifuged for 30 minutes at 4 C and 4000 rpm. Filtering the supernatants via a 0.22 mm filter sterilized them (Millipore, Billerica, MA, United States).

3.3.6.3 Well Diffusion Assay

To identify antibacterial properties of supernatant isolated from Lactobacillus isolated, the agar disc diffusion technique (Tagg and McGiven, 1971) was adapted. First, Bacterial Suspension 0.5 McFarland have been swabbed on the surfaces of MH agar plates. Cell-free supernatant from isolated lactobacilli were then put into 6 mm diameter wells (100 l/well), and after a 24- hours incubated at 37°C, inhibitory zone was noted.

3.3.6.4 Preparation of Bacteriocin

By centrifuging *Lactobacillus* cultures cultured in MRS medium at 37 C for 24 hours at 10,000 grams, 10 minutes, cell free cultures supernatant (CFCS) have been created.

3.3.6.5 Antibacterial activity

Utilizing the well diffusing technique described earlier, the antibacterial activity of the *Lactobacillus spp.* isolate strain against certain pathogenic bacteria was evaluated (Davoodabadi *et al*.,2015b). Bacterial strains have been injected into nutritional broth, comprising *E. coli* and *S. saprophyticus*. The bacterial concentration was increased to 0.5 McFarland after an incubation time at 37°C. use Densicheck and Muller Hinton agar for cultures. The Muller Hinton agar plate were therefore pierced with 8 mm diameter wells, and 100 mL of the CFCS were added to every well. The plate has been then incubated at 37 C for 18–24 hours. Therefore, the well's surround growth-free inhibitory zones were used to test the antibacterial activity.

3.3.7 Parathyroid hormone ELISA assay

3.3.7.1 Principles:

Method of Sandwich Equilibrium (TYPE 2), High affinity and specificity antibody (enzyme and immobilization), with varied and unique epitope identification, in excess, and native antigens are the key reagent needed for an immune-enzymometric test. In this method, the x-PTH antibodies (C terminal epitope) covered on the well interacts with the assays via immobilization at the surface of a microplate well. The native antigen and the antibodies react without competition or steric hindrance to create a sandwich complex when the enzyme-labeled antibodies (N-terminal epitope) is combined with a serum containing the native antigens.

The antibody-bound fractions are isolated from unbound antigens using decantation or evacuation after enough time has passed. The native antigens concentrations directly correlate with the enzymatic activity in the antibody-bound fraction. A dosage response curve may be produced and used to determine the antigens concentrations of an unknown by using many distinct serum standards for known antigens value.

3.3.7.2 Standard curve and result calculation

- Standard curves were plotted using Microsoft Excel by plotting absorbance on vertical axis (Y-axis) versus concentration on horizontal (X-axis) in pg/ml. (figure 3.2)
- Absorbance of unknown sample were located on vertical axis to find the concentration on horizontal axis.

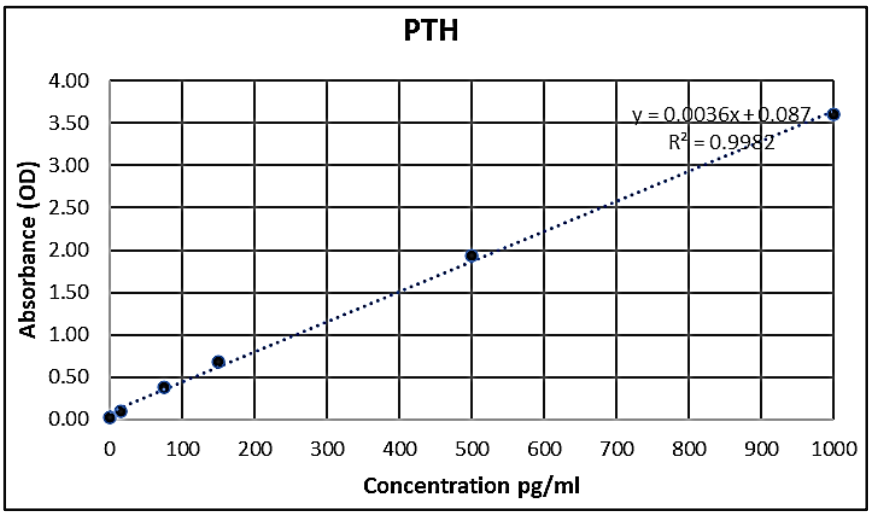

Figure (3.2) Parathyroid hormone standard curve

3.3.8 Vitamin D ELISA assay

3.3.8.1 Principle

Sequential Competitive Method: Immobilized antibodies, enzyme-antigen combination, and natural antigen are necessary components for a solid state sequence enzyme - linked immunosorbent assay. Native antigens and a small number of insolubilized binding sites bind together when immobilized antibody is combined with whole blood that contains the native antigen. The enzyme-conjugated antigens is introduced after any unreacted native antigen has been removed during a wash phase. The compound interacts with antibodies regions that the natural antigens did not occupy.

The antibody-bound fractions and unattached antigens are isolated by decantation or evacuation following a brief second incubation. The natural antigens concentrations and the enzymatic activities in the antibody-bound

fractions are inversely related. A dose response curve may be produced and used to determine the antigens concentrations of an unidentified by using a variety of calibrators with known antigens concentrations.

3.3.8.2 Standard curve and result calculation

- Standard curve were plotted using Microsoft Excel by plotting absorbance on vertical axis (Y-axis) versus concentration on horizontal (X-axis) in pg/ml. (figure 3.3)
- Absorbance of unknown sample were located on vertical axis to find the concentration on horizontal axis.

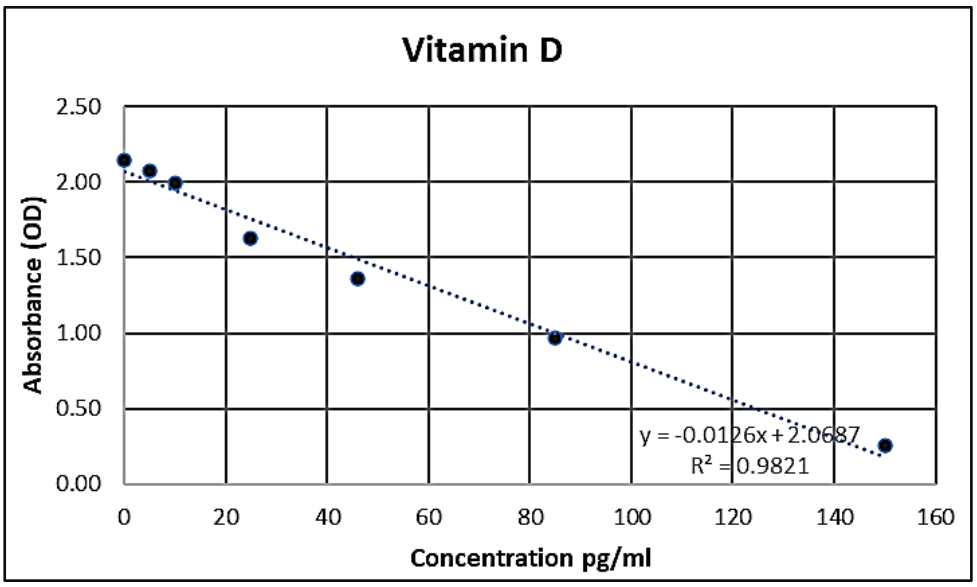

Figure (3.3) Vitamin D standard curve

3.3.9 Complete Blood Count (CBC) assay

Whole blood sample in EDTA tube was used immediately to get complete blood count using automated 3-part hematology autoanalyzer, samples were swirled several times to mix the sample and then processed in the autoanalyzer to get result within 60 seconds, result printed out and recorded.

3.4 Ethical approval

1- The study was done, and the cases were collected after getting the agreement of the patients (verbal acceptance).
2- Approval of the ethical committee of Babylon Science for Women's College.
3- The Babylon Health Department gave authorization before the trial began.

3.5 Statistical analysis

Results of the present study were illustrated as means ± standard deviation (SD). The values were statistically analyzed by using SPSS 20 program and analysis of variance were explained. The lowest significant difference (LSD) was ($P<0.05$) (Daniel, 1999).

Chapter Four

Results

4.1 General demographical data of pregnant women

Table (4-1): General demographical data of pregnant women

Parameter		Groups of study	
		BV **F (%)**	non-BV **F (%)**
Age	Mean	27.34	25.50
	S.D	6.886	6.312
	16-25	25 (50%)	21 (52.5%)
	26-35	20 (40%)	15 (37.5%)
	36-45	5 (10%)	4 (10%)
	Total	50 (100%)	40 (100%)
Type of delivery	Normal delivery	28 (56%)	19 (47.5%)
	Cesarean	9 (18%)	8 (20%)
	Both	3 (6%)	3 (7.5%)
	No previous birth	10 (20%)	10 (25%)
	Total	50 (100%)	40 (100%)
No. of previous births	0	10 (20%)	10 (25%)
	1	14 (28%)	8 (20%)
	2	4 (8%)	12 (30%)
	3	8 (16%)	4 (10%)
	4	5 (10%)	3 (7.5%)
	5	5 (10%)	2 (5%)
	6	4 (8%)	1 (2.5%)
	Total	50 (100%)	40 (100%)
Residence	Urban	27 (54%)	18 (45%)
	Rural	23 (46%)	22 (55%)
	Total	50 (100%)	40 (100%)

F: Frequency

Table (4-1) showed that 25 (50%) of BV group and 21 (52.5%) of non-BV group age were between (16-25) years, while 20 (40%) of BV group and 15 (37.5%) of non-BV group age between (26-35) years, the lowest percentage in BV group was 5 (10%) and non-BV group 4 (10%) age were (36-45) years.
The highest frequency of delivery type in BV and non-BV groups were normal delivery 28 (56%) and 19 (47.5%) respectively, the frequency of other types of delivery (Cesarian, Both, no previous birth) in BV group were 9 (18%), 3 (6%), and 10 (20%) respectively, and for non-BV group were 8 (20%), 3 (7.5%), and 10 (25%) respectively.

Regarding the number of previous births the highest frequency in BV group (14 (25%)) was single previous birth, while in non-BV group (12 (30%)) was two previous births, other previous births in BV group were ranging from 2-6 previous births with frequency 4 (8%), 8 (16%), 5 (10%), 5 (10%), and 4 (8%) respectively, and for non-BV group were ranging from 1-6 with frequency 8 (20%), 12 (30%), 4 (10%), 3 (7.5%), 2 (5%), 1 (2.5%) respectively.

For the residency place the highest percentage in BV group was urban 27 (54%), then rural 23 (46%), while in non-BV group the highest was rural 22 (55%), then urban 18 (45%).

4.2 Relation of Body Mass Index (BMI) with Bacterial Vaginosis

Results that explained in the table (4-2) shows that the body mass index of pregnant women with an overweight than normal is more susceptible to infection with bacterial vaginosis

Table (4-2): Percentage of body mass index of study groups.

BMI	Groups of study		P-value
	BV	Non-BV	
Healthy	35 (70%)	33 (82.5%)	0.083
Overweight	15 (30%)	7 (17.5%)	
Total	50 (100%)	40 (100%)	

Table (4-2) showed that 35 (70%)of BV group BMI were within Healthy categories, while 33 (82.5%) of non-BV group BMI were within Healthy categories, and the overweight was 15 (30%) in BV group and 7 (17.5%) in non-BV group.

4.3 Identification of bacterial isolates with VITEK2

Table (4-3) showed no significant difference between the study groups according to VIKEK2 identification of *Lactobacillus* spp. and shows two types of *Lactobacillus, L. gasseri, L. casei.*

Table (4-3): Result of identification on bacterial isolates with VITEK2 in BV and non-BV groups

Vitek2	Groups of study				P-value
	BV		Non-BV		
	F	%	F	%	
L. casei	3	6.0	11	27.5	0.769
L. gasseri	4	8.0	19	47.5	
No growth	43	86.0	10	25.0	
Total	50	100.0	40	100.0	

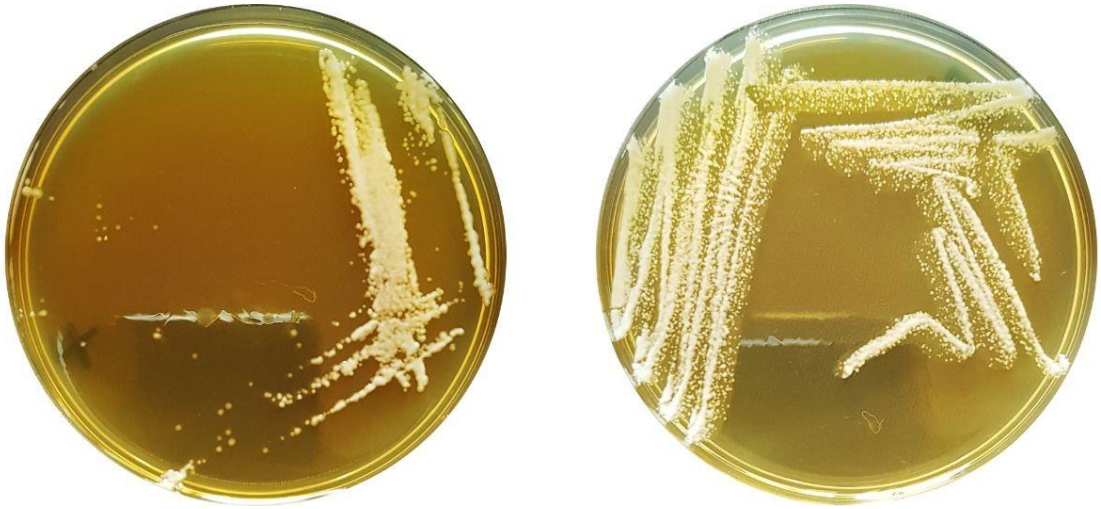

Figure (4-1): Anaerobic bacterial isolates on MRS

4.4 Antibiotics susceptibility of Lactobacillus

Figures (4-2) and (4-3) in these figures to some antibiotics were used to show the effects on *Lactobacillus* species in healthy women and women with vaginosis

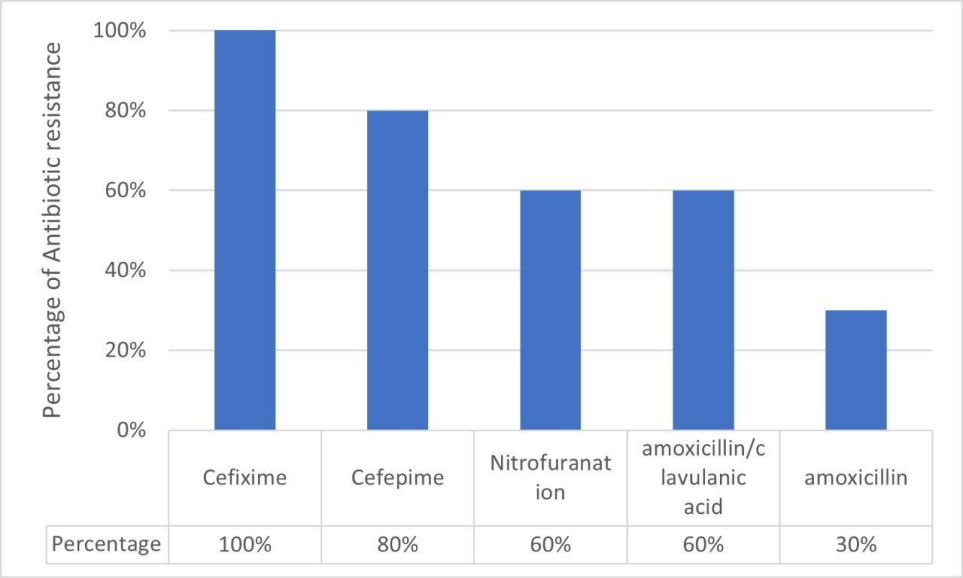

Figure (4-2): Antibiotic Resistant among *Lactobacillus gasseri* Isolates in healthy pregnant women

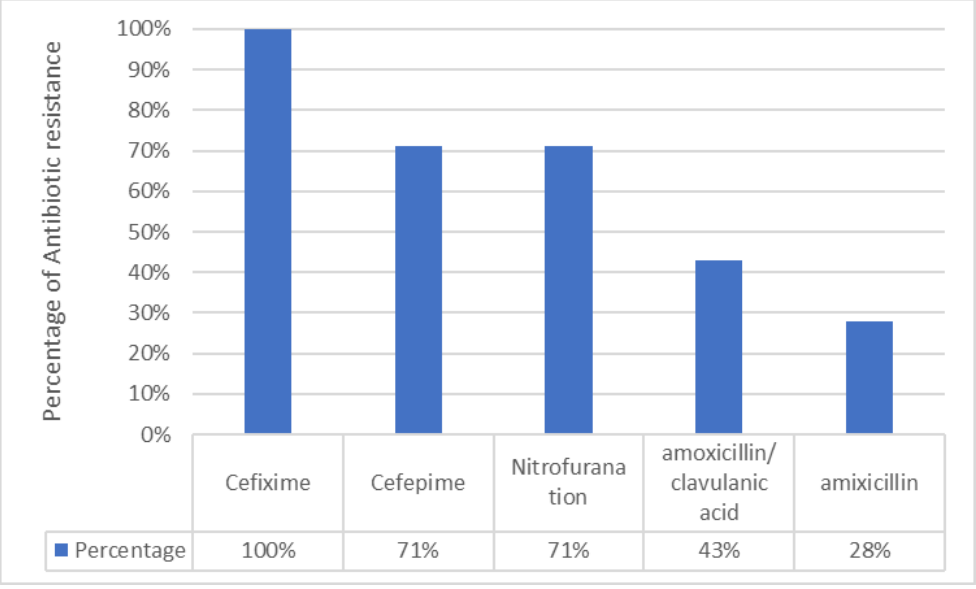

Figure (4-3): Antibiotic Resistant among *Lactobacillus gasseri* Isolates in pregnant with bacterial vaginosis

Figure (4-4): Antibiotics susceptibility of *Lactobacillus gasseri* on Muller Hinton agar

4.5 Result of Antibacterial Activity

In table (4-4) the results showed that the CFS of *Lactobacillus gasseri* from healthy, where in women were more effective against the pathogenic bacterial isolates than the CFS of Lactobacillus spp. from women with vaginosis

Table (4-4) Antibacterial activity

Pathogenic bacteria	*Lactobacillus gasseri* (Isolated from BV)	*Lactobacillus gasseri* (Isolated from non-BV)
E. coli	**14mm**	**13mm**
S. aureus	**19mm**	**22mm**

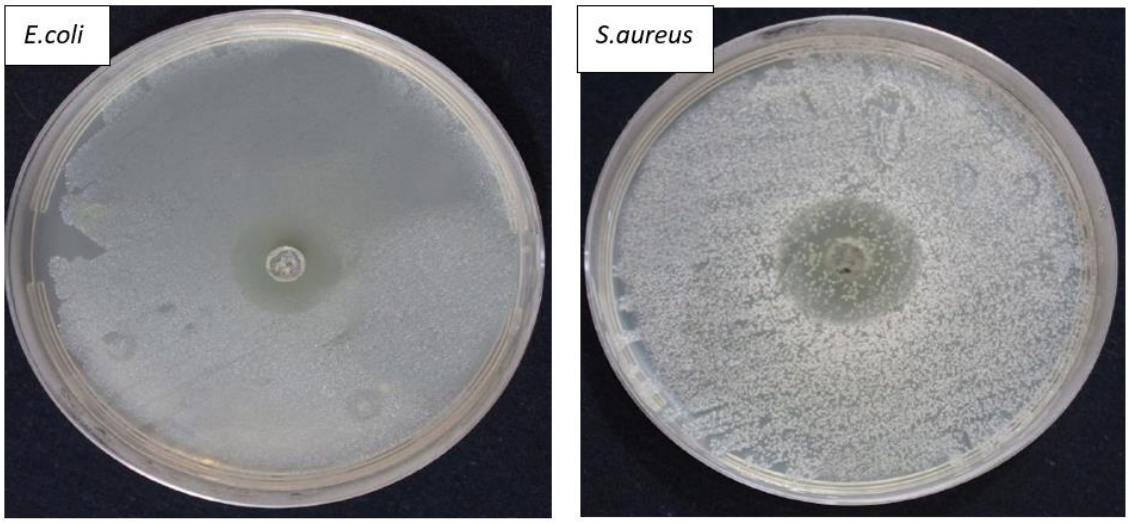

Figure (4-5) Antibacterial activity of *Lactobacillus gasseri*

4.6 Level of Vitamin D in BV and non-BV pregnant women

Statistical analysis reveals a highly significant differences at level P<0.05 in the level of Vitamin D between BV (19.91±11.2) during first trimester of pregnant women and non-BV(33.7±14.8). Table (4-3) showed the level of vitamin D in women with BV and non BV.

Table (4-5): Level of vitamin D in BV and non-BV groups

Vitamin D (pg\ml)	Groups of study		p-value
	BV	Non-BV	
Mean	19.91*	33.71	**0.001**
S.D	11.202	14.89	**H.S**

4.7: Level of Parathyroid hormone in BV and non-BV pregnant women

The table below shows there is significant increase at level P<0.05 of parathyroid hormone in pregnant women in the first trimester with bacterial vaginosis (23.7±13.6) compared to healthy women (26.5±10.9).

Table (4-6): Level of Parathyroid hormone in BV and non-BV pregnant women

PTH (pg\ml)	Groups of study		p-value
	BV	Non-BV	
Mean	32.7240*	26.5025	0.021
S.D	13.58816	10.89355	Sig

4.8 The total number of White blood cells in BV and non-BV groups

The figure (4-6) below shows the white blood cell count compared to pregnant women infected with bacterial vaginosis, where there was a significant increase (P=0.01) in infected women (7.77±2.74) compared to healthy ones (6.47±1.64).

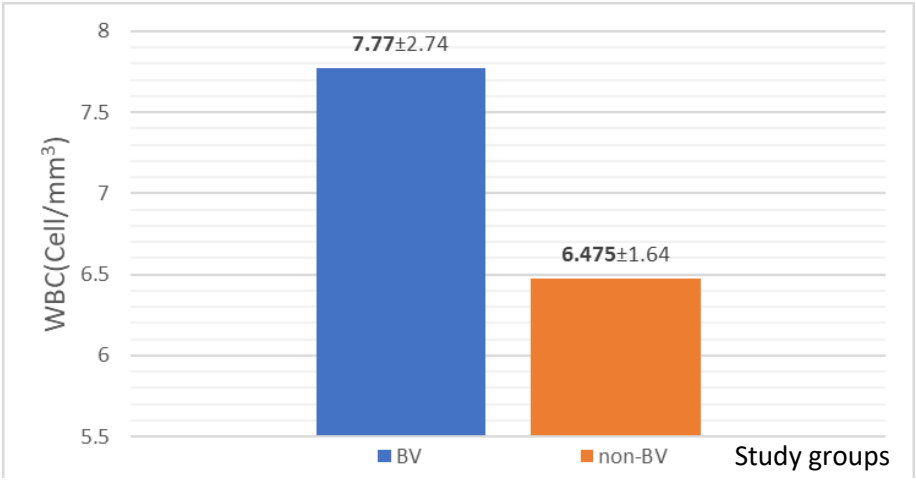

Figure (4-6) The total number of White blood cells (WBcs cells\mm^3) in patient with BV and healthy control.

4.9: Packed Cell Volume (PCV) value in BV and non-BV groups

The figure (4-7) shows the level of packed cell volume were there is no significant deference (P=0.104) between pregnant women with bacterial vaginosis (36.9±4.35) compared to healthy women (35.9±2.91).

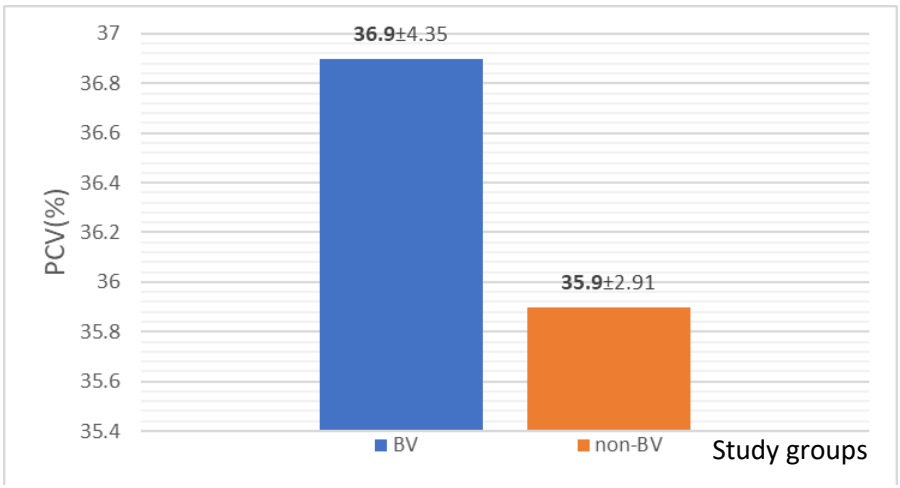

Figure (4-7): The level of Packed cell volume (PCV%) in patients with BV and healthy control.

4.10 Platelet count in BV and non-BV groups

The figure (4-8) below shows a significant increase (P=0.009) in the number of blood platelets in pregnant women in the first trimester with bacterial vaginosis (230.24±77.6) compared to healthy women (191.72±53.39).

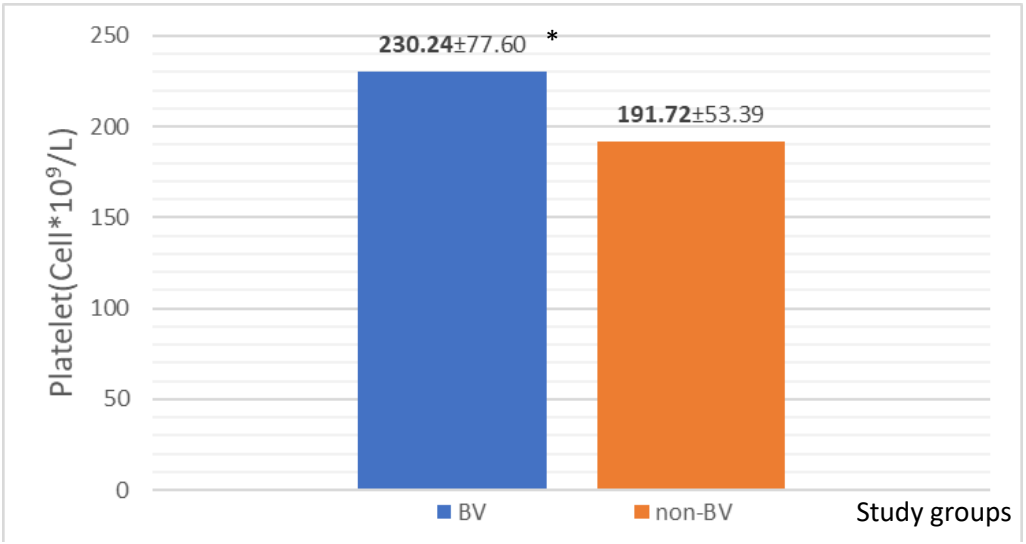

Figure (4-8):The level of blood platelets (cells\mm^3) in patients with BV and those healthy control.

‘

4.11 Correlation between demographical data and measured parameters in BV group

Table (4-7) showed significant the relationship between all parameters in bacterial vaginosis

Table (4-7): correlation between demographical data and measured parameters in BV group

		BMI	Vit.D	PTH	WBC	Hb	PLT
Age	Pearson Correlation	.667**	-.331-*	.351*	.050	.438**	.025
	Sig. (2-tailed)	.000	.019	.012	.729	.001	.863
BMI	Pearson Correlation		-.231-	.262	-.043-	.418**	-.069-
	Sig. (2-tailed)		.107	.066	.768	.003	.635
Vit.D	Pearson Correlation			-.519-**	-.284-	.202	-.266-
	Sig. (2-tailed)			.000	.076	.210	.098
PTH	Pearson Correlation				.176	.005	.193
	Sig. (2-tailed)				.276	.975	.234
WBC	Pearson Correlation					.226	.981**
	Sig. (2-tailed)					.160	.000
Hb	Pearson Correlation						.312
	Sig. (2-tailed)						.050
**Correlation is significant at the 0.01 level.							
*Correlation is significant at the 0.05 level.							

The table (4-6) showed there was a significant positive correlation between age and BMI (r=0.667), age and PTH (r=0.351), age and Hb (r=0.438), BMI and Hb (r=0.418), and between WBC and platelet (r=0.981), while there was a significant negative correlation between age and vitamin D (r=-0.331), and between vitamin D and PTH (r=-0.519),

Figure (4-9) showed there was a significant negative correlation (r=-0.519,sig=0.001) between vitamin D and parathyriod hormone.

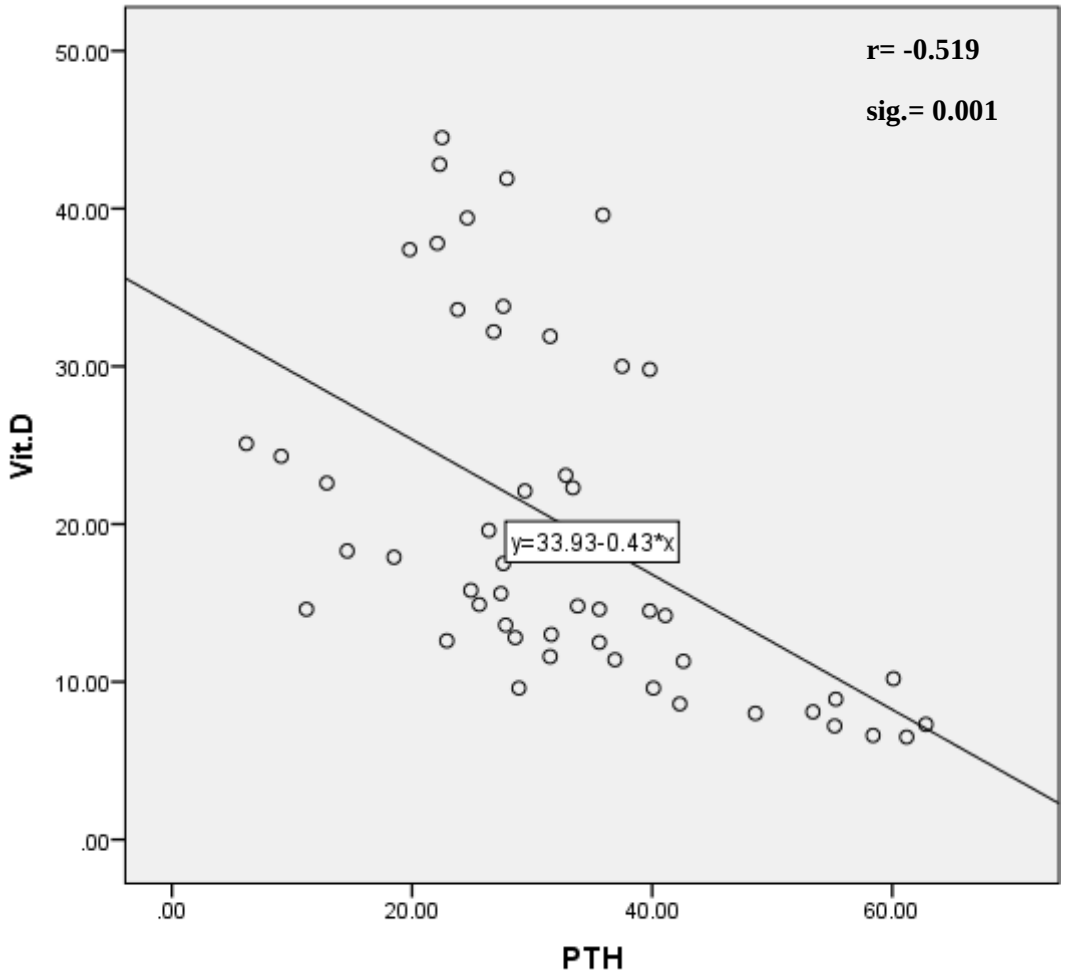

Figure (4-9) The correlation coffecients occurring between PTH and Vit-D in patients with BV.

Chapter Five

Discussion

5.1 Bacteriological study

5.1.1 Isolation and Phenotypic Identification of *Lactobacillus spp.*

The female genital tract is dominated by *Lactobacillus* spp. in approximately 70% of women (Petrova *et al*.,2015) . *Lactobacillus* species play an essential role in maintaining the ecosystem of the vagina *Lactobacillus* (Amabebe and Anumba,2018). us has proven to be efficient in treating BV by producing organic acids, hydrogen peroxide, bacteriocins, and adhesion inhibitors (Tachedjian *et al*.,2017). Furthermore, the presence of *Lactobacillus* alongside other opportunistic pathogens could be due to a number of factors, including antibiotic effects, incubation type (as some *Lactobacillus* species are unable to produce certain defense factors under anaerobic incubation), and antagonism among *Lactobacillus* species to maintain dominance (Razzak *et al*., 2011).

From the results, the *lactobacillus* was isolated on MRS agar (7) isolate from BV group and (30) isolate from Non-BV group and other the plates from both groups no *lactobacillus* bacteria growth was observed . *Lactobacillus* colony morphology on MRS agar was examined under the microscope and revealed a circular, big, smooth, glistering, white, colorless, and creamish colony showed in figure (4-1). After incubating, fungi *Candida albicans* growth was observed in some plats.

Lactobacillus was isolated from pregnant women with Bacterial Vaginosis and non-Bacterial Vaginosis in the first trimester and was isolated anaerobically.. Sexual activity is linked to bacterial vaginosis (Koumans *et al*., 2007). *Lactobacillus* has the capacity to protect the vaginal area from disease, which is why it is now employed as a probiotic (Andreu, 2004). *Lactobacillus* species dominate normal vaginal flora in the vast majority of cases. Normalcy can be maintained in the absence of *lactobacilli* by other, more rabid lactic acid generating bacteria (Lamont *et al.*, 2011).

5.1.2 Direct rapid identification of *Lactobacillus sp.* By VITEK 2 compact.

The VITEK2 technology is a simple and quick procedure, and the results demonstrated that isolated strains may be used bacterial species diagnosis (Al Masoudi & Algboory, 2021). The table (4-3) Result findings revealed two Lactobacillus species were found they were *Lactobacillus casei* and *Lactobacillus gasseri.*

The *L. gasseri* was the dominant species in healthy women group and BV group in this study (47.5% and 8% respectively) in comparison to *L. casei* (27.5% and 6% respectively) and the remaining samples (25% and 86% respectively) were didn't grow. Unlike this study (Mahmood & Hameed, 2018) found that *Lactobacillus casei* was shown to be the most common Lactobacillus species in healthy women's vaginal samples.

Other researchers discovered *Lactobacillus* spp. in vaginal samples (Mohammed & Kadhim., 2020) *Lactobacillus acidophilus, L. crispatus, L. gasseri,* and *L. iners* were the vaginal *Lactobacillus* species studied in this study. *Candida albicans, Gardenella vaginalis, Staphylococcus aureus,* and *Escherichia coli* were among the urogenital pathogens isolated from vagina.

5.1.3 Antibiotics Susceptibility of *Lactobacillus spp*.

In healthy women and women with vaginosis, antibiotics were tested to see how they affected *Lactobacillus* species. In this study, five antibiotics (Cefixime CFM(5), Cefepime FEP(10), Nitrofurantoin F(100), Amoxicillin/Clavulanic acid AMC(30), and Amoxillin AX(25) were used to investigate the effects of antibiotics on Lactobacillus isolates that gave positive pure cultures from women suffering from vaginitis and healthy women , these antibiotics selected based on the most common antibiotics administrated by gynecologists for treatment of bacterial infections in pregnant women . The results showed that all *Lactobacillus* isolates (10 isolates) from non-BV group and (7 isolates) from

BV group were resist to Cefixime, for other antibiotics Cefepime, Nitrofurantoin, Amoxicillin/Clavulanic acid and Amoxillin the results for resistance were (80%, 60%, 60%, and 30% respectively) for non-BV group and (71%, 71%, 43%, 28% respectively) for BV group showed in figure (4-2) and figure (4-3). Showed Figure (4-4): Antibiotics susceptibility of on Muller Hinton agar Where was the presence of inhibition zone indicated on sensitivity to antibiotics and absence indicates antibiotic resistance

The high rates of recurrence of BV after treatment with currently approved antibiotics has always been a source of contention. These findings could be explained by one of two ways. To begin, BV-associated bacteria and multispecies BV biofilms may be implicated in the disease's possible pathogenic process (Swidsinski *et al.,*2014). When mutations or intrinsic resistance mechanisms are responsible for the resistance phenotype, antibiotic resistance in *Lactobacillus* does not indicate safety concern. Indeed, some probiotic strains with inherent antibiotic resistance may be advantageous for re-establishing the microbiota of the host after antibiotic treatment (Gueimonde *et al*., 2013). Abdul – Sattar *et al*., (2011) isolated 22 *Lactobacillus* isolates from healthy women's vagina and studied the effects of drugs on *Lactobacillus* isolates, finding that all isolates were resistant to Metronidazole, the most generally used antibiotic for bacterial vaginosis treatment. These findings suggest that certain strains could be used for therapy restoration in conjunction with antimicrobial bacterial vaginosis treatment and resistance to Ciprofloxacin, as well as Nalidxic acid, which aids in the selection of proposed probiotics that patients should take during antimicrobial therapy .

The effect of antimicrobial therapy on *Lactobacillus* was studied by Razzak *et al*., (2011), and it was discovered that the antibiotic had an observable effect against the presence of Lactobacillus; these antibiotics can indiscriminately destroy both beneficial and pathogenic bacteria in the body; also, antibiotics can

cause a microflora imbalance with very negative consequences, as they cause the microflora to remove from epithelial cells of the vagina, thus allopathogenic. As a result, patients' usage of antibiotics to treat vaginitis should be highly selective in order to avoid killing *lactobacilli.*

Although antimicrobial agents are generally effective at eradicating the infection, there is a high incidence of recurrence, which affects the patient's quality of life, and many women become frustrated by the cycle of reported antimicrobial agents, whose effectiveness is diminishing due to the increasing development of microbial resistance, in addition, the use of antimicrobial agents can disrupt the body's balance by killing off beneficial bacteria (Reid, 2008) .

5.1.4 Antibacterial Activity

In this study the antibacterial activity of *Lactobacillus spp*. CFS was done and the results showed that the CFS of *Lactobacillus spp*. from healthy women were more effective against the pathogenic bacterial isolates than the CFS of *Lactobacillus* spp. from women with vaginosis, table (4-4). Showed Figure (4-5) Antibacterial activity of *Lactobacillus gasseri*

The vaginal microbiome is incomplete without vaginal *Lactobacilli*. The vaginal microbiota is thought to protect against opportunistic infections. Probiotic *Lactobacillus*, for example, can effectively treat bacterial vaginosis. *Lactobacillus vaginalis* supports the vaginal epithelium's innate immune activity (Ventolini & Sawyer, 2015).

The mechanisms by which *Lactobacillus* controls the colonization of other bacterial species in the vagina decide whether or not probiotics can be used to treat vaginal dysbacteriosis in combination with other pathogenetic or aetiological therapies (Kovachev & VatchevaDobrevska, 2014; Deidda *et al.*, 2016).

Mahmood & Hameed, 2018, found that the ability to produce bacteriocin (as antibacterial) appears to be rare among *L. casei* isolated from vagina, and the agar well diffusion assay and disc method were considered sufficient for detecting bacteriocin production, indicating that bacteriocin production was best in broth medium compared to solid medium. The highest activity was observed against *C.urealyticum* and *S.aureus*, with no activity observed against *E.coli* and *Ps.aeruginosa. Lb.casei* was the greatest producer isolate, producing it in the broth media (MRS) used in this study.

Lactobacillus plays an important role in maintaining vaginal health by producing defense factors, some of which have an inhibitory effect on opportunistic pathogens (*S. aureus, E. coli)*. This fact was confirmed by Ronquist *et al*.(2006) who reported that the normal vagina of reproductive age women is predominately colonized with *Lactobacillus*, which produce hydrogen peroxide, bacteriocins, and lactic acid. When the amount of *Lactobacillus* bacteria decreases, the pH rises, favoring an overgrowth of anaerobic and facultative bacteria, which can lead to virginities and vaginosis.

The *G. vaginalis* growth was suppressed by vaginal and probiotic *Lactobacillus* strains in a strain-specific manner. (Teixeira *et al*., 2012) reported that metronidazole-resistant *G. vaginalis* strains were less responsive to *Lactobacillus spp*. inhibition . Happel *et al*., (2020) studied the probiotic properties of a variety of vaginal *Lactobacillus* strains from South African women. The probiotic potential of these isolates was discovered to be strain-specific, with vaginal *Lactobacillus* strains outperforming probiotic strains already utilized in vaginal health probiotics marketed internationally and in South Africa. The development of a probiotic for vaginal health is now being considered using some of the best-performing, sequenced vaginal *Lactobacillus* isolates. A study found that Vaginal *Lactobacillus* strains performed

significantly better than probiotic strains currently utilized in vaginal health probiotics.

5.2 Physiological study

5.2.1 Level of Vitamin D

The familiar action of vitamin D is to regulate calcium and phosphate homeostasis, which are essential for bone formation and resorption. Vitamin D promotes the intestinal absorption of phosphate and calcium, it stimulates the differentiation of progenitor cells to osteoclasts, and it recovers calcium from.bones and encourages bone matrix mineralization (Rui Lin, 2016).

Vitamin D is important for women especially for pregnant women because to regulate and balance calcium and phosphate ions , in this study vitamin D deficiency because pregnant women don't exposure to sunlight , good diet and the fetus need vitamin D for body's skeleton from the mother.

Through the current study, a decrease in Vitamin D was observed in the blood samples of pregnant women in the first trimester, as this study agrees with many studies , including (Bodnar .,2009, Hu *et al*.,2022) pointed out that vitamin D deficiency is associated with BV at <16 wk of pregnancy. A better understanding of vitamin D-BV relation will be ascertained with prospective studies of "incident" BV infections, persistent infections, and infections that spontaneously resolve. The majority of studies have shown that vitamin D deficiency is highly prevalent among pregnant women and the general population (Thippeswamy *et al*.,2021).

In this study, it was found that there is an inverse relationship between vitamin D deficiency an bacterial vaginosis in pregnant women in first trimester where it was in group BV (19.91±1102) ,and Non BV (33.71±14.9), significant. An inverse association was found between bacterial vaginosis and 25(OH)-vitamin D levels in pregnancy, the risk of bacterial vaginosis increasing

in case of vitamin D deficiency (Bodnar *et al.*,2007, Bodnar *et al*.,2009Jefferson *et al*.,2019; Várbíró *et al*., 2022). vitamin D deficiency among pregnant women has been associated with elevated risk for other pregnancy complications such as preeclampsia) and bacterial vaginosis Vitamin D deficiency may have the following effects on pregnancy: increased risk for preeclampsia, GDM, spontaneous premature birth and bacterial vaginosis. may also conclude that vitamin D demand increases in pregnancy: optimal vitamin D levels decrease both maternal and fetal risks.

The effect of vitamin D on BV elimination can be explained by the impact of vitamin D on the immune system, especially in the local immunity of the vagina. Cytokines were considered the main factor linking BV and vitamin D (Yudin *et al*., 2003; Bodner *et al*.,2009).

In this study showed that there is an inverse(R =-.231-) relationship between vitamin D and body mass index, and there was a difference but no significant shows in the table (4-2) as in the following study. Vitamin D deficiency also affects BMI and during seasons. The present results indicated the presence of a significant seasonal variability of vitamin D status. It also found an inverse relationship between vitamin D status and BMI, which is explained by a smaller skin surface area for vitamin D synthesis in comparison to the total body volume (Lagunova *et al*.,2009). adequate vitamin D supplementation from conception to birth and in postpartum is increasingly important both for the mother and newborn, although currently there are no uniform professional recommendations regarding the exact amount of vitamin D supplementation during pregnancy (Wierzejska and Wajda,2022).

5.2.2 Parathyroid Hormone

Through the results which are from obtained PTH, it was found that there are significant differences between BV and non-BV group, at level $P<0.05$ PTH

levels were significantly higher in BV group in comparison to non-BV group shown in table (4-6). PTH is important during pregnancy within the normal level because it is responsible for regulating the level of calcium .It's rise leads to what was mentioned in the study of parathyroid hormone (PTH), are essential to increase maternal calcium absorption during pregnancy. Through the course of pregnancy, PTH concentrations increase to reach a mid-normal range by the third trimester .The secretion of PTH is regulated primarily by extracellular calcium concentrations; lower amounts of circulating calcium trigger an increase in PTH (Kovacs *et al*.,2006 ; Marbut *et al*., 2017) . A rise in PTH-related protein (PTHrP) and calcitriol in the first trimester, and the flux of other hormones during pregnancy, such as estradiol, progestins, placental lactogen, and insulin-like growth factor I, may have direct or indirect effects on maternal calcium (Kovacs.,2016). In response to placental calcium transfer as well as an expanding extracellular volume and increased urinary calcium loss, maternal PTH concentrations rise during pregnancy (Kovacs *et al*., 2006).

The levels of vitamin D in this study is inversely proportional to the concentration of parathyroid hormone as shown in figure (4-9). Many studies have shown that there is an inverse relationship between Vitamin D and the parathyroid hormone thus the result agreed with those studies, including studies of (; Haddow *et al*.,2011; Ustuner *et al*., 2011 Kramer *et al*., 2016; Jääskeläinen *et al*.,2013; Naugler *et al*., 2013 Hysaj *et al*., 2021) . In other studies) elevated PTH concentrations are an indicator of vitamin D deficiency; nevertheless, there are other factors that are involved in the physiological changes in PTH. Herein, An investigated other potential factors determining PTH concentrations using multiple regression models. These models indicated that 25(OH)D concentrations are a statistically significant determinant of PTH levels in both early and late pregnancy. Education of the partner, multiparity, season, and outdoor activity were also statistically significant in early pregnancy, whereas

country of origin was a statistically significant determinant of PTH in late pregnancy. Studies in the general population and in pregnant women have demonstrated an association between education and vitamin D levels, that better education is associated is with higher levels of vitamin D.

Parathyroid hormone is associated with immunity according to the conclusion of the following study. Since the early 1980s, PTH receptors were found on most immunologic cells (neutrophils, B and T cells) (Geara *et al*.,2010).

5.2.3 Blood Parameters in Pregnant Women with BV and Healthy Women (Non BV).

The changes of CBC parameters during pregnancy are described in this study. There are significant differences between pregnant with bacterial vaganosis and pregnant without bacterial vaganosis .

The estimation of anemia prevalence is an important step for health policy makers, as hemoglobin estimation is most important parameter to start supplementation of iron or removing other causes of anemia to prevent unfavorable outcome (Dhariwal *et al.*,2016).

5.2.3.1 The White Blood Cell (WBC)

A significant increase in the number of white blood cells was observed. The cause of this increase was due to inflammation that found in vaginal tract , the results which are compatible with study of (Stock and Hoffman, 2000) which found White blood cells are an important component of the host defense system, responsible for protection against bacteria, fungi, viruses, and invading parasites, it's level elevated during infection, Neutrophils was frequently increased in infection and in any acute WBC that primary respond to a bacterial infection (Anjali *et al.*, 2020). The increase observed in WBC count from the first to third trimester(Akingbola *et al*.,2006, Akinbami *et al*., 2013, Patel *et al*., 2014).

5.2.3.2 :Packed Cell Volume (PCV)

In this study it was observed that non-significant in PCV values which is an indication of Pregnancy causes significant changes in metabolism, fluid balance, organ function and blood circulation which are driven by hormones and the presence of the feto - placental unit, these dramatic changes influence a wide variety of hematological parameters, decreasing in RBC, Hb, PCV some of these changes which due to increasing plasma volume (Elgari, 2013) . All above studies support result of PCV in current study.

5.2.3.3 The Platelet Count (PLT)

The value of platelets pregnant women with bacterial vaginosis .(7.77±2.74) was high when compared to healthy control (6.475 ±1.64).observed high significant , P=0.009 , this result corresponds to the result of Talukdar et al. (2017), the latter study found that the average number of blood platelets is associated with increased disease effectiveness. Platelets interact with all kingdoms of microbial pathogens, including viruses, bacteria, fungi, and protozoa (Yeaman, 2010). Platelet releases soluble immunomodulatory factors upon stimulation by bacterial components (Hamzeh-Cognasse *et al.*, 2015). There is a growing recognition of the critical role of platelets in inflammation and immune responses (Thomas & Storey, 2015).

Conclusions and Recommendations

Conclusions

From data that were obtained from the present study, one can be concluded the following facts :-

1-Vitamin D decreased concentration in pregnant women with bacterial vaginosis.

2-There were among physiological parameter with bacterial vaginosis.

3-The *Lactobacillus casei* and *Lactobacillus gasseri* from BV and Non-BV was most common.

4-Antibacterial secretion from *Lactobacillus casei* and *Lactobacillus gasseri* had effective.

Recommendations

1-Study the concentration of Calcium and phosphorus with BV.

2-Study the parathyroid hormone and Thyroid hormone and correlation with *Lactobacillus* .

3-Study the toxicity of *Lactobacillus* spp. CFS on Lab animals .

4-Molucular study of *Lactobacillus* Spp.

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الخلاصة

هناك مدى واسع ومتنوع من العوامل المُمْرضة تتضمن البكتريا والفطريات والفيروسات يمكنها ان تسبب التهاب المهبل وخصوصاً خلال الحمل، صممت الدراسة الحالية لفحص وعزل وتشخيص *Lactobacillus* spp. من النساء المصابات بالتهاب المهبل البكتيري خلال الأشهر الثلاثة الأولى من الحمل وكذلك إيجاد نقص فيتامين د والهرمون جار الدرقية مصحوبة ببعض معلمات الدم.

تضمنت هذه الدراسة عدد من النساء (٩٠)، (٥٠) منهن كانت مصابة بالتهاب المهبل البكتيري خلال الأشهر الثلاثة الأولى من الحمل، وما تبقى (٤٠) امرأة حامل في الأشهر الثلاثة الأولى تم استخدامها كمجموعة سيطرة، كانت اعمار مجموعات الدراسة بين (١٦-٤٥) سنة. تم جمع العينات خلال الفترة من تشرين الثاني ٢٠٢١ ولغاية شباط ٢٠٢٢ في محافظة بابل، مستشفى الامام علي (ع) العام والعيادات الخاصة، تم جمع العينة بواسطة الطبيبة اختصاص الامراض النسائية، وتم جمع عينة دم ومسحة مهبلية عليا لكل امرأة حامل. تم قياس مستوى فيتامين د والهرمون جار الدرقية باستخدام تقنية المقايسة الامتصاصية المناعية للانزيم المرتبط (ELISA) وكذلك تم فحص عد الدم الكامل (CBC) باستخدام جهاز عد الدم الاوتماتيكي، اما بالنسبة للمسحة المهبلية العليا تم زرعها على الوسط الزرعي (MRS) لعزل بكتريا *Lactobacillus* وتشخيصها بإستخدام جهاز الـ (VITIK-2) وتم استخدام الوسط الزرعي (MHA) لفحص فعالية المضادات الحياتية والمضادات البكتيرية.

أظهرت بيانات هذه الدراسة بأن مؤشر كتلة الجسم كان اعلى في مجموعة النساء المصابات بالتهاب المهبل البكتيري مقارنة بمجموعة النساء الاصحاء. اظهر متوسط مستوى فيتامين د في مصل الدم ارتفاعاً معنويا ($P<0.05$) في مجموعة النساء المصابات بالتهاب المهبل البكتيري (١٩,٩١±١١,٢) مقارنة بمجموعة السيطرة (٣٣,٧١±١٤,٩) كلما انخفض مستوى فيتامين د ازداد خطر الإصابة بالتهاب المهبل البكتيري. كان موسط مستوى الهرمون جار الدرقية بارتفاع معنوي ($P=0.21$) في مجموعة النساء المصابات بالتهاب المهبل البكتيري (٣٢,٧±١٣,٦) مقارنة بمجموعة السيطرة (٢٦,٥±١٠,٩).

علاوة على ذلك، اظهر متوسط عدد كريات الدم البيضاء ارتفاع معنوي ($P<0.05$) في مجموعة النساء المصابات بالتهاب المهبل البكتيري (٧,٧٧±٢,٧) مقارنة بمجموعة السيطرة (٦,٤٧±١,٦)، وكذلك بالنسة للصفيحات الدموية أظهرت ارتفاع معنوي ($P<0.05$) في مجموعة النساء المصابات بالتهاب المهبل البكتيري (٢٣٠,٢±٧٧,٦) مقارنة بمجموعة السيطرة (١٩١,٧±٥٣,٤)، ولكن بالنسبة لحجم كريات الدم المظغوط (PCV) لم يكن هنالك فرق معنوي بين مجموعات الدراسة.

تم زرع جميع الـ٩٠ مسحة مهبلية على الوسط الزرعي (MRS agar and broth)، للتحري عن *Lactobacillus* spp. كمؤشر عكسي للاتهاب المهبل البكتيري خلال الأشهر الثلاثة الأولى من الحمل، ٧ (١٤٪) من المسحات المهبلية لمجموعة الدراسة تم تشخيصها كـ *Lactobacillus* بينما ٤٣ (٨٦٪) اظهرت نتيجة سالبة للزرع البكتيري، و ٣٠ (٧٥٪) من المسحات المهبلية لمجموعة السيطرة تم تشخيصها كـ *Lactobacillus* ، بينما ١٠ (٢٥٪)

اظهرت نتيجة سالبة للزرع البكتيري تم التشخيص بجهاز VITIK-2 وتم تشخيص نوعان من بكتريا الـ *Lactobacillus* : *Lactobacillus gasseri* و *Lactobacillus case* .

أظهرت النتائج ان جميع عزلات الـ*Lactobacillus* spp. التي كانت من النساء الاصحاء أظهرت مقاومة اعلى للمضادات الحيوية المستخدمة في الدراسة cefixime وcefepime وNitrofurantoin وAmoxicillin/clavulanic acid وAmoxicillin بنسبة مقاومة (١٠٠٪ و ٨٠٪ و ٦٠٪ و ٦٠٪ و ٣٠٪ على التوالي) في مجموعة النساء الاصحاء و(١٠٠٪ و ٧١٪ و ٧١٪ و ٤٣٪ و٢٨٪ على التوالي) في مجموعة النساء المصابات بالتهاب المهبل البكتيري.

أظهرت هذه الدراسة ان الراشح الخالي من الخلايا (CFS) المستخلص من *Lactobacillus* spp من النساء الاصحاء كان أكثر تأثيراً ضد عزلات البكتريا المرضية من CFS من L. spp. المعزولة من النساء المصابات بالتهاب المهبل البكتيري.

وزارة التعليم العالي والبحث العلمي
جامعة بابل
كلية العلوم للبنات
قسم الاحياء

العلاقة بين الهرمون جار الدرقية وفيتامين د مع العصيات اللبنية المهبلية في الاشهر الثلاثة الاولى من الحمل لدى الحوامل في مدينة الحلة

رسالة مقدمة
الى مجلس كلية العلوم للبنات/ جامعة بابل
كجزء من متطلبات نيل درجة الماجستيرفي علوم الحياة

من قبل الطالبة
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بكالوريوس علوم حياة كلية العلوم للبنات/ جامعة بابل (٢٠١٦)

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٢٠٢٢ م ١٤٤٣ هـ

Ministry of Higher Education & Scientific Research

University of Babylon

College of Science for Women

Department of Biology

Association of Parathyroid hormone and vitamin D with vaginal *Lactobacillus* in First Trimester Pregnant in Hilla City

A thesis submitted to the Council of the College of women Science, University of Babylon. As a partial requirement of the fulfillment of the Degree of Master Biology

Submitted by

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2022 A.D **1444 A.H.**

«وَقُلِ اعْمَلُوا فَسَيَرَى اللَّهُ عَمَلَكُمْ وَرَسُولُهُ وَالْمُؤْمِنُونَ وَسَتُرَدُّونَ إِلَى عَالِمِ الْغَيْبِ وَالشَّهَادَةِ فَيُنَبِّئُكُمْ بِمَا كُنْتُمْ تَعْمَلُونَ»

صدق الله العلي العظيم

سورة التوبة (الآية ١٠٥)

Certification of Examination Committee

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Dedication

To

All my dreams my daughter Ruqaya.

My parents.

My brothers and sisters.

My love and support Hayder.

My second family.

My friend Reema.

Acknowledgment

First and foremost, my gratitude to Allah.

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Summary

A wide variety of pathogenic agents including bacteria, fungi, and virus can cause infections vaginitis especially during pregnancy. The present study is designed to investigate the isolation and identification of *Lactobacillus* spp. from women affected with bacterial vaginosis during first trimester of pregnancy and also determination of Vitamin D level, Parathyroid hormone, and some hematological parameters.

The number of pregnant women that included in the present study were ninety (90) women, (50) women were infected with bacterial vaginosis (BV) in first trimester of pregnancy, the remaining (40) women were used as a control group (non-BV) and they were also pregnant in first trimester. Age of the pregnant women were (16-45) years. All samples were collected during a period ranged from November (2021) to February (2022). Samples collection were done in Babylon province, Imam Ali general hospital and private clinics, sample collection done by Gynecologists physician , for each participant blood sample and high vaginal swab (HVS) were collected. All samples tested for serum level of Vitamin D and Parathyroid hormone (PTH) using the enzyme linked immunosorbent assay technique (ELISA) and also tested for complete blood count (CBC) by automated hematology autoanalyzer, and for HVS were cultured on De man, Rogosa and Sharpe (MRS) agar and broth for isolation of *Lactobacillus* spp. then identified using VITIK-2 device, and for antibiotic and antibacterial activity Muller-Hinton Agar (MHA) was used.

The data of this study showed that the body mass index (BMI) of BV group have higher BMI compared to those healthy women. The mean vitamin D levels in serum were significantly lower (P=0.001) in BV group (19.91±11.2) than control group (33.71±14.9) The lower the vitamin D, the greater the risk of developing BV. The mean levels of PTH were significantly higher (P=0.021) in BV group (32.7±13.6) than control group (26.5±10.9).

Moreover, the mean count of White blood cells (WBCs) were significantly higher (P=0.01) in BV group (7.77±2.7) than control group (6.47±1.6), and for platelet were also signficantly higher (P=0.009) in BV group (230.2±77.6) than control group (191.7±53.4), But for Packed cell

volume (PCV) there were no significant diffrence (P=0.230) between groups.

All 90 vaginal swabs were cultured on MRS agar and broth, were investigated on *Lactobacillus* spp. bacteria as a reverse indicator of bacterial vaginosis in first trimester pregnant and healthy women, 7 (14%) vaginal swab of BV group diagnosed as *Lactobacillus* whereas 43 (86%) vaginal swab showed no growth result, and 30 (75%) vaginal swab of control group diagnosed as *Lactobacillus* whereas 10 (25%) showed No growth result, then the identification was made with VITEK-2 device, and two types of *Lactobacillus* were detected: *Lactobacillus gasseri* and *Lactobacillus casei*.

The results showed that all *Lactobacillus* spp. isolates were from healthy women showed higher resistance to antibiotics used in this study cefixime, cefepime, Nitrofurantoin, Amoxicillin/clavulanic acid, and Amoxicillin with percentage of resistance (100%, 80%, 60%, 60%, 30% respectively) in non-BV group and (100%, 71%, 71%, 43%, 28% respectively) in BV group.

In this study, the cell free supernatant (CFS) extracted from *Lactobacillus* spp. from healthy women were more effective against the pathogenic bacteria isolates than the CFS from *Lactobacillus*. spp. from women with vaginosis.

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List of Abbreviations

Abbreviation	Meanings
1,25(OH)2D	1,25-Dihydroxy vitamin D
AMC	Amoxicillin/Clavulanic acid
AR	Antibiotic resistance
ATP	Adenosine triphosphate
AX	Amoxicillin
BMD	Bone mineralization density
BV	Bacterial vaginosis
CaSR	Calcium-sensing receptor
CBC	Complete blood count
CFM	Cefixime
CFS	Cell- free- supernatant
EDTA	Ethylenediaminetetra acetic
ER s	Endoplasmic reticulum
F	Nitrofurantoin
FBC	Full Blood count
FEP	Cefepime
GBS	Group B streptococci
GDM	Gestational diabetes mellitus
Hb	Hemoglobin
HGT	Horizontal gene transfer

HPV	Human papillomavirus
Hsv-2	Herps simplex virus type 2
HVS	High vaginal swab
LAB	Lactic acid bacteria
MCV	Mean corpuscular volume
MHA	Muller-Hinton Agar
MRS	Deman , Rogosa and Sharpe
NaPi2a	Type II sodium phosphate cotransporter
NaPi2c	Type IIc Sodium–Dependent Phosphate Transporter
non- BV	Non- bacterial vaginosis
PCV	Packed cell volume
PHPT	Hyperparathyroidism
PID	Pelvic Inflammatory Disease
PLT	Platelet
PTB	preterm birth
PTGs	Parathyroid glands
PTH	Parathyroid hormone
PTHrp	Parathyroid hormone-relater protein
PTHrP	PTH-related protein
QVS	quantitative ultrasound
RBCs	Red blood cells
RER	Rough endoplasmic reticulum
RP	Related peptide
RTI s	Reproductive tract infections
SHPT	Secondary hyperparathyroidism
STI	Sexually transmitted
UTI	Urinary Tract Infection
V-D3	Vitamin D3
VDD	Vitamin D deficiency
VDR	Vitamin D receptor
Vit A	Vitamin A
Vit D	Vitamin D
VVC	Vulvovaginal candidiasis
WBcs	White blood cells
WRA	Women of reproductive age

Chapter One

Introduction

1.1-Intoduction

Vaginal symptoms are one of the most prevalent causes for visits in clinical practice, and they arise in women throughout their reproductive lives, leading to numerous consultations with an obstetrician or gynecologist (Muvunyi *et al*.,2009). Vaginitis can take many different forms, including irritating, sexually transmitted illnesses, hormonal, foreign body, and infective vaginitis. There is a variety of things that can cause pain to the sick women (Kerry *et al*., 2018). *Trichomoniasis, Vulvovaginal candidiasis,* and bacterial vaginosis are the 3 most prevalent causes of vaginitis, in 40 to 50 % of cases with a recognized cause, bacterial vaginosis is to blame, following by *trichomoniasis* (20 to 25 % of cases) and *vulvovaginal candidiasis* (15 % to 20% of cases) Only 5 to 10 percent of vaginal infections are caused by atrophic, irritant, allergic, and inflammatory vaginitis (Workowski *et al*.,2015).

Bacteria, fungi, parasites, and viruses all have the infectious vaginitis is a possibility. Numerous bacteria which are characterized by proliferation and lacking Lactobacilli, which produce hydrogen peroxide, are associated with vaginitis. *Staphylococcus aureus*, *Klebsiella pneumoniae*, Group B *Streptococci* (GBS), *Escherichia coli, Listeria monocytogenes*, *Acinetobacter spp*., *Neisseria gonorrhoeae,* and bacteria species produce bacterial vaginosis are only a few of the bacteria that may induce vaginitis. The most critical component for *Lactobacillus spp*. to keep the vaginal flora in equilibrium. *Lactobacillus spp.* functions as a probiotics and preventive agent in the treatment and prevention of vaginal infection by producing antagonistic chemicals such as hydrogen peroxide, lactic acid, and bacteriocin (Jomehzadeh *et al*.,2020). ,

The probability of Preterm birth probability is doesn't decrease although the symptoms improve with treatments for vaginitis throughout gestation (Koss *et al*.,2012, Brocklehurst *et al*.,2013). Increased vaginal secretions, which often smell like fish due to the presence of amine compounds, and a white to

yellowish white color accompanied by a burning sensation when urinating and the appearance of Clue-cell in the secretions, which are torn cells of the vaginal epithelium accompanied by a raise in pH up to 4.5, which is known as Amsel Criteria, are common symptoms of the disease (DeCherney *et al*.,2013)

Probiotics are *Lactobacillus* spp. that are non-pathogenic and generate antibacterial compounds. Living microorganism which, if administered in the right concentrations, improve the host's health (World Health Organization, 2005).

Lactobacilli spp. are the most common type of bacteria in the vaginal tracts of healthy women . An imbalance in the local microbiota can make women more susceptible to diseases like bacterial vaginosis (BV) and *vulvovaginal candidiasis* (VVC). Despite the fact that antimicrobial therapy is often effective, repeated use of antimicrobials leads to a high rate of recurrence and a rise in microbial resistance. (Santos *et al*., 2016).

PTH (parathyroid hormone, also known as the peptide hormone parathormone (also known as parathyrin) is produced by the parathyroid glands and regulates serum calcium levels through acting on bones , kidneys, and the gut (Coetzee *et al*.,2004).

Vitamin D is a fat-soluble, which is essential for both children and adults' calcium metabolism. A sufficient intake of vitamin D is essential for maternal health, fetus skeleton development, and the best results for both the mother and the fetus is when calcium requirements rise in the 3rd trimester of pregnancy. The metabolites of this vitamin play an essential role in control of mineral metabolism by altering calcium absorption in tissue of intestinal and having additional effect on the bones and many other tissue of body (Mulligan *et al*., 2010).

Vitamin D and parathyroid hormone (PTH) are two hormones that control the body's serum calcium levels (Holick, 2013). The calcium absorption is improved when 1,25(OH)2D enters the bloodstream and goes to the small intestines. In the kidney, the most conspicuous endocrine effects of 1,25(OH)2D are tight controls of their own equilibrium by suppressing 1-hydroxylase and stimulating 24-hydroxylase at the same time. An unbroken 1,25(OH)2D-vitamin D receptors scheme is essential for both baseline and PTH-induced osteo-clastogenesis. Mature osteoclast remove phosphorus and Ca from the bones, preserving optimum blood level of the 2 elements (Dusso *et al*.,2005; Holick ,2007).

Aim of the study

The aim of the present study was employed to explore the interaction between vaginal *Lactobacillus* and vitamin D deficiency of pregnant women in first trimester

To carry out these aims the following parameters were followed :.

- Investigation on *Lactobacillus* spp. bacteria as a reverse indicator of bacterial vaginosis in first trimester pregnant and healthy women
- Diagnosis of BV in first trimester pregnancy.
- Isolation and identification of *Lactobacillus spp*. from BV and Non-BV pregnant women.
- Determination of the antibacterial activity of Cell- Free Supernatants (CFS) extracted from *Lactobacillus spp.*
- Estimation of vitamin D ,parathyroid hormone level, and Count Blood Cells (CBC) assay.
- Estimation of the correlation between Vitamin D level and parathyroid hormone (PTH) with BV in first trimester pregnancy .

Chapter Two

Literature Review

2.1: Vaginitis

Definition and Symptoms of Vaginitis

Vaginitis defined as vaginal symptoms such as itching, burning, irritation, odor, and vaginal discharge is among the most prevalent causes for women seeking medical treatment is vulvovaginal pain (Hainer and Gibson ,2011). The pH of the vaginal fluid has been demonstrated to have an important function in determining vaginal health. During the reproductive years, the vaginal pH should be less than 4.5, but it should be higher than 4.5 before menarche and after menopause. Vaginal epithelial cells grow in response to estrogen, their glycogen levels rise, and *lactobacillus* manufactures lactic acid from glycogen, resulting in an acidic pH (pH 2.8–4.2) that promotes vaginal health. A vaginal pH greater than 4.5 may indicate bacterial vaginosis, *Trichomonas vaginalis*, group B *streptococcus*, or other pathogenic organisms in the case of vaginitis. As a result of epithelial cells' enhanced proliferation rate, the thickness of epithelial cell layers has grown (Patton *et al.,* 2000).

Some symptoms related to vaginal are inflammation caused by *streptococcus*, *staphylococcus*, and *E. coli* as 'aerobic vaginitis.' Vaginal *Streptococcus agalactiae* (also known as Group B *streptococcus*) and *E. coli* have long been linked with invasive maternal and newborn infections (van de Wijgert *et al.,* 2014).

Clinicians consider vaginal discharge as a common presenting sign in a variety of conditions (primary care, gynecology, family planning, and department of genitourinary medicine). Vaginal discharge is likely to be either useful or harmful. Despite the fact that atypical vaginal discharge frequently leads to sexually transmitted infections (STI) testing, vaginal discharge is a poor predictor of STI presence. It is typical for bacterial vaginosis to return following treatment, and this may be made worse by personal hygiene routines like

vaginal douching that disturb the normal flora in the vagina. STIs and bacterial vaginosis are commonly linked (Mitchell, 2004).

Reproductive tract infections (RTI's) cover three types of infections: sexually transmitted infections (STIs), infections caused by overgrowth of organisms which are commonly found in the reproductive system, and infections connected with medical operations, such as abortion and the implantation of intrauterine devices are all covered by the term "reproductive tract infections" Female RTIs, such as vaginitis and cervicitis, frequently start in the lower genital tract and cause symptoms such abnormal vaginal discharge, genital pain, itching, and a burning sensation when urinating (Rabiu *et al.*, 2010).

Infectious vaginal discharge in pregnant women can lead to abortion, premature rupture of membranes, chorioamnionitis, preterm, low birth weight, and postpartum endometritis, among other issues (Passini *et al*., 2010). *Lactobacillus* species dominate the healthy human vagina, they perform an important role in preventing women from genital infection. Lactobacilli deficiency can disrupt the microbial equilibrium in the vaginal area, leading to bacterial vaginosis (BV) syndrome. (Aroutcheva *et al*., 2001), which is linked to a quantitative and qualitative shift from naturally occurring *lactobacilli* to a mixed microflora dominated by anaerobic bacteria (Forsum *et al* ,2005).

2.2 Bacterial Vaginosis

Bacterial vaginosis is a bacterial imbalance in the vaginal flora caused by an overabundance of anaerobic bacteria and a deficiency of Lactobacillary flora. Women may experience signs of a typical vaginal discharge, but they are frequently asymptomatic. (Brocklehurst *et al*., 2013)

Among women of reproductive age, (BV) is a common infection of the genital tract that's been related to neonatal deaths, earlier miscarriage, repeated

abortion, premature delivery, and post-abortal infection. Following delivery, infection that result in preterm labor as well as other pregnancy complications might still exist, putting the infant at significant life-threatening danger. The public knowledge of the function of BV in premature births (PTB) is limited and inconsistent since many infection are subclinical (Africa *et al*., 2014)

Clinical symptoms, such as exfoliated epithelial cell containing Gram-variable polymorphic rod-shaped bacteria clinging to their surface, and a fishy odor, are all indicators of bacterial vaginosis (Turovskiy *et al*., 2011). The most common anaerobic microorganisms detected in BV are *Prevotella* species, *Gardnerella vaginalis*, *Mycoplasma homins*, and *Mobiluncas* species (Rampersaud, 2012; Zarbo *et al*., 2013). BV is related to an increased risk of Pelvic Inflammatory Disease (PID), STIs, and prematurity (Fredrick *et al.*, 2011; Tomusiak *et al.,* 2013). Vaginal discharge is typically caused by infection with *Neisseria gonorrhoeae* and/or *Chlamydia trachomatis*, while cervical discharge is usually caused by infection with *Neisseria gonorrhoeae* and/or *Chlamydia trachomatis*. (Zemouri *et al*., 2016)

Non-infectious reasons of vaginal secretions include vaginal douching, utilizing scented soap for cleansing, certain laundry detergent, toilet paper, personal care products, as well as other feminine care items that may irritate and generate unusual discharge. Foreign body (like a stuck tampons, condoms, or toilet paper) can sometimes cause a foul-smelling or bloody discharge (Le Doare and Heath, 2013).

Lactobacillus colonization requires attachment to epithelial surfaces, and one of the proposed mechanisms by which they could defend the vagina against pathogen colonization is through competing for adhesion receptor sites (Monteagudo-Mera *et al*., 2019). Interactions between specific adhesins on the bacterial surface and receptors on host tissues are part of bacterial adherence to mucosal surfaces (An and Friedman, 2000). Low-birth-weight newborns,

preterm delivery, and pelvic inflammatory illness have all been linked to bacterial vaginosis. Human Papillomavirus (HPV), Herpes Simplex Virus type 2 (HSV-2), *Trichomonas vaginalis*, *Neisseria gonorrhoeae,* and HIV are all more likely in women with BV. Given the high prevalence and severity of related morbidity, it was vital to properly diagnose and treat BV-affected women, particularly pregnant women (Hemalatha *et al.,* 2013).

Although bacterial vaginosis (BV) is associated with an increased risk of premature birth during pregnancy, treatment with metronidazole was not effective and, in fact, hazardous to the pregnancy. Although broader spectrum antibiotics, such as clindamycin, and combination antibiotherapy have demonstrated superior benefits, not all research agree (Gilbert *et al*., 2017). BV-associated bacteria toxin (lipopolysaccharide) can cross the placenta and injure fetuses' brains. Toxins have the potential to induce irreversible neurological brain damage like cerebral palsy, an increased risk of Parkinson's disease, and/ or schizophrenia (Hashemi *et al*., 2000). BV has also been related to an increased possibility of HIV infection (Urakubo *et al.,* 2001; Sha *et al.,* 2005), as well as herpes simplex virus type 2 infection (Sha *et al*., 2005; Eriksson *et al.,* 2005). Antibiotics, such as clindamycin or metronidazole, are commonly used to treat BV, and can be either orally or administered topically Antibiotics, on the other hand, do not eliminate all vaginal harmful microorganisms. About 60% of BV patients respond to this therapy, and there is a significant recurrent rate of 30% to 40% (Ling *et al.,* 2004; Larsson and Forsum, 2005). Furthermore, antibiotics disrupt the healthy vaginal microbiome, and the risk of acquiring antimicrobial drug resistance rises rapidly as the overall usage of anti- microbial (feminine hygiene and treatments) preparation rises. Metronidazole and Clindamycin inhibit *Lactobacillus spp*. in vitro at concentration lesser than those used topically to treat the bacteria (Aroutcheva *et al*., 2001; Macklaim *et al.,* 2015).

2.3 *Lactobacillus*

Lactobacilli are the most common bacterial genus found in the healthy vaginal tract of women of reproductive age (Witkin *et al.*, 2007). By regulating the environments as well as other bacterial via the formation of lactic acid, bacteriocins, and competitive exclusion, are believed to improve vaginal hygiene (Zhou *et al*., 2004;Fredricks *et al*., (2005), stated that the two main species present are *L. crispatus* and *L. iners*, and *L. crispatus* or *L. gasseri*.

The other organisms *L. jensenii*, *L. gallinarum*, or *L. vaginalis* may be presented in many instances as well (Pavlova *et al*., 2002). However a modified microbiota, like the lack of *lactobacilli*, may accompany vaginosis, the existence of lactobacilli also isn't required for vaginosis prevention (Witkin *et al*., 2007). *Lactobacillus* is a phylogenetically diverse genus that currently has over 125 species (Dellaglio *et al*., 2005). Probiotics are *Lactobacillus spp*. that are non-pathogenic and generate antibacterial compounds. This is described as a living microorganism which, when administered at the right concentration, boost the host's health (World Health Organization, 2005). As a result, they can be discovered in human body places where dietary substrates are easily available 126 (O'Callaghan and Toole, 2011).

The mechanisms by which *Lactobacillus* controls the colonizations of other bacterial species in the vagina decide whether or not pathogenetic or etiological treatments (Li *et al.*, 2005; Kovachev and VatchevaDobrevska, 2014).

2.4 Characteristics of *Lactobacillus* SPP.

Lactobacillus spp. are Gram-positive rods that are catalase negative, nonsporing, and facultative anaerobic, though they prefer to grow in microaerophilic conditions ,their Gram stain morphology can range from short, plump rods to long slender rods, in chain or palisades, and their colony

morphology can range from small to medium gray colonies on MRS that usually exhibit alpha hemolysis on blood agar (Dover *et al.,* 2008; Razzak *et al.,* 2011).

The pathogenicity of commensal *Lactobacillus* is undetermined, although the ability to create acids and the ability to grow and survive in an acidic environment are the most known predictors of cariogenicity of *lactobacilli* (Edelman, 2005; Badet and Thebaud, 2008). Other chemicals released by *Lactobacillus* are vital for maintaining vaginal microbiota balance (Shopova 2003; Stoyancheva *et al.,* 2014). Another defense component generated from Lactobacillus vaginalis is H2O2 (Vallor et al. 2001; Kovachev, 2018). *Lactobacilli* have a strong oxi-reduction potential in the vaginal environments, which prevents anaerobic bacteria from colonizing (Zarate and Nader-Macias, 2006; Gil *et al.,* 2010). *Lactobacillus* adheres strongly to the stratified, nonkeratinizing vaginal epithelium, causing pathogenic bacteria like Candida albican, Gonococcus vaginalis, E. coli, Strep. agalactiae, and Strep. aureus to be excluded and rejected (Parolin *et al*., 2015).

It is most likely the same method, *Lactobacillus* adheres to the vaginal wall predominantly by adhesins, which are produced as fine fimbriae on the cell surfaces and bind extracellular matrix ingredients such as laminin, collagen, and fibrokinetin that surround the host epithelium (Antikainen *et al.,* 2002; Younes *et al*., 2016). The varying affinity of adhesion for certain receptor, or their relative locations in the case of steric hindrance, are blamed for changes in vaginal epithelial cell adhesions (Zarate and Nader-Macias, 2006). After engaging and activating plasminogen system receptors, *Lactobacillus* is incapable to keep the produced cells plasmin activity (Lahteenmaki *et al*., 2001; Lorca *et al.,* 2002). Physiological antiproteases, unlike pathogenic microorganisms, are thought to be unable to combat the plasminogen proteolysis mechanism (Styriak *et al*., 2003; Buck *et al.,* 2005*).*

Lactobacillus contributes to vaginal health by producing chemicals (mostly organic acid) that acidify the environments and hinder the growth of infections (Tachedjian *et al*., 2017). To defend their ecological niche against competing organisms, *Lactobacillus* spp. as well as other bacteria produce antimicrobial peptides (bacteriocins) that are applied topically (Sablone *et al.*, 2000; Diep and Nes, 2002). The transmembrane potential and pH gradients are depleted as a result of their antimicrobial action, which is often brought on by the cellular membranes being permeable, which results in an efflux of ATR amino acids and ion. (Oscáriz and Pisabarro, 2001)

2.5 Bacteriocin as Antibacterial

Bacteriocins are multifunctional proteinaceous compounds generated by ribosomes that have strong antibacterial activity at specific quantities. They are created by bacteria and archaea to prevent the growth of bacteria that are similar or closely related. Antimicrobial action against pathogenic and degrading microorganisms is demonstrated by these compounds, indicating their biotechnological potential. (Negash and Tsehai, 2020). As previously mentioned, bacteriocin production was tested using both an agar spot test and a well-diffusion experiment (Delgado *et al*., 2007).

Bacteriocins have antibacterial properties against Gram-positive and Gram-negative bacteria, as well as fungus (Stoyancheva *et al.*, 2014). Bacteriocin kill pathogen by compromising cell wall integrity and inhibiting DNA and synthesis of proteins (Dover *et al.*, 2008). This results in a lake of adenosine-triphosphate (ATP). Bacteriocins have inhibitory effects similar to antibiotics, however they differ in terms of production, toxicity, method of action, and resistance mechanism (Kaur *et al.*, 2013).

Bacteriocin resistance is not commonly found in antibiotic-resistant bacteria, nor it is genetically determined. Furthermore, bacteriocin is more

active when the vaginal pH is between 4.0 and 7.0, and their effectiveness diminishes rapidly outside this range. According to toxicology studies, vaginal Lactobacillus spp. produces antimicrobial peptides that are non-haemolytic, don't really irritate the vaginal endothelium, and therefore do not affect the viability of the vaginal *Lactobacillus* spp. (Li *et al.*, 2005). This suggests that bacteriocins and the Lactobac from which they originate are safe (Stoyancheva *et al*., 2014).

2.6 Antibiotic and *Lactobacillus Spp.*

Due to the extensive use of antibiotics, pathogenic and non-pathogenic microorganisms linked to people, animal, and the environmental have developed and transmitted antibiotic resistance (AR) (Hernando-Amado *et al.,* 2019). The rapid spread of AR among bacteria, along with a dearth of new antibiotics, is a huge public health problem around the world (WHO, 2014). The selection pressure imposed by antibiotic in the environments has aided the spread of AR through horizontal gene transfer (HGT) (von Wintersdorff *et al.*,2016).

Antibiotic that is used to treat infection in people is frequently the same as that utilized in veterinary medicine, leading to the rapid spread of AR gene among bacteria in the food chain (EFSA, 2016). *Lactobacillus* spp. Are found in the native microbiota of food, as well as human and animal mucosa. Some Lactobacillus strains are utilized as starter cultures and supplement cultures to manage food fermentation, while others are used as probiotic to prevent and treat intestinal disorders (Sáez-Lara *et al.*, 2015).

Lactobacillus is found in the native microbiota of food, as well as human and animal mucosa. Some Lactobacillus strains are utilized as starter cultures and supplement cultures to manage food fermentation, while others are used as probiotics to prevent and treat intestinal disorders (Sáez-Lara *et al.*, 2015;

EFSA, 2018). In reality, most Lactobacillus species have been found to be resistant to these antibiotics (Abriouel *et al.*, 2015). Lactobacilli susceptibility to antibiotics must be determined, and the lack of transferrable AR genes in selected strains must be ensured (Salvetti and O'Toole, 2017). Over the last 15 years, studies have been published that show bifidobacteria and lactobacilli are typically sensitive to tetracycline, erythromycin, chloramphenicol, ampicillin, penicillin, quinupristin-dalfopristin, clindamycin, linezolid, and clarithromycin (Abriouel *et al.*, 2015). Resistance to aminoglycosides can develop through a variety of pathways, (Doi *et al.*, 2016) including

I. Aminoglycoside acetyltransferases, nucleotidyltransferases, or phosphotransferases which are responsible for the enzyme modifications and deactivation of the antibiotic.

II. A higher efflux

III. Reduced permeability

IV. Modification to the 30S ribosomal subunit that prevent this family of antibiotic from adhering.

Some phylogroups of *Lactobacillus* spp. as well as other LAB species, including *Leuconostoc* spp., have a built-in strong resistance to vancomycin (Goldstein *et al*.,2015).

2.7 Probiotic

Probiotic are described as living microorganism ,if these probiotic were given in the right amounts, help the host's health (Reid, 2008).

Probiotics have been suggested as an alternative therapy for BV and may be applied locally or orally to the vaginal system in the form of pantyliners, suppositories, or tampons. Oral probiotics may enhance the long-term outcomes of BV therapy with vaginal suppositories including *L. acidophilus* by

harmonizing the vaginal and gastrointestinal flora (Antonio and Hillier, 2003; Delia *et al.*, 2006). By colonizing the vaginal system, the newly imported *Lactobacillus* spp. would beat off vaginal infections and stop the introduction of new illnesses. Despite the fact that the majority of trials were small and sometimes lacked control group, several studies on the treatments of BV with probiotic have shown mainly favorable outcomes (Reid *et al.*, 2001; Uehara *et al.*, 2006)

In vitro properties such as generation of antimicrobial agents (H_2O_2, bacteriocins), ability to adhere to vaginal epithelial cells, and antimicrobial effectiveness against pathogens have all played a role in the selection of *Lactobacillus* spp.-based probiotics. Other desirable criteria for probiotics include their ability to strongly acidify the vaginal environment with lactic acid in order to have beneficial effects on the host, such as immunomodulatory activities, the ability to survive and outcompete other bacteria, coaggregation, and antibiotic susceptibility other than those used to treat BV. (MacPhee *et al.*, 2010; Santos *et al.*, 2016).

Probiotics are becoming more widely accepted as an alternative to antibiotic therapy for controlling some infections and reestablishing the human bacterial ecology. *Lactobacillus* spp. protect the vaginal epithelium from pathogen colonization through two main mechanisms: blocking pathogen attachment and producing substances that inhibit pathogen multiplication. Various pathogens may respond differently to the same *Lactobacillus* strain in terms of blocking and inhibitory activities. This emphasizes the necessity of recognizing *Lactobacillus* spp. strains prior administering them humans as probiotics. The main indications for probiotic *Lactobacillus* use in urogenital tract infection include bacterial vaginosis, repeated vaginal candidiasis, post-infection prophylactic by re-establishing the vaginal flora to prevent any future

episode, and repeated Urinary tract infection (UTI) in healthy pre-menopausal and post-menopausal women. (Andreu, 2004).

2.8 Vagina Anatomy and Sex hormones

The vagina (meaning sheath in Latin) is an important part of the female reproductive tract or reproductive organs located in the pelvis. It is a thin-walled fibromuscular tube (approximately 8-10 cm in length) connecting the uterine cervix internally and the vestibule externally. A stratified, squamous and nonkeratinized epithelium is placed on a dense connective tissue that contains a vascular venous plexus, and act as an erectile tissue (Sato *et al*.,2018)

Bacterial communities in the human vagina are thought to have a critical role in protecting the host against infectious disease. In reproductive age women, it is thought they do so through the production of lactic acid resulting in a low pH environment that restricts the growth of pathogens and other opportunistic organisms (O'Hanlon *et al*., 2011)

During pregnancy, the placenta is the primary endocrine organ for maintaining pregnancy and fetal growth. The placenta releases hormones, including androgens, estrogens, and progestogens, and these hormones occur at extremely high concentrations in the maternal circulation. These hormones are important paracrine and autocrine regulators affect the growth and differentiation of the placental trophoblast, growth and maturation of the placental vascular tree, and uterine endovascular invasion by the extravillous cytotrophoblast (Armaly *et al*.,2018). Progesterone and estrogens also function as modulators of uterine vessels in that they decrease the resistance of the spiral uterine arteries and modulate the synthesis and release of angiogenic factors by placental cells (Berkane *et al*.,2017, Kumar *et al*.,2018)

Sex hormones contribute to the development and activity of the immune system, accounting for differences in gender-related immune responses. Both innate and

adaptive immune systems bear receptors for sex hormones and respond to hormonal cues (Moulton ,2018). It is well known that estrogen suppresses T and B cell lymphopoiesis and activates B cell function. ERs are found on thymocytes as well as thymic epithelial cells (Savino *et al*.,2016) Estrogen influences T cell development and lymphopoiesis, its effects on the thymus are complex (Zoller and Kersh,2006)

2.9 Complete Blood Count (CBC):-

2.9.1 Blood

In humans, blood is a biological fluid in circulatory systems that moves transporting metabolic waste products out from cells while providing or supplying crucial nutrients oxygen and nutrients. The produced constituents include RBCs, WBCs (neutrophil, monocyte, lymphocyte, eosinophil, and basophil), and platelets. Plasma is mostly water, with 7% ,the remainder is made up of protein (globulin, albumin, and fibrinogen) and 2% other substances (electrolyte, nutrient, breathing gases, waste product, and regulatory compound) (Thakare, 2021). Plasma volume increases in the first weeks of pregnancy, with the steepest increase occurring during the second trimester, after which it continues to increase further in the third trimester (De Haas *et al*.,2017) . During pregnancy, 25(OH)D level may be influenced by physiological hemodilution. Also, blood cell count (RBC), hemoglobin (Hb), and hematocrit could be the indicators of hemodilution. Thus, examined the 25(OH)D level as well as the red blood cell count (RBC), hemoglobin (Hb), and hematocrit across all the three trimesters among pregnant women (Takaoka *et al*.,2020)

Red blood cells, white blood cells, and platelets are all produced in the human body constituents of blood. The majority of cells in the blood (erythrocytes) are RBCs. Their primary function is to transport O_2 from the lung to the tissue and return CO_2 to the lungs. This is because haemoglobin, a

protein that binds to oxygen quickly and reversibly, is present. Under specific conditions, haemoglobin's affinity for oxygen varies, allowing for higher oxygen offloading at the respiratory tissues. The body's defense against pathogens is made up of white blood cells (leucocytes) ,Granulocytes and agranulocytes are two types of cells that kill infections via separate ways (Glenn and Armstrong, 2019).

Despite this diversity of responsibilities, the available platelet function tests focus on changes that are directly related to the hemostatic process (Harrison, 2009) .The typical platelet count in a mm3 of blood is between 150,000 and 300,000. (Des, 2020)

The complete blood count (CBC) is a widely ordered test that is routinely conducted on large, automated hematology analyzers in central laboratories. The results of the CBC are used to make critical and clinical choices like whether to transfuse, provide chemotherapy, or give antibiotics (Anthony, 2015)

The RBCs, WBCs (eosinophil, lymphocyte, neutrophil, monocyte, and basophil), and platelet make up the formed constituents. Plasma is mostly made up of water, with 7% of protein (globulin, albumin, and fibrinogen) and 2% of miscellaneous chemicals (electrolyte, nutrient, respiratory gases, waste product, and regulatory substance) (Thakare *et al.*, 2021). Erythrocytes have a role in cardiovascular system modulation through mechanisms that include their interaction with the endothelium. Bacteria, viruses, parasites, poisons, and tumors are all examples of invading microorganisms, and leukocytes operate to eliminate dead cells and detritus from the body. WBCs are divided into five types, each of them is classified as granulocytes or agranulocytes based on the presence or lack of granules and the staining properties of their cytoplasm (Des, J. T. R, 2020).

When a blood cell passes through a small hole surrounded by electrodes, the equipment generates an electrical pulse. As a result, the electronic counter not only counts the total number of cells, but also assesses the average of cell volume and cell size fluctuation. These metrics are known as the mean corpuscular volume (MCV) and the RBC distribution width, respectively, in the context of RBCs. Modern electronic counters may also analyze cell size and content in multiple ways, giving you more information on the different types of WBCs like neutrophils, lymphocytes, monocytes, eosinophils, and basophils (Tefferi *et al.,* 2005).

2.9.2 Leucocytes

In medical practice, alterations in peripheral leucocyte count can be very useful, and the cells line implicated can be particular to certain conditions. Neutrophilia is a type of bacterial infection that is usually detected in individuals. The most serious infections are linked to increased neutrophilia and, in some cases, a degree of myeloid left shift (the existence of immature myeloid cell in the peripheral circulation) as well as 'toxic' neutrophil granulation. Non-infectious illnesses can also cause neutrophilia. It's a common side effect of steroid therapy, strenuous activity, and surgery or splenectomy, but it can also happen with systemic vasculitis, tissue necrosis/burn, and as a result of some cancers. Lympopenia is a typical side effect of steroid and other immunosuppressive medications, such as azathioprine. It is related with rheumatoid arthritis, systemic lupus, and sarcoidosis, and it can be a presenting characteristic in patient with Hodgkin lymphoma. Mild lymphopenia is a fairly typical finding in a routine FBC and should not prompt further inquiry in the absence of any additional symptoms(Yedgar *et al*., 2002; Poz *et al*., 2018).

In experience, investigating moderate isolated lymphopenia rarely yields positive results. Lympocytosis is a typical side effect of viral infection, it's often accompanied by a modest, self-limiting neutropenia. Stress lymphocytosis is

brought on by acutely occurring disorders such heart attack, severe trauma, and epileptic seizures, which is a typical occurrence in hospital patients. The lymphocytosis arises suddenly and lasts only a few days after the trauma. Mild lymphocytosis can occur after a splenectomy and can also be caused by smoking (Leach, 2014).

2.9.3 Hemoglobin: -

A 50 % reduction in anemia in women of reproductive age (WRA) is a World Health Assembly Global Nutrition Target for 2025, and accurate hemoglobin measurement is a global priority (WHO, 2020). The use of hemoglobin measurements as a screening approach for donor eligibility in the blood banking context is a must. Anemia is a disorder caused by a lack of circulating RBC, which limits their ability to transport O_2 throughout the body (McLean *et al.,* 2009).

Hb is a protein found in red blood cells that transports oxygen to the tissues. Hb concentrations below a certain threshold (70–130 g/L dependent on age, gender, pregnancy status, and degree of severity) are considered anemia (WHO, 2018). Anemia affects roughly 1.93 billion people, or 27% of the global populations, which is a serious public health issue, with WRA having the greatest frequency in low-income and middle-income nations and preschoolers (Kassebaum, 2016).

Hemoglobin levels in men range from 13.5 to 17.5, whereas women's levels range from 12 to 16 (Turgeon, 2005, Mairabäurl and Weber, 2011). Both normal hemostasis and pathological bleeding and thrombosis need the involvement of human platelets. These cells play an important role in tumor growth and metastasis, as well as vascular constriction and repair (George, 2000) Platelets also have a role in inflammation, associated diseases, and the advancement of atherosclerosis when they collaborate with other cells such as

white, endothelial, or smooth muscle cells. (Katz *et al.,* 2011; Badimon and Vilahur, 2014; Katz *et al.,* 2011).

2.9.4 Platelets

Megakaryocytes, which are found largely in the bone marrow, create circulating platelets. Megakaryocytes, like all other blood cells, are produced by a master stem cell (Michelson, 2019). In individuals with ongoing chronic infection, inflammation, or cancer, thrombocytosis is usually encountered as a reactive phenomena. The longer these disease processes go on, the more possible it is that thrombocytosis will develop. Other inflammatory indicators are frequently elevated in these patients, and the blood film tend to show small homogeneous platelet with minimal differences in size. Thrombocytosis is commonly connected with chronic bleeding and iron deficiency anemia, and it will go away after the bleeding source and iron shortage are addressed. The majority of instances observed in general medical practice are reactive thrombo-cytosis and thrombocytosis following splenectomy. Thrombo- cytosis is a symptom of a number of myelo-proliferative illnesses, and it is frequently associated with hemoglobin or platelet count abnormalities. Inflammatory indicators will not be elevated in these circumstances, and the blood film will often reveal big platelets with a wide range of individual sizes (Leach, 2014).

2.10 Parathyroid glands

PTGs (parathyroid glands) are nodular structures that are commonly seen along the thyroid's posterior wall. They produce parathyroid hormone (PTH), which is critical for maintaining calcium homeostasis in the body. Primary hyperparathyroidism (PHPT) is caused by an adenomatous, hyperplastic, or occasionally carcinomatous gland overproducing PTH, while secondary hyperparathyroidism is caused by hypocalcemia (SHPT). Chronic renal disease or malabsorption can cause hypocalcemia. (Apetrii, 2017).

The parathyroid gland is one of the extrarenal structures that produces calcitriol, it plays a important role in Ca and phosphate homeostasis via Ca, phosphate, and vitamin D feedback loops. Segersten *et al*. were the first to discover that human parathyroid glands produce 1OHase protein and mRNA, the enzyme is more abundantly expressed in hyperplastic glands from patients with primary and secondary hyperparathyroidism than in normal glands (Segersten *et al*., 2002). There are three forms of hyperparathyroidism: primary, secondary, and tertiary. In the absence of a known stimulus, primary hyperparathyroidism is defined by excessive secretion of the parathyroid hormone (PTH) from larger parathyroid glands, resulting in hypercalcemia. (DeLellis *et al*., 2008; Carlson ,2010).

The parathyroid gland is made up of four tiny glands that are implanted in the back of the thyroid gland. Its primary role is to produce and secrete parathyroid hormone (PTH), a polypeptide hormone it keeps serum calcium levels in check. PTH and level of serum calcium are inversely proportional. PTH, in concert with vitamin D, mobilizes calcium reserves and rises calcium absorption and re- absorption in the body when serum calcium levels are low. The parathyroid gland have a negative feedback from vitamin D and Calcium, which binds to a receptor in the gland when levels increase, inhibiting PTH production and release. (Schwartz *et al*., 2019; Demir *et al.,* 2020; Ilahi *et al*., 2021). Chief cells and oxyphil cells are the two main cell types found in the parathyroid glands. (Hakami and Khan, 2019; Khan *et al*., 2021).

PTH, an 84-amino-acid straight-chain polypeptide, is synthesized and secreted by the parathyroid glands' main cells. A bigger precursor is generated in RER ribosome and transported to the Golgi apparatus via the ER's cisternal channels, where it is membranes-bound glandular or storage granule after being enzymatically converted into a prohormone precursor. The amino-terminals end of the 90-amino-acid pro- hormone is stripped of a hexapeptide to create the

physiologically active hormone. During production, the granule and cellular membranes fusion releases PTH into the interstitium, in which it spreads into blood capillaries. PTH along with calcitonin and calcitriol, is involved in calcium homeostasis regulation (the active form of vit. D) (Mense and Rosol, 2018). Oxyphil cells are substantially larger (12–20 mm) than chief cells (6–8 mm) and feature an eosinophilic cytoplasm due to their high mitochondrial contents. Oxyphil cells are generated from transitional cells, which are small like chief cells but eosinophilic similar chief cells. The finding that glial cells do not express the 2 gene and that transitory and oxyphil cells both produce parathyroid hormone (PTH) strengthens the case for a chief-to-oxyphil cells trans- differentiation (Ritter *et al.,* 2012).

2.11 Parathyroid hormone

The parathyroid gland produces parathyroid hormone, which is a polypeptide that is produced and cleaved into an active form. A pre-pro-PTH, a 115-amino-acid polypeptide, is synthesized first, then cleaved to generate pro-PTH, which is 90-amino-acids long. It is subsequently cleaved a second time, this time at the amino-terminal end, to yield 84 amino acid active parathyroid hormone. This is the most important hormone in the body, since it is stored, secreted, and operated. Synthesis, cleavage, and storage are all expected to take less than an hour. When low serum calcium is observed, active PTH production can occur in as little as a few seconds. The method of secretion is exocytosis, in which the hormones are secreted through membranes vesicles carried to the cellular membranes and discharged after the vesicles merge with the outside membranes. Activated PTH has a short serum half-life and is swiftly eliminated from the bloodstream by the kidneys and liver. (Hans and Levine, 2021; Goyal *et al*., 2021).

Hypoparathyroidism is defined by a decrease in the actions of parathyroid hormone (PTH), which can be caused by either decreased secretion

or resistance to PTH. Studies in genetic anomalies of hypoparathyroidism patients have substantially increased understanding of the etiology of hypoparathyroidism. A new hypoparathyroidism classification based on these genetic anomalies is proposed, as well as a diagnostic method for hypoparathyroidism patients. Endo et al. (Endo *et al.,* 2007).

PTH receptors from a variety of other species have been cloned. Due to the diverse action of PTH in a number of different body tissues and the in vitro studies that have shown for numerous second messengers of hormones discharge from research findings with cell membrane shards improved in the PTH receptor, it was initially hypothesized that many various receptors would've been found to facilitate some of the pleotropic actions of this peptide hormones. Because of this, it came as a bit of a surprise when early cloning efforts in a variety of species only produced one G proteins-coupled receptors, now recognized as the common PTH/PTHrP receptors. This receptor is involved in the majority of PTH's classical functions in mineral ion homeostasis, as well as its effects on bones and kidneys. There are other receptor for both PTHrP and PTH, which are beyond the focus of this review; however, they have a variety of biological functions ascribed to them, most of which include contact with parts of either PTHrP and PTH beyond the amino terminal 34 residue (Jüppner *et al*., 2000). There are numerous ways to assess PTH in the blood: intact PTH, N-terminal PTH, mid-molecule PTH, and C-terminal PTH, and different assays are employed in different clinical settings. The typical PTH concentration is 8–51 pg/mL. (Longo *et al.*,2012).

PTH production is closely controlled on a transcriptional of post-transcriptional and levels by extracellular calcium concentrations. Hypocalcemia, glucocorticoids, and estrogen all stimulate PTH gene transcription. PTH intracellular breakdown can also be accelerated by hypercalcemia. Hypocalcemia, dopamine, adrenergic agonists, and

prostaglandin E2 all stimulate PTH secretion. The CaSR, which is found in the cell membrane of parathyroid cells, detects changes in serum calcium. The receptor allows the parathyroid gland to detect changes in plasma calcium concentration, resulting in desired changes in PTH release (Goodman and Puarles, 2008).

PTH reduces the quantity of NaPit2c and NaPit2a, in the renal proximal tubule brush border membranes, resulting in reduced phosphate reabsorption and phosphaturia. The sodium phosphate cotransporter responses to PTH involves a number of kinases, include proteins kinases A and C, mitogen-activated proteins kinase extracellular signal-regulated kinase 1/2, and a myosin motors (myosin VI) (Lederer *et al.,* 2003; Blaine *et al.,* 2009).

The rise in osteoclastic activity induced by PTH results osteopenia and osteoporosis are caused by localized bone weakening and a broad reduction in bone mineral density (BMD). Deficit calcium phosphorus intake results in a skeletal mineralization deficiency. (Holick ,2007).

This disorder, known as rickets, causes a variety of skeletal abnormalities in young children who have insufficient mineral in their skeleton. Muscle weakness is another symptom of VDD with affected youngsters having trouble standing and walking (Holick,2006, Gordon *et al*.,2008), and the elderly experiencing increased sway and more frequent falls. As a result, the risk of fracture increases. Vitamin D Deficiency was linked to a nearly 3-fold greater incidence of Bacterial Vaginosis in pregnant women, based on the National Health and Nutrition Examination Survey data BV (Hensel *et al*.,2011).

The diagnosis of parathyroid dysfunction has gotten a lot easier since the emergence of reliable and precise PTH testing (Marx, 2000). Several investigations, however, have shown that PTH assays are not interchangeable (Cantort *et al*., 2006; Souberbielle *et al*., 2006). Furthermore, preanalytic

factors and method variations can have a negative impact on clinical decision-making. (Torres,2006; Cantort *et al*.,2006; Joly *et al*.,2008).Variability can be influenced by a number of factors, including the test employed, the population studied, vitamin D status, and a number of pre-analytical circumstances (Joly *et al.*,2008; Parent *et al*.,2008).

PTH also modulates fetoplacental calcium homeostasis and skeletal development in pregnant women (prentice, 2000;Kovacs and Fuleihan, 2006). As a result, it's critical to comprehend the factors that influence PTH levels during pregnancy. 25(OH)D, Dietary calcium and blood calcium have all been examined in relation to PTH (Green *et al*.,2008;Kramer *et al*.,2016). However, the available information regarding the effects of calcium supplementation on PTH is debatable, because of the lack of agreement on the recommended calcium intake during pregnancy (Hemmingway *et al*.,2018; Cormick *et al*.,2019). Other factors such as socioeconomic and lifestyle characteristics have previously been suggested to PTH levels can be predicted in non-pregnant adults, but data on pregnant women is few (Jääskeläinen, *et al*.,2013;Hysaj *et al*.,2021).

Furthermore, despite the fact that quantitative ultrasound (QUS) is a valuable, safe, and inexpensive technology, data on bone quality in the research on pregnant women and PTH is limited (Brandao *et al.*,2013; Hellmeyer *et al.*,2015).

2.12 Vitamin D :-

Vitamin D a lipid-soluble substance that promotes bone metabolism and helps maintain calcium and phosphorus balance. The role of vitamin D in extracellular health has received a lot of attention. When it comes to metabolism during pregnancy, there is a lot of research and disagreement about the impact of vitamin D levels on fetuses and neonates (Salle, 2000; Wagner, 2012).

Vitamin D deficiency is common in pregnant women all over the world (Wagner ,2012).

Vitamin D is important for bones well-being and plays a key role in the mineralization of bones. Vitamin D3 is essential to the maintenance of a constant relationship between calcium and phosphate ions, which are key factors in strengthening and protecting teeth. (Brito *et al.,* 2004). In human skin, solar rays facilitate the formation of vitamin D3 from 7-dehydrocholesterol. Vitamin D3 undergoes two-step hydroxylation to form 25-hydroxy vitamin D3 (25-OHD3) and biologically active 1,2-dihydroxyvitamin D3 (1,25-(OH)2D3) ((Suda *et al.,* 2003; Bikle, 2014).

Vitamin D insufficiency in pregnancy causes newborn vitamin D deficit, which increases the risk of rickets since newborn vitamin D reserves rely on maternal vitamin D levels (Dawodu *et al*., 2005). Vitamin D regulates calcium and phosphate equilibrium, as well as bone mineralization (Holick, 2003). Serum 25(OH)D levels of 80 nmol/L are now widely accepted as a marker of vitamin D insufficiency (Dawson-Hughesb *et al*.,2005). Vitamin D production in the skin is seasonal; vitamin D shortage is frequent in winter.1,25(OH)2D increases the development of antimicrobial peptides in respiratory epithelial cells, protecting the lungs against infection. It has been claimed that vitamin D insufficiency is a "seasonal stimulant," explaining pandemic influenza's exceptional seasonality (Cannell *et al.,* 2006; Bischoff-Ferrari *et al.,* 2006). The ideal vitamin D content throughout pregnancy and in newborn is still up for debate (Marshall *et al.,* 2013).

A serum level of 11 ng/mL is assumed to be enough to prevent rickets in babies, while a serum level of >30 ng/mL is needed to control secondary rickets. Hyper- parathyroidism and increased calcium absorption deficient values are less than 20 ng/mL, and the optimal concentration is >30 ng/mL (Holick *et al*., 2011). Vitamin D insufficiency was linked to issues with

embryonic bone formation and growth. (Cavalier *et al*., 2008). It may impair newborn skeletal development, bones growth, and insufficient bone mass development, and also cause osteomalacia during gestation, skeletal abnormalities, and fetus minerals bones acquisitions in infant (Dawodu & Akinbi, 2013; Viljakainen *et al.*, 2010).

Extraskeletal effects of vitamin D insufficiency in pregnancy includes an increased risk of gestational diabetes, hypertension, and maternal bacterial vaginosis, but not caesarean section delivery. Infants are more likely to experience intrauterine development retardation, low birth weight, and early-onset sepsis as a result of it. Furthermore, adequate vitamin D levels during pregnancy are thought to benefit both the mother and the child's immune systems. (Pooraziz, 2015; Aghajafari *et al.,* 2013).

Early-onset infection in term neonate can be prevented with enough vitamin D treatment during pregnancy (Cetinkaya *et al.*, 2014). Inadequate vitamin consumption, a lack of fortified foods, lifestyle, seasonal change, color of skin, low supplement complain, premature delivery, body covering, obesity, and living at high latitudes are all causes of vitamin D deficiency (Maghboli *et al.,* 2006; Hagenau *et al*., 2009).

In several countries, vitamin D insufficiency was documented in large proportions among pregnant women and babies. According to a large study, the occurrence of vitamin D insufficiency differs between 18 and 84 percent in different countries, depending on ethnicity, geography, culture, and customs. (Kovacs *et al.,* 2001). Measuring vitamin D insufficiency in Iran, and varied figures for pregnant women and neonate have been reported (Maghbooli *et al.*, 2007). Vitamin D insufficiency is rather common in healthy pregnant mothers and newborns. Vitamin D is involved in calcium and phosphorus metabolism, which is important for bones health, as well as the physiopathology of various

autoimmune diseases, therefore finding techniques to minimize vitamin D deficiency looks to be key (Hashemipour *et al.*, 2004).

The American Academy of Pediatrics recommends that all breastfed infants born to mother with vitamin D deficiency receive 400 IU/day of oral vitamin D supplementation, which should be continued until they reach the age of six. The dose must be 600 IU/day in situations of dark skin (Wagner & Greer, 2008). A healthy lifestyle should be encouraged, with outdoor activities and sun exposure, as well as the use of fortified foods (Dovnik *et al.*, 2014). In 90 percent of newborns, Shakiba found that a four-month gap in vitamin D sufficiency had clinical effects (Shakiba *et al.*, 2014).

Vitamin D is necessary for bones health it plays an important role in bone mineralization (Suda *et al.*,2006), Vitamin D deficiency in the mother causes vitamin D deficit in the baby, increasing the risk of rickets (Dawodu A *et al.*, 2005). A more recent evidence reveals that deficiency of vitamin D is linked to non-skeletal health effects in addition to impaired skeleton mineralisation (Holick, 2004; Pasco *et al*., 2008). Vitamin D is stored in the form of 25-hydroxyvitamin D (25(OH)D) and circulating plasma concentration of 25(OH)D is a good predictor of vitamin D level. There is no consensus on what constitutes appropriate vitamin D level, therefore, many cut-off points have been employed to determine degrees of deficiency: either a severe deficit of 12•5 nmol/l, an inadequacy of 50 nmol/l, or an inadequacy of 80 nmol/l (Lips,2004; Hollis, 2007; De Ronne and De Schepper, 2013).

The definitions and importance of vit D deficiency are still up for debate. 40 percent of European are vitamin D insufficient, with 13 percent having severe deficiencies, according to new big observed data (Cashman *et al*., 2016) It has been questioned if this widespread shortage is significant and whether supplement is necessary (Zhang *et al*., 2019)

This was also evident in the topic's meta-analyses, which was conducted with inadequate methodological standards (Amrein *et al.,* 2017). Low vitamin D levels are becoming more frequent around the world, and various research ranging from basic science to clinical application have found a strong link between chronic diseases and acute situations. Furthermore, pathological connections of vitamin D with energy balance and immunological and endocrine system modulation are accompanied by the huge amount of observational data presently available (Bouillon *et al.,* 2013). Vitamin D supplementation appears to ameliorate three poor pregnancy outcomes: 40 percent fewer preterm births, 50 percent fewer cases of gestational diabetic, and 60 percent fewer cases of preeclampsia (Rostami *et al.,* 2018).

These findings are in line with past research on the subject (Wagner *et al.,* 2009). Furthermore, similar to the genomic and epigenetic consequences of vitamin D supplementations, vitamin D insufficiency during pregnancy appears to be able to trigger certain genomic pathway linked to autoimmune illness in infancy and later life (Hossein-nezhad *et al.,* 2015; Novakovic *et al.,* 2009). Because The placenta, is like the kidneys, may convert 25(OH)D to the active form 1,25(OH) 2D, further basic study on the particular vitamin D metabolism in pregnancy is needed in the future (Hossein-nezhad *et al.,* 2012).

In 2016, conducted a comprehensive review of the relationship between vitamin D deficit during pregnancy and PTB, finding that pregnant women with vitamin D deficiency had a higher risk of PTB According to an update in 2017 update on this issue's comprehensive study (Qin *et al*., 2016; Zhou *et al*.,2017). Vitamin D supplementation alone during pregnancy can minimize the risk of PTB, whereas circulatory 25-OH D insufficiency in pregnant women can raise the risk of PTB. Despite systematic reviews examining the link between vitamin D deficit and PTB during pregnancy, no research has found a relationship between vitamin D insufficiency and PTB during different pregnancies.

Furthermore, high-quality meta-analysis is becoming more widely recognized as one of the most important strategies for obtaining evidences (Yao *et al*.,2016; Tian *et al.*,2017).

When exposed to UV radiation, the skin produces vitamin D3 (cholecalciferol), a fat-soluble steroid derived from 7-dehydrocholestrol. The hepatic enzyme 25-hydroxylase catalyzes the hydroxylation of vitamin D at position 25 to generate 25-hydroxyvitamin D, also known as calcidiol. Before reaching the kidneys, 25-hydroxyvitamin D is produced in the liver and enters the circulation, where it is bound to vitamin D binding protein. Two enzymes 1-hydroxylase and 24-hydroxylase found in tubular cells of the kidney can further hydroxylate calcidiol, resulting in 1,25(OH)2D, the most active form of vitamin D, or 24,25-dihydroxyvitamin D, an inactive metabolite (Dusso et al.,2005). As a result, the vitamin D hormonal system can take many different forms, ranging from cutaneous precursors through dietary components to the most active metabolite, 1,25(OH)2D, which regulates calcium homeostasis and bone health by acting on target organ receptors. The serum concentration of 25(OH)D, the precursor form of physiologically active vitamin D, is the most accurate indicator of total vitamin D storage or status (Holick ,2007). It has been related to an increased risk of preeclampsia, gestational diabetes, low birth weight, and PTB in both mothers and babies (Agarwal *et al.*, 2018).

Vitamin D Deficiency was found to reduce the link between smoking and BV in non-pregnant women. Disorders of the pelvic floor as people get older, illnesses of the pelvic floor, such as urine and fecal incontinence, are becoming more common. With a 30% failure rate, pelvic floor issues continue to be among the greatest prevalent indications for gynecologic surgeries. They were associated to osteoporosis and low BMD. Low levels of 25(OH)D are public in women, and lower level has been associated to an increased risk of pelvic floor disorders (Badalian and Rosenbaum, 2010) .

Complications of Vitamin D Deficiency Calcium, bone metabolism and phosphorus, are disrupted as a result of VDD. The absorption of dietary calcium and phosphorus is reduced by VDD, leading in an increase in PTH levels (Lips *et al*.,2006; Heaney, 2004).

Chapter Three

Materials and Methods

3. Material and Methods

3.1 Material

3.1.1 Laboratory Equipment and Instrument

The laboratory equipment and instrument used in the present study have been recorded in the table (3-1) below.

Table (3-1): Equipment and Instruments

No	Laboratory Equipment and Instrument	Manufacturer/Origin
1	Anaerobic jar	Fisher(UK)
2	Autoclave	Hirayama (Japan)
3	Benze burner	Amal (Turkish)
4	Centrifuge	Hettich (Germany)
5	Compound light microscope	Olympus(Japan)
6	Densichek Turbidity meter	Biomerieux (France)
7	Disposable and glassware	Afco(Jordan)
8	Distillator	Deltalab(Spain)
9	Electric sensitive balance	Sarteorius ag (Germany)
10	ELISA	BioTeck (USA)
11	Hematology Auto analyzer	Dymind (China)
12	Hot plate with magnetic stirrer	IKA (Germany)
13	Incubator	Memmert (Germany)
14	Inoculation loop	Himedia(India)
15	Micropipette	Dragon (china)
16	Refrigerator	Concord (Lebanon)
17	Safety hood	EuroClone (Italy)
18	VITEK 2	Biomerieux (France)

3.1.2 Chemical Materials Utilized in the Study

The biological and chemical materials that were used in the study have been itemized in the table below.

Table (3-2) Biological and Chemicals Material

No	Biological and Chemical Materials	Manufacturer
1	Glycerol ($C_3H_8O_3$)	Merck(England)
2	Gram stain	Spectrum (Germany)

3.1.3 Commercial kit

The commercial kits that were used in this study were illustrated in the table (2-3).

Table (3-3): Commercial kit

No	Type of Kit	Origin
1	Vitamin D Elisa	Monobind (USA)
2	PTH Elisa	Monobind (USA)
3	VITIK 2 ANC Card	Biomerieux (France)

3.1.4 Culture Media

Table(3-4) contains a list of the cultural media employed in this study.

Table(3-4): Culture Media and the Purpose of Use

No	Culture Media	Company(Origin)	Purpose of use
1	**De man, Rogosa and Sharpe (MRS) agar & MRS broth**	Himedia(India)	For *Lactobacillus* spp isolation
2	**Muller-Hinton Agar (MHA)**	Himedia(India)	Antibiotic susceptibility

3.1.5 Antibiotic Discs

Table(3-5) provides a list of the antibiotics utilized in this study.

Table (3-5): Antibiotic Discs, Symbols and Potency

No	Anti- biotic disc	Symbols	µg/disk	Manufacture /Origin
1	Cefixime	CFM	5	Bioanalyse (Turkey)
2	Cefepime	FEP	10	
3	Nitrofuranation	F	100	
4	Amoxicillin/ Clavulanic acid	AMC	30	
5	Amoxicillin	AX	25	

3.2 Study Design

Study design shown in figure (3-1).

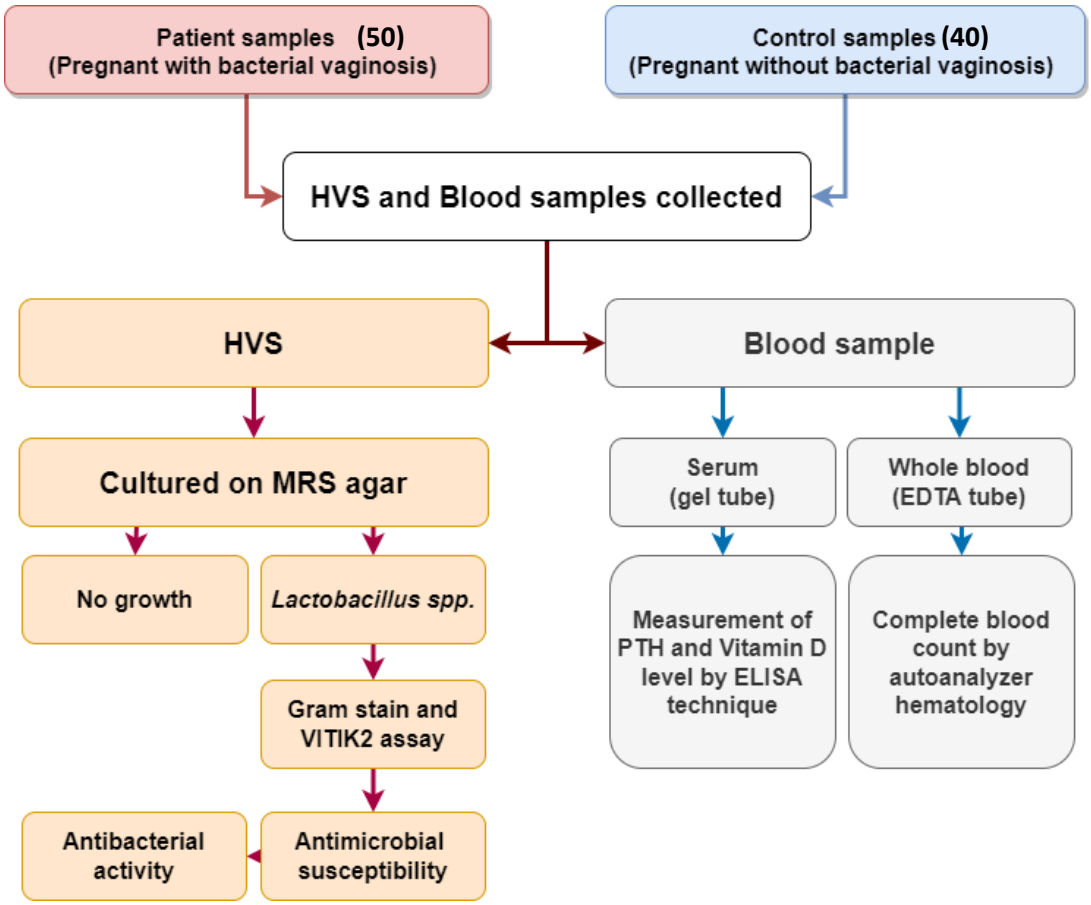

Figure (3-1): Study design

3.3 Methods

3.3.1 Specimens Collection

Ninety High vaginal swab (HVS) and blood sample were collected during four months from November 2021 until February 2022, they were collected from admitted patients to Imam Ali general hospital and private clinics, HVS swab collected using sterile cotton swab without media, and 4 ml of blood were collected and separated into two tubes, 2 ml for each tube, one tube with anticoagulant (Ethylene diamine tetra acetic acid (EDTA)) were used to get whole blood sample and the other was gel tube to get serum after centrifugation.

Samples were distributed as (50) sample from first trimester pregnant women suffering from bacterial vaginosis, and (40) from healthy first trimester pregnant women as control group, based on the Amesl criteria (Thin, gray/white vaginal discharge, Malodorous “fishy” discharge upon adding 10% potassium hydroxide, High vaginal pH (>4.5), Identification of vaginal epithelial cells heavily coated with bacteria (clue cells)) (DeCherney *et al*., 2013).

3.3.2 Culture Media Preparation and Sterilization Technique

3.3.2.1 De man, Rogosa and Sharpe (MRS) agar

MRS agar was used to isolation of lactic acid bacteria and it was prepared as in the following procedure:

To completely dissolve the medium, 65.13 grams had been mixed with 1000 ml of distilled water and heated to boiling. After that, it was autoclaved at 15 lbs of pressure (121° C) for fifteen minutes before being permitted to cooling to 45–50° C. Poured onto sterilized Petri plates after thoroughly mixing.

3.3.2.2 De man, Rogosa and Sharpe (MRS) broth

MRS broth was used for activation of lactic acid bacteria and it was prepared as in the following procedure:

54.3 grams were suspended in 1000 ml of distilled water, heated until completely dissolved and autoclaved at 121ºC for 15 minutes. Mixed well and poured into sterile test tubes.

3.3.2.3 Muller-Hinton Agar (MHA)

MHA were used for antibiotic susceptibility and antibacterial activity and prepared in the following procedure:

To completely dissolve the medium, 38.0 grams had been placed in 1000 ml of distilled water and then heated to boiling. After that, it was autoclaved at 15 lbs of pressure (121° C) for fifteen minutes before being left to cool to 45–50° C. Poured onto sterilized Petri plates after thoroughly mixing.

3.3.3 Preparation of Solution

3.3.3.1 Normal Saline Solution

Suitable to use sterile for all tests and investigations that required it, normal saline was utilized.

3.3.4 Preparation and method Gram stain

Gram stain was provided from spectrum company ready to use. The solution was used to study Gram stain reaction and their arrangement (Forbes *et al.*, 2007). Gram stain of isolates done using the following procedure:(Smith and Hussey, 2005)

- A cells smears that had been air-dried, heat-fixed, and flooded for 1 minutes with crystals violet staining solution.
- Slides cleaned for two seconds in a light, indirect stream of water.
- Slides covered with Gram's iodine, a mordant. one minutes of wait
- The slide washed for two seconds in a light, indirect spray of water from the faucet.
- Decolorizing agent drenched the slide. fifteen second wait
- Slides covered with safranin and counterstain. waiting between 30 and 60 secs. Slides should then be gently and indirectly washed with tap water, then dried with absorbent paper.
- A microscope used to see the outcomes of the staining technique while submerged in oil.

3.3.5 Laboratory Diagnosis

3.3.5.1 Isolation and Identification of Bacterial Isolates

All vaginal swabs were cultured on MRS agar and MRS broth under anaerobic conditions at 37 °C for 24- 48 hours. If a colony appeared, it was recognized based on it's own morphological (colony form, shape, colour, boundaries, type of pigment, elevations, and textures), and after being stained with Gram's stains, it was inspected using a light microscope, Isolates with growth of *Lactobacillus spp*. Stored in glycerol 40%. (Rogosa and Sharpe,1963; Winn *et al*. , 2006).

3.3.5.2: VITEK 2 assay

Isolated *lactobacillus spp*. was identified using VITEK2 ANC card for further identification by adding isolated colonies from a plate to 3 ml saline in clear plastic test tube to reach density equivalent to a McFarland No. 2.70 to 3.30 (according to manufacture instructions) metered by DensiCHEK Plus, to run the assay in VITEK2, final result appeared after approximately 6 hours.

3.3.5.3 Maintenance and preservation of isolated microorganism

This procedure was done according to (Harely and Prescott, 2002) as follows:

3.3.5.3.1 Short-term preservations

Isolate had been subcultured on the surfaces of suitable agar media after being kept for a few weeks. Parafilm was used to firmly wrap the plates, and they were kept at 4 °C.

3.3.5.3.2 Long-term preservations

By adding 0.4 ml of sterile glycerol to 2 ml of an exponentially bacterial growth in a sterile Micro- centrifuge tube and storing the mixture at -20 °C, isolate have been able to be kept for an extended amount of time in MRS broth media, which contains 20 percent glycerol.

3.3.6 Preparations of Bacterial Inoculum

MRS broth was used to activate the isolate for 18 hours at 37°C, and the proliferation were regulated to 0.5 McFarland using Densicheck device.

3.3.6.1 Antibiotic susceptibility test

In accordance with guidelines from the Clinical and Laboratory Standards Institute, the in vitro susceptibility of lactobacillus isolated to five antimicrobial drugs often prescribed by gynecologists to pregnant women was assessed using the disc - diffusion technique (CLSI, 2017). Using a clean cotton swab, bacterial inoculum was applied to Muller Hinton agar (MHA). In order to fully touch the agar inoculated with bacteria, antibiotic disks were put over MHA and carefully pushed down to achieve thorough contact. Following 24 hours. of incubation at 37°C, the diameter of the inhibition area in millimeters (mm). Results were interpreted as either sensitive or resistant in accordance with (CLSI, 2017).

3.3.6.2 Cell-Free Supernatants (CFS) preparations

Cell-free *lactobacilli* supernatant had been grown in MRS medium for 24 hours at 37 C. After that, the culture had been centrifuged for 30 minutes at 4 C and 4000 rpm. Filtering the supernatants via a 0.22 mm filter sterilized them (Millipore, Billerica, MA, United States).

3.3.6.3 Well Diffusion Assay

To identify antibacterial properties of supernatant isolated from Lactobacillus isolated, the agar disc diffusion technique (Tagg and McGiven, 1971) was adapted. First, Bacterial Suspension 0.5 McFarland have been swabbed on the surfaces of MH agar plates. Cell-free supernatant from isolated lactobacilli were then put into 6 mm diameter wells (100 l/well), and after a 24- hours incubated at 37°C, inhibitory zone was noted.

3.3.6.4 Preparation of Bacteriocin

By centrifuging *Lactobacillus* cultures cultured in MRS medium at 37 C for 24 hours at 10,000 grams, 10 minutes, cell free cultures supernatant (CFCS) have been created.

3.3.6.5 Antibacterial activity

Utilizing the well diffusing technique described earlier, the antibacterial activity of the *Lactobacillus spp.* isolate strain against certain pathogenic bacteria was evaluated (Davoodabadi *et al*.,2015b). Bacterial strains have been injected into nutritional broth, comprising *E. coli* and *S. saprophyticus*. The bacterial concentration was increased to 0.5 McFarland after an incubation time at 37°C. use Densicheck and Muller Hinton agar for cultures. The Muller Hinton agar plate were therefore pierced with 8 mm diameter wells, and 100 mL of the CFCS were added to every well. The plate has been then incubated at 37 C for 18–24 hours. Therefore, the well's surround growth-free inhibitory zones were used to test the antibacterial activity.

3.3.7 Parathyroid hormone ELISA assay

3.3.7.1 Principles:

Method of Sandwich Equilibrium (TYPE 2), High affinity and specificity antibody (enzyme and immobilization), with varied and unique epitope identification, in excess, and native antigens are the key reagent needed for an immune-enzymometric test. In this method, the x-PTH antibodies (C terminal epitope) covered on the well interacts with the assays via immobilization at the surface of a microplate well. The native antigen and the antibodies react without competition or steric hindrance to create a sandwich complex when the enzyme-labeled antibodies (N-terminal epitope) is combined with a serum containing the native antigens.

The antibody-bound fractions are isolated from unbound antigens using decantation or evacuation after enough time has passed. The native antigens concentrations directly correlate with the enzymatic activity in the antibody-bound fraction. A dosage response curve may be produced and used to determine the antigens concentrations of an unknown by using many distinct serum standards for known antigens value.

3.3.7.2 Standard curve and result calculation

- Standard curves were plotted using Microsoft Excel by plotting absorbance on vertical axis (Y-axis) versus concentration on horizontal (X-axis) in pg/ml. (figure 3.2)
- Absorbance of unknown sample were located on vertical axis to find the concentration on horizontal axis.

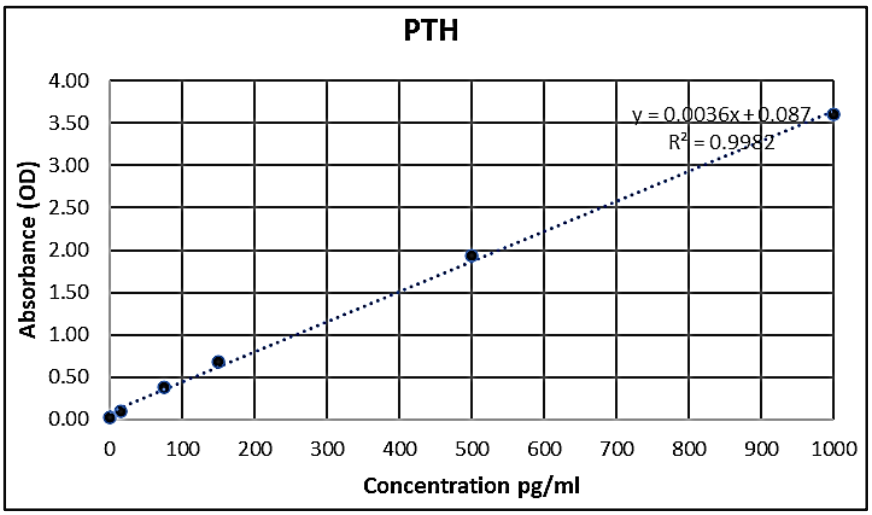

Figure (3.2) Parathyroid hormone standard curve

3.3.8 Vitamin D ELISA assay

3.3.8.1 Principle

Sequential Competitive Method: Immobilized antibodies, enzyme-antigen combination, and natural antigen are necessary components for a solid state sequence enzyme - linked immunosorbent assay. Native antigens and a small number of insolubilized binding sites bind together when immobilized antibody is combined with whole blood that contains the native antigen. The enzyme-conjugated antigens is introduced after any unreacted native antigen has been removed during a wash phase. The compound interacts with antibodies regions that the natural antigens did not occupy.

The antibody-bound fractions and unattached antigens are isolated by decantation or evacuation following a brief second incubation. The natural antigens concentrations and the enzymatic activities in the antibody-bound

fractions are inversely related. A dose response curve may be produced and used to determine the antigens concentrations of an unidentified by using a variety of calibrators with known antigens concentrations.

3.3.8.2 Standard curve and result calculation

- Standard curve were plotted using Microsoft Excel by plotting absorbance on vertical axis (Y-axis) versus concentration on horizontal (X-axis) in pg/ml. (figure 3.3)
- Absorbance of unknown sample were located on vertical axis to find the concentration on horizontal axis.

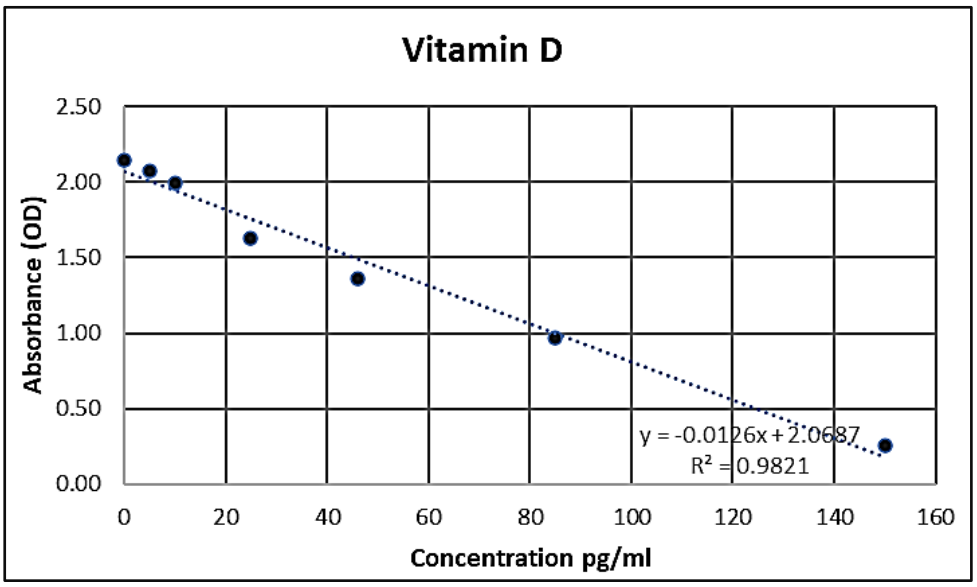

Figure (3.3) Vitamin D standard curve

3.3.9 Complete Blood Count (CBC) assay

Whole blood sample in EDTA tube was used immediately to get complete blood count using automated 3-part hematology autoanalyzer, samples were swirled several times to mix the sample and then processed in the autoanalyzer to get result within 60 seconds, result printed out and recorded.

3.4 Ethical approval

1- The study was done, and the cases were collected after getting the agreement of the patients (verbal acceptance).
2- Approval of the ethical committee of Babylon Science for Women's College.
3- The Babylon Health Department gave authorization before the trial began.

3.5 Statistical analysis

Results of the present study were illustrated as means ± standard deviation (SD). The values were statistically analyzed by using SPSS 20 program and analysis of variance were explained. The lowest significant difference (LSD) was ($P<0.05$) (Daniel, 1999).

Chapter Four

Results

4.1 General demographical data of pregnant women

Table (4-1): General demographical data of pregnant women

Parameter		Groups of study	
		BV **F (%)**	non-BV **F (%)**
Age	Mean	27.34	25.50
	S.D	6.886	6.312
	16-25	25 (50%)	21 (52.5%)
	26-35	20 (40%)	15 (37.5%)
	36-45	5 (10%)	4 (10%)
	Total	50 (100%)	40 (100%)
Type of delivery	Normal delivery	28 (56%)	19 (47.5%)
	Cesarean	9 (18%)	8 (20%)
	Both	3 (6%)	3 (7.5%)
	No previous birth	10 (20%)	10 (25%)
	Total	50 (100%)	40 (100%)
No. of previous births	0	10 (20%)	10 (25%)
	1	14 (28%)	8 (20%)
	2	4 (8%)	12 (30%)
	3	8 (16%)	4 (10%)
	4	5 (10%)	3 (7.5%)
	5	5 (10%)	2 (5%)
	6	4 (8%)	1 (2.5%)
	Total	50 (100%)	40 (100%)
Residence	Urban	27 (54%)	18 (45%)
	Rural	23 (46%)	22 (55%)
	Total	50 (100%)	40 (100%)

F: Frequency

Table (4-1) showed that 25 (50%) of BV group and 21 (52.5%) of non-BV group age were between (16-25) years, while 20 (40%) of BV group and 15 (37.5%) of non-BV group age between (26-35) years, the lowest percentage in BV group was 5 (10%) and non-BV group 4 (10%) age were (36-45) years.
The highest frequency of delivery type in BV and non-BV groups were normal delivery 28 (56%) and 19 (47.5%) respectively, the frequency of other types of delivery (Cesarian, Both, no previous birth) in BV group were 9 (18%), 3 (6%), and 10 (20%) respectively, and for non-BV group were 8 (20%), 3 (7.5%), and 10 (25%) respectively.

Regarding the number of previous births the highest frequency in BV group (14 (25%)) was single previous birth, while in non-BV group (12 (30%)) was two previous births, other previous births in BV group were ranging from 2-6 previous births with frequency 4 (8%), 8 (16%), 5 (10%), 5 (10%), and 4 (8%) respectively, and for non-BV group were ranging from 1-6 with frequency 8 (20%), 12 (30%), 4 (10%), 3 (7.5%), 2 (5%), 1 (2.5%) respectively.

For the residency place the highest percentage in BV group was urban 27 (54%), then rural 23 (46%), while in non-BV group the highest was rural 22 (55%), then urban 18 (45%).

4.2 Relation of Body Mass Index (BMI) with Bacterial Vaginosis

Results that explained in the table (4-2) shows that the body mass index of pregnant women with an overweight than normal is more susceptible to infection with bacterial vaginosis

Table (4-2): Percentage of body mass index of study groups.

BMI	Groups of study		P-value
	BV	Non-BV	
Healthy	35 (70%)	33 (82.5%)	0.083
Overweight	15 (30%)	7 (17.5%)	
Total	50 (100%)	40 (100%)	

Table (4-2) showed that 35 (70%)of BV group BMI were within Healthy categories, while 33 (82.5%) of non-BV group BMI were within Healthy categories, and the overweight was 15 (30%) in BV group and 7 (17.5%) in non-BV group.

4.3 Identification of bacterial isolates with VITEK2

Table (4-3) showed no significant difference between the study groups according to VIKEK2 identification of *Lactobacillus* spp. and shows two types of *Lactobacillus, L. gasseri, L. casei.*

Table (4-3): Result of identification on bacterial isolates with VITEK2 in BV and non-BV groups

Vitek2	Groups of study				P-value
	BV		Non-BV		
	F	%	F	%	
L. casei	3	6.0	11	27.5	0.769
L. gasseri	4	8.0	19	47.5	
No growth	43	86.0	10	25.0	
Total	50	100.0	40	100.0	

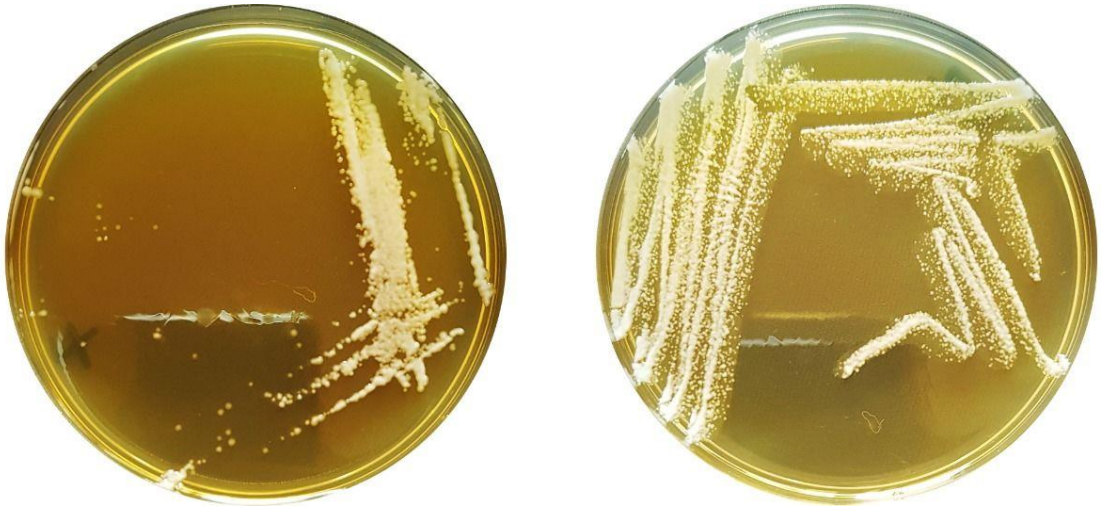

Figure (4-1): Anaerobic bacterial isolates on MRS

4.4 Antibiotics susceptibility of Lactobacillus

Figures (4-2) and (4-3) in these figures to some antibiotics were used to show the effects on *Lactobacillus* species in healthy women and women with vaginosis

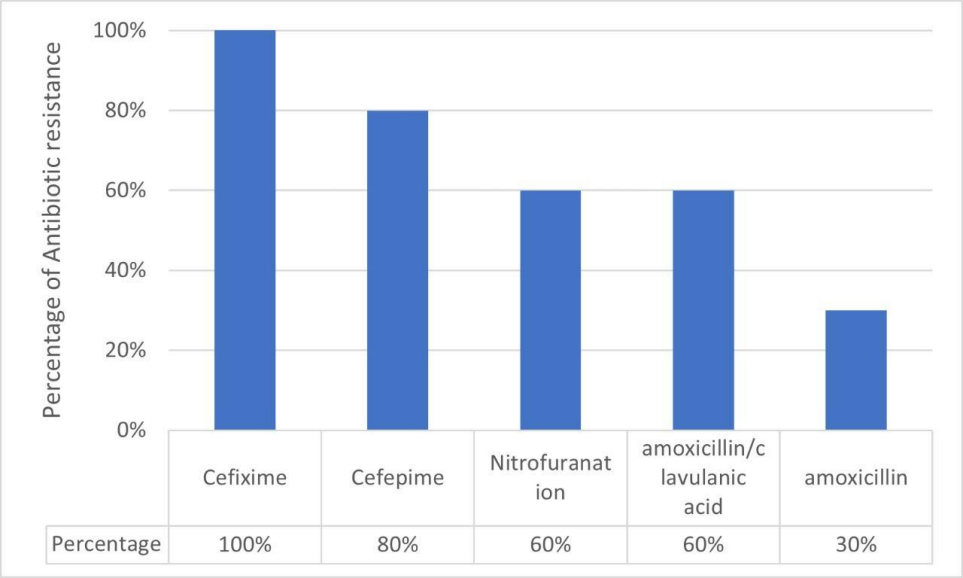

Figure (4-2): Antibiotic Resistant among *Lactobacillus gasseri* Isolates in healthy pregnant women

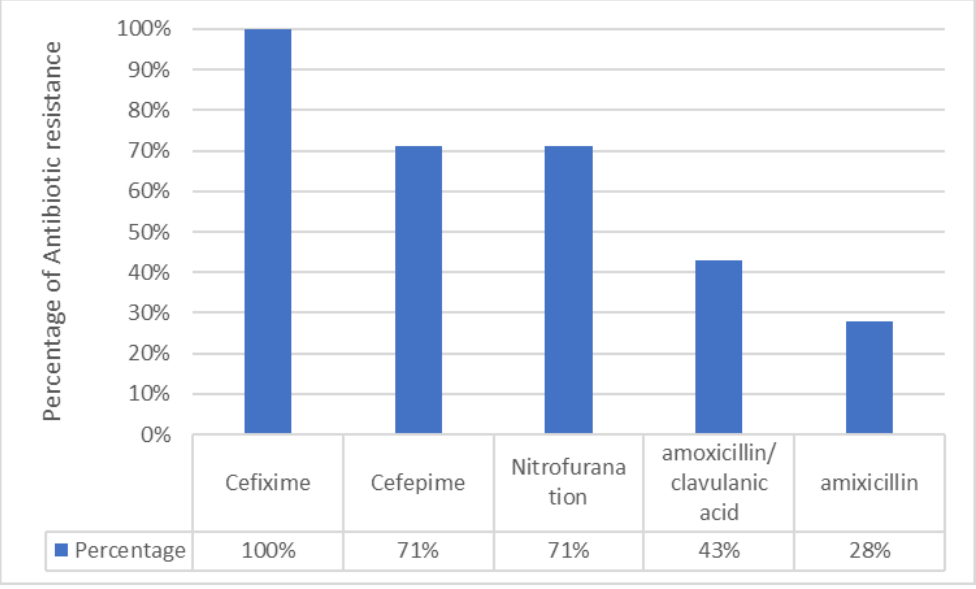

Figure (4-3): Antibiotic Resistant among *Lactobacillus gasseri* Isolates in pregnant with bacterial vaginosis

Figure (4-4): Antibiotics susceptibility of *Lactobacillus gasseri* on Muller Hinton agar

4.5 Result of Antibacterial Activity

In table (4-4) the results showed that the CFS of *Lactobacillus gasseri* from healthy, where in women were more effective against the pathogenic bacterial isolates than the CFS of Lactobacillus spp. from women with vaginosis

Table (4-4) Antibacterial activity

Pathogenic bacteria	*Lactobacillus gasseri* (Isolated from BV)	*Lactobacillus gasseri* (Isolated from non-BV)
E. coli	**14mm**	**13mm**
S. aureus	**19mm**	**22mm**

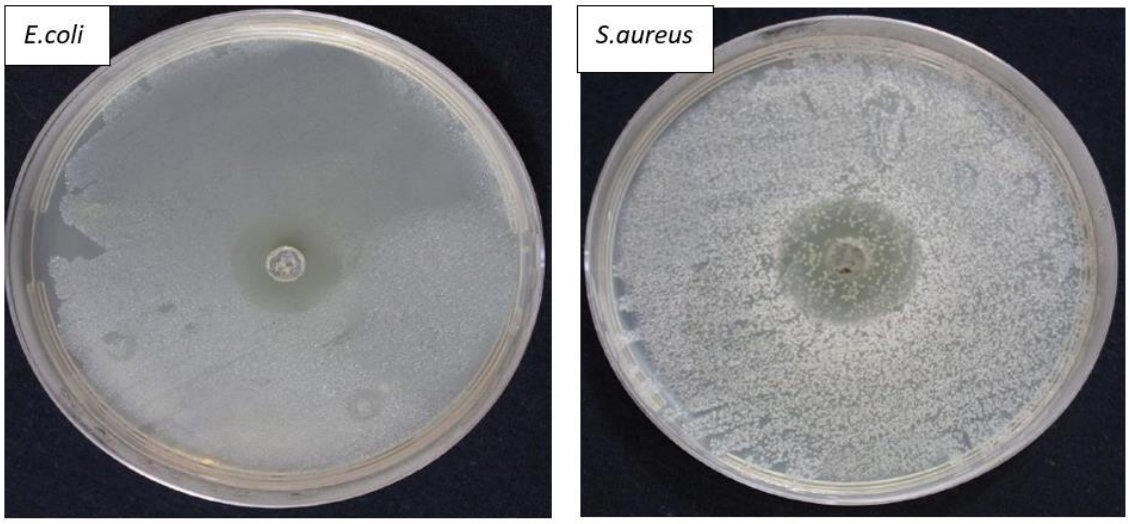

Figure (4-5) Antibacterial activity of *Lactobacillus gasseri*

4.6 Level of Vitamin D in BV and non-BV pregnant women

Statistical analysis reveals a highly significant differences at level P<0.05 in the level of Vitamin D between BV (19.91±11.2) during first trimester of pregnant women and non-BV(33.7±14.8). Table (4-3) showed the level of vitamin D in women with BV and non BV.

Table (4-5): Level of vitamin D in BV and non-BV groups

Vitamin D (pg\ml)	Groups of study		p-value
	BV	Non-BV	
Mean	19.91*	33.71	**0.001**
S.D	11.202	14.89	**H.S**

4.7: Level of Parathyroid hormone in BV and non-BV pregnant women

The table below shows there is significant increase at level P<0.05 of parathyroid hormone in pregnant women in the first trimester with bacterial vaginosis (23.7±13.6) compared to healthy women (26.5±10.9).

Table (4-6): Level of Parathyroid hormone in BV and non-BV pregnant women

PTH (pg\ml)	Groups of study		p-value
	BV	Non-BV	
Mean	32.7240*	26.5025	0.021
S.D	13.58816	10.89355	Sig

4.8 The total number of White blood cells in BV and non-BV groups

The figure (4-6) below shows the white blood cell count compared to pregnant women infected with bacterial vaginosis, where there was a significant increase (P=0.01) in infected women (7.77±2.74) compared to healthy ones (6.47±1.64).

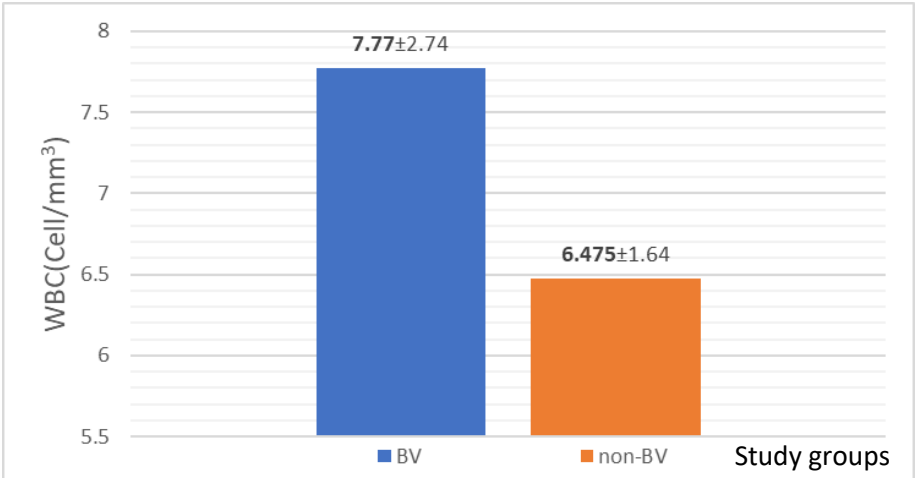

Figure (4-6) The total number of White blood cells (WBcs cells\mm^3) in patient with BV and healthy control.

4.9: Packed Cell Volume (PCV) value in BV and non-BV groups

The figure (4-7) shows the level of packed cell volume were there is no significant deference (P=0.104) between pregnant women with bacterial vaginosis (36.9±4.35) compared to healthy women (35.9±2.91).

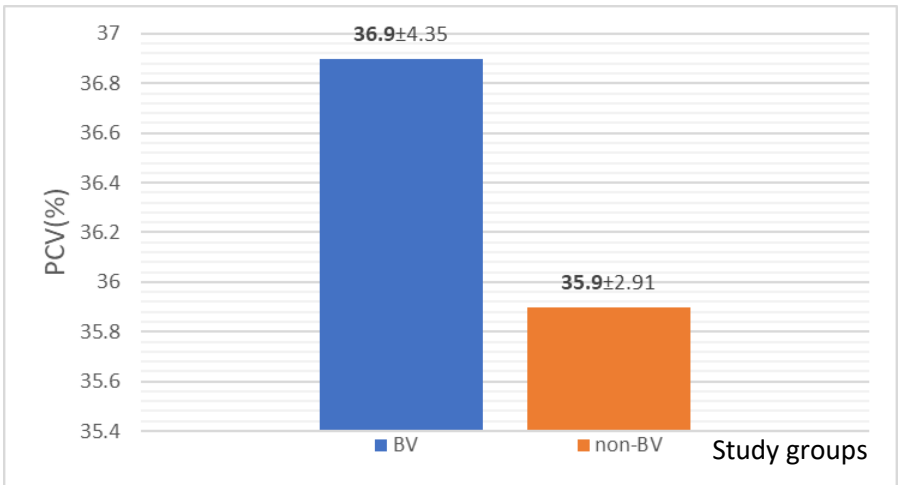

Figure (4-7): The level of Packed cell volume (PCV%) in patients with BV and healthy control.

4.10 Platelet count in BV and non-BV groups

The figure (4-8) below shows a significant increase (P=0.009) in the number of blood platelets in pregnant women in the first trimester with bacterial vaginosis (230.24±77.6) compared to healthy women (191.72±53.39).

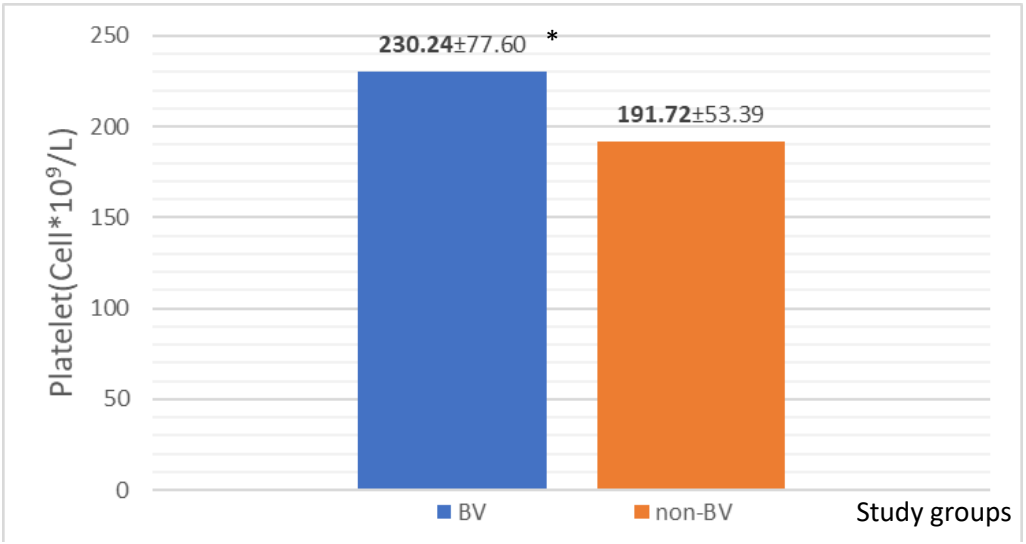

Figure (4-8):The level of blood platelets (cells\mm^3) in patients with BV and those healthy control.

‘

4.11 Correlation between demographical data and measured parameters in BV group

Table (4-7) showed significant the relationship between all parameters in bacterial vaginosis

Table (4-7): correlation between demographical data and measured parameters in BV group

		BMI	Vit.D	PTH	WBC	Hb	PLT
Age	Pearson Correlation	.667**	-.331-*	.351*	.050	.438**	.025
	Sig. (2-tailed)	.000	.019	.012	.729	.001	.863
BMI	Pearson Correlation		-.231-	.262	-.043-	.418**	-.069-
	Sig. (2-tailed)		.107	.066	.768	.003	.635
Vit.D	Pearson Correlation			-.519-**	-.284-	.202	-.266-
	Sig. (2-tailed)			.000	.076	.210	.098
PTH	Pearson Correlation				.176	.005	.193
	Sig. (2-tailed)				.276	.975	.234
WBC	Pearson Correlation					.226	.981**
	Sig. (2-tailed)					.160	.000
Hb	Pearson Correlation						.312
	Sig. (2-tailed)						.050
**Correlation is significant at the 0.01 level.							
*Correlation is significant at the 0.05 level.							

The table (4-6) showed there was a significant positive correlation between age and BMI (r=0.667), age and PTH (r=0.351), age and Hb (r=0.438), BMI and Hb (r=0.418), and between WBC and platelet (r=0.981), while there was a significant negative correlation between age and vitamin D (r=-0.331), and between vitamin D and PTH (r=-0.519),

Figure (4-9) showed there was a significant negative correlation (r=-0.519,sig=0.001) between vitamin D and parathyriod hormone.

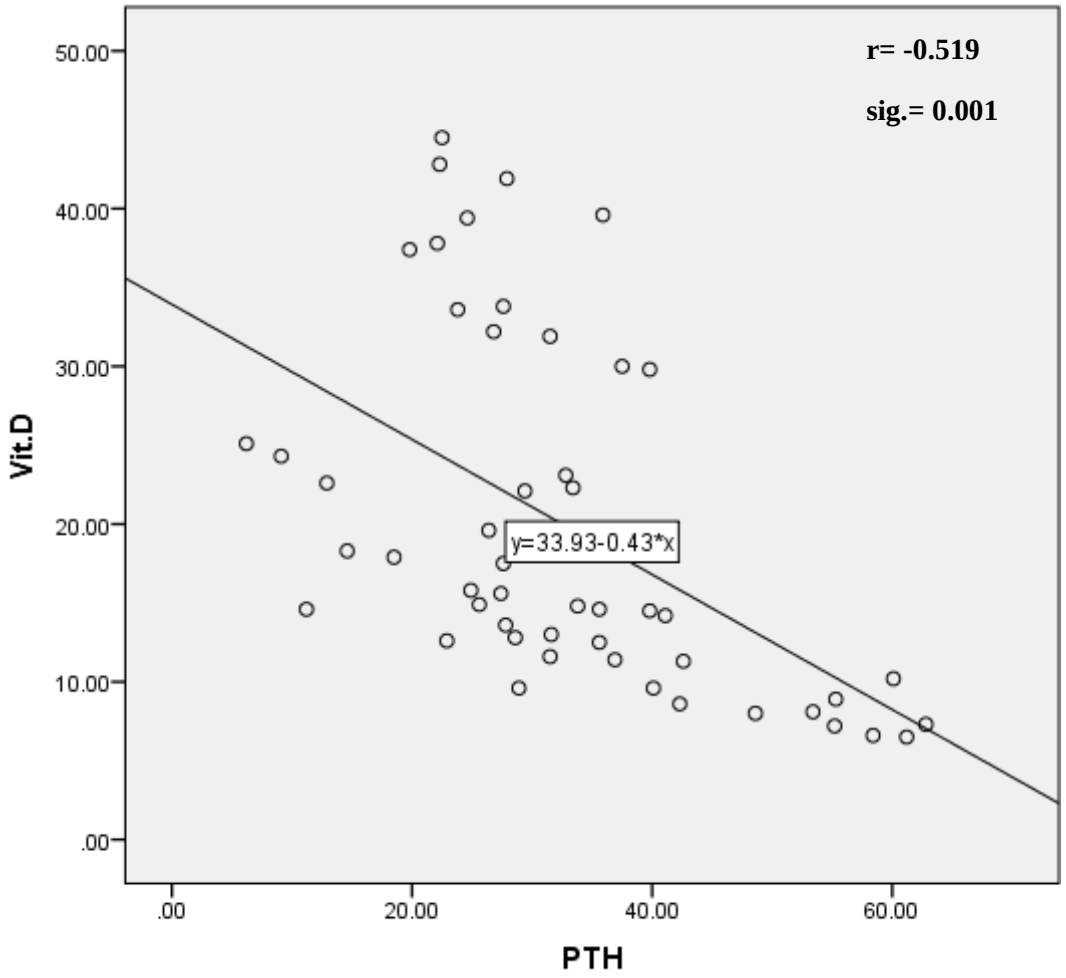

Figure (4-9) The correlation coffecients occurring between PTH and Vit-D in patients with BV.

Chapter Five

Discussion

5.1 Bacteriological study

5.1.1 Isolation and Phenotypic Identification of *Lactobacillus spp.*

The female genital tract is dominated by *Lactobacillus* spp. in approximately 70% of women (Petrova *et al*.,2015) . *Lactobacillus* species play an essential role in maintaining the ecosystem of the vagina *Lactobacillus* (Amabebe and Anumba,2018). us has proven to be efficient in treating BV by producing organic acids, hydrogen peroxide, bacteriocins, and adhesion inhibitors (Tachedjian *et al*.,2017). Furthermore, the presence of *Lactobacillus* alongside other opportunistic pathogens could be due to a number of factors, including antibiotic effects, incubation type (as some *Lactobacillus* species are unable to produce certain defense factors under anaerobic incubation), and antagonism among *Lactobacillus* species to maintain dominance (Razzak *et al*., 2011).

From the results, the *lactobacillus* was isolated on MRS agar (7) isolate from BV group and (30) isolate from Non-BV group and other the plates from both groups no *lactobacillus* bacteria growth was observed . *Lactobacillus* colony morphology on MRS agar was examined under the microscope and revealed a circular, big, smooth, glistering, white, colorless, and creamish colony showed in figure (4-1). After incubating, fungi *Candida albicans* growth was observed in some plats.

Lactobacillus was isolated from pregnant women with Bacterial Vaginosis and non-Bacterial Vaginosis in the first trimester and was isolated anaerobically.. Sexual activity is linked to bacterial vaginosis (Koumans *et al*., 2007). *Lactobacillus* has the capacity to protect the vaginal area from disease, which is why it is now employed as a probiotic (Andreu, 2004). *Lactobacillus* species dominate normal vaginal flora in the vast majority of cases. Normalcy can be maintained in the absence of *lactobacilli* by other, more rabid lactic acid generating bacteria (Lamont *et al.*, 2011).

5.1.2 Direct rapid identification of *Lactobacillus sp.* By VITEK 2 compact.

The VITEK2 technology is a simple and quick procedure, and the results demonstrated that isolated strains may be used bacterial species diagnosis (Al Masoudi & Algboory, 2021). The table (4-3) Result findings revealed two Lactobacillus species were found they were *Lactobacillus casei* and *Lactobacillus gasseri.*

The *L. gasseri* was the dominant species in healthy women group and BV group in this study (47.5% and 8% respectively) in comparison to *L. casei* (27.5% and 6% respectively) and the remaining samples (25% and 86% respectively) were didn't grow. Unlike this study (Mahmood & Hameed, 2018) found that *Lactobacillus casei* was shown to be the most common Lactobacillus species in healthy women's vaginal samples.

Other researchers discovered *Lactobacillus* spp. in vaginal samples (Mohammed & Kadhim., 2020) *Lactobacillus acidophilus, L. crispatus, L. gasseri,* and *L. iners* were the vaginal *Lactobacillus* species studied in this study. *Candida albicans, Gardenella vaginalis, Staphylococcus aureus,* and *Escherichia coli* were among the urogenital pathogens isolated from vagina.

5.1.3 Antibiotics Susceptibility of *Lactobacillus spp*.

In healthy women and women with vaginosis, antibiotics were tested to see how they affected *Lactobacillus* species. In this study, five antibiotics (Cefixime CFM(5), Cefepime FEP(10), Nitrofurantoin F(100), Amoxicillin/Clavulanic acid AMC(30), and Amoxillin AX(25) were used to investigate the effects of antibiotics on Lactobacillus isolates that gave positive pure cultures from women suffering from vaginitis and healthy women , these antibiotics selected based on the most common antibiotics administrated by gynecologists for treatment of bacterial infections in pregnant women . The results showed that all *Lactobacillus* isolates (10 isolates) from non-BV group and (7 isolates) from

BV group were resist to Cefixime, for other antibiotics Cefepime, Nitrofurantoin, Amoxicillin/Clavulanic acid and Amoxillin the results for resistance were (80%, 60%, 60%, and 30% respectively) for non-BV group and (71%, 71%, 43%, 28% respectively) for BV group showed in figure (4-2) and figure (4-3). Showed Figure (4-4): Antibiotics susceptibility of on Muller Hinton agar Where was the presence of inhibition zone indicated on sensitivity to antibiotics and absence indicates antibiotic resistance

The high rates of recurrence of BV after treatment with currently approved antibiotics has always been a source of contention. These findings could be explained by one of two ways. To begin, BV-associated bacteria and multispecies BV biofilms may be implicated in the disease's possible pathogenic process (Swidsinski *et al.,*2014). When mutations or intrinsic resistance mechanisms are responsible for the resistance phenotype, antibiotic resistance in *Lactobacillus* does not indicate safety concern. Indeed, some probiotic strains with inherent antibiotic resistance may be advantageous for re-establishing the microbiota of the host after antibiotic treatment (Gueimonde *et al*., 2013). Abdul – Sattar *et al*., (2011) isolated 22 *Lactobacillus* isolates from healthy women's vagina and studied the effects of drugs on *Lactobacillus* isolates, finding that all isolates were resistant to Metronidazole, the most generally used antibiotic for bacterial vaginosis treatment. These findings suggest that certain strains could be used for therapy restoration in conjunction with antimicrobial bacterial vaginosis treatment and resistance to Ciprofloxacin, as well as Nalidxic acid, which aids in the selection of proposed probiotics that patients should take during antimicrobial therapy .

The effect of antimicrobial therapy on *Lactobacillus* was studied by Razzak *et al*., (2011), and it was discovered that the antibiotic had an observable effect against the presence of Lactobacillus; these antibiotics can indiscriminately destroy both beneficial and pathogenic bacteria in the body; also, antibiotics can

cause a microflora imbalance with very negative consequences, as they cause the microflora to remove from epithelial cells of the vagina, thus allopathogenic. As a result, patients' usage of antibiotics to treat vaginitis should be highly selective in order to avoid killing *lactobacilli.*

Although antimicrobial agents are generally effective at eradicating the infection, there is a high incidence of recurrence, which affects the patient's quality of life, and many women become frustrated by the cycle of reported antimicrobial agents, whose effectiveness is diminishing due to the increasing development of microbial resistance, in addition, the use of antimicrobial agents can disrupt the body's balance by killing off beneficial bacteria (Reid, 2008) .

5.1.4 Antibacterial Activity

In this study the antibacterial activity of *Lactobacillus spp*. CFS was done and the results showed that the CFS of *Lactobacillus spp*. from healthy women were more effective against the pathogenic bacterial isolates than the CFS of *Lactobacillus* spp. from women with vaginosis, table (4-4). Showed Figure (4-5) Antibacterial activity of *Lactobacillus gasseri*

The vaginal microbiome is incomplete without vaginal *Lactobacilli*. The vaginal microbiota is thought to protect against opportunistic infections. Probiotic *Lactobacillus*, for example, can effectively treat bacterial vaginosis. *Lactobacillus vaginalis* supports the vaginal epithelium's innate immune activity (Ventolini & Sawyer, 2015).

The mechanisms by which *Lactobacillus* controls the colonization of other bacterial species in the vagina decide whether or not probiotics can be used to treat vaginal dysbacteriosis in combination with other pathogenetic or aetiological therapies (Kovachev & VatchevaDobrevska, 2014; Deidda *et al.*, 2016).

Mahmood & Hameed, 2018, found that the ability to produce bacteriocin (as antibacterial) appears to be rare among *L. casei* isolated from vagina, and the agar well diffusion assay and disc method were considered sufficient for detecting bacteriocin production, indicating that bacteriocin production was best in broth medium compared to solid medium. The highest activity was observed against *C.urealyticum* and *S.aureus*, with no activity observed against *E.coli* and *Ps.aeruginosa. Lb.casei* was the greatest producer isolate, producing it in the broth media (MRS) used in this study.

Lactobacillus plays an important role in maintaining vaginal health by producing defense factors, some of which have an inhibitory effect on opportunistic pathogens (*S. aureus, E. coli)*. This fact was confirmed by Ronquist *et al*.(2006) who reported that the normal vagina of reproductive age women is predominately colonized with *Lactobacillus*, which produce hydrogen peroxide, bacteriocins, and lactic acid. When the amount of *Lactobacillus* bacteria decreases, the pH rises, favoring an overgrowth of anaerobic and facultative bacteria, which can lead to virginities and vaginosis.

The *G. vaginalis* growth was suppressed by vaginal and probiotic *Lactobacillus* strains in a strain-specific manner. (Teixeira *et al*., 2012) reported that metronidazole-resistant *G. vaginalis* strains were less responsive to *Lactobacillus spp*. inhibition . Happel *et al*., (2020) studied the probiotic properties of a variety of vaginal *Lactobacillus* strains from South African women. The probiotic potential of these isolates was discovered to be strain-specific, with vaginal *Lactobacillus* strains outperforming probiotic strains already utilized in vaginal health probiotics marketed internationally and in South Africa. The development of a probiotic for vaginal health is now being considered using some of the best-performing, sequenced vaginal *Lactobacillus* isolates. A study found that Vaginal *Lactobacillus* strains performed

significantly better than probiotic strains currently utilized in vaginal health probiotics.

5.2 Physiological study

5.2.1 Level of Vitamin D

The familiar action of vitamin D is to regulate calcium and phosphate homeostasis, which are essential for bone formation and resorption. Vitamin D promotes the intestinal absorption of phosphate and calcium, it stimulates the differentiation of progenitor cells to osteoclasts, and it recovers calcium from.bones and encourages bone matrix mineralization (Rui Lin, 2016).

Vitamin D is important for women especially for pregnant women because to regulate and balance calcium and phosphate ions , in this study vitamin D deficiency because pregnant women don't exposure to sunlight , good diet and the fetus need vitamin D for body's skeleton from the mother.

Through the current study, a decrease in Vitamin D was observed in the blood samples of pregnant women in the first trimester, as this study agrees with many studies , including (Bodnar .,2009, Hu *et al*.,2022) pointed out that vitamin D deficiency is associated with BV at <16 wk of pregnancy. A better understanding of vitamin D-BV relation will be ascertained with prospective studies of "incident" BV infections, persistent infections, and infections that spontaneously resolve. The majority of studies have shown that vitamin D deficiency is highly prevalent among pregnant women and the general population (Thippeswamy *et al*.,2021).

In this study, it was found that there is an inverse relationship between vitamin D deficiency an bacterial vaginosis in pregnant women in first trimester where it was in group BV (19.91±1102) ,and Non BV (33.71±14.9), significant. An inverse association was found between bacterial vaginosis and 25(OH)-vitamin D levels in pregnancy, the risk of bacterial vaginosis increasing

in case of vitamin D deficiency (Bodnar *et al.*,2007, Bodnar *et al*.,2009Jefferson *et al*.,2019; Várbíró *et al*., 2022). vitamin D deficiency among pregnant women has been associated with elevated risk for other pregnancy complications such as preeclampsia) and bacterial vaginosis Vitamin D deficiency may have the following effects on pregnancy: increased risk for preeclampsia, GDM, spontaneous premature birth and bacterial vaginosis. may also conclude that vitamin D demand increases in pregnancy: optimal vitamin D levels decrease both maternal and fetal risks.

The effect of vitamin D on BV elimination can be explained by the impact of vitamin D on the immune system, especially in the local immunity of the vagina. Cytokines were considered the main factor linking BV and vitamin D (Yudin *et al*., 2003; Bodner *et al*.,2009).

In this study showed that there is an inverse(R =-.231-) relationship between vitamin D and body mass index, and there was a difference but no significant shows in the table (4-2) as in the following study. Vitamin D deficiency also affects BMI and during seasons. The present results indicated the presence of a significant seasonal variability of vitamin D status. It also found an inverse relationship between vitamin D status and BMI, which is explained by a smaller skin surface area for vitamin D synthesis in comparison to the total body volume (Lagunova *et al*.,2009). adequate vitamin D supplementation from conception to birth and in postpartum is increasingly important both for the mother and newborn, although currently there are no uniform professional recommendations regarding the exact amount of vitamin D supplementation during pregnancy (Wierzejska and Wajda,2022).

5.2.2 Parathyroid Hormone

Through the results which are from obtained PTH, it was found that there are significant differences between BV and non-BV group, at level $P<0.05$ PTH

levels were significantly higher in BV group in comparison to non-BV group shown in table (4-6). PTH is important during pregnancy within the normal level because it is responsible for regulating the level of calcium .It's rise leads to what was mentioned in the study of parathyroid hormone (PTH), are essential to increase maternal calcium absorption during pregnancy. Through the course of pregnancy, PTH concentrations increase to reach a mid-normal range by the third trimester .The secretion of PTH is regulated primarily by extracellular calcium concentrations; lower amounts of circulating calcium trigger an increase in PTH (Kovacs *et al*.,2006 ; Marbut *et al*., 2017) . A rise in PTH-related protein (PTHrP) and calcitriol in the first trimester, and the flux of other hormones during pregnancy, such as estradiol, progestins, placental lactogen, and insulin-like growth factor I, may have direct or indirect effects on maternal calcium (Kovacs.,2016). In response to placental calcium transfer as well as an expanding extracellular volume and increased urinary calcium loss, maternal PTH concentrations rise during pregnancy (Kovacs *et al*., 2006).

The levels of vitamin D in this study is inversely proportional to the concentration of parathyroid hormone as shown in figure (4-9). Many studies have shown that there is an inverse relationship between Vitamin D and the parathyroid hormone thus the result agreed with those studies, including studies of (; Haddow *et al*.,2011; Ustuner *et al*., 2011 Kramer *et al*., 2016; Jääskeläinen *et al*.,2013; Naugler *et al*., 2013 Hysaj *et al*., 2021) . In other studies) elevated PTH concentrations are an indicator of vitamin D deficiency; nevertheless, there are other factors that are involved in the physiological changes in PTH. Herein, An investigated other potential factors determining PTH concentrations using multiple regression models. These models indicated that 25(OH)D concentrations are a statistically significant determinant of PTH levels in both early and late pregnancy. Education of the partner, multiparity, season, and outdoor activity were also statistically significant in early pregnancy, whereas

country of origin was a statistically significant determinant of PTH in late pregnancy. Studies in the general population and in pregnant women have demonstrated an association between education and vitamin D levels, that better education is associated is with higher levels of vitamin D.

Parathyroid hormone is associated with immunity according to the conclusion of the following study. Since the early 1980s, PTH receptors were found on most immunologic cells (neutrophils, B and T cells) (Geara *et al*.,2010).

5.2.3 Blood Parameters in Pregnant Women with BV and Healthy Women (Non BV).

The changes of CBC parameters during pregnancy are described in this study. There are significant differences between pregnant with bacterial vaganosis and pregnant without bacterial vaganosis .

The estimation of anemia prevalence is an important step for health policy makers, as hemoglobin estimation is most important parameter to start supplementation of iron or removing other causes of anemia to prevent unfavorable outcome (Dhariwal *et al.*,2016).

5.2.3.1 The White Blood Cell (WBC)

A significant increase in the number of white blood cells was observed. The cause of this increase was due to inflammation that found in vaginal tract , the results which are compatible with study of (Stock and Hoffman, 2000) which found White blood cells are an important component of the host defense system, responsible for protection against bacteria, fungi, viruses, and invading parasites, it's level elevated during infection, Neutrophils was frequently increased in infection and in any acute WBC that primary respond to a bacterial infection (Anjali *et al.*, 2020). The increase observed in WBC count from the first to third trimester(Akingbola *et al*.,2006, Akinbami *et al*., 2013, Patel *et al*., 2014).

5.2.3.2 :Packed Cell Volume (PCV)

In this study it was observed that non-significant in PCV values which is an indication of Pregnancy causes significant changes in metabolism, fluid balance, organ function and blood circulation which are driven by hormones and the presence of the feto - placental unit, these dramatic changes influence a wide variety of hematological parameters, decreasing in RBC, Hb, PCV some of these changes which due to increasing plasma volume (Elgari, 2013) . All above studies support result of PCV in current study.

5.2.3.3 The Platelet Count (PLT)

The value of platelets pregnant women with bacterial vaginosis .(7.77±2.74) was high when compared to healthy control (6.475 ±1.64).observed high significant , P=0.009 , this result corresponds to the result of Talukdar et al. (2017), the latter study found that the average number of blood platelets is associated with increased disease effectiveness. Platelets interact with all kingdoms of microbial pathogens, including viruses, bacteria, fungi, and protozoa (Yeaman, 2010). Platelet releases soluble immunomodulatory factors upon stimulation by bacterial components (Hamzeh-Cognasse *et al.*, 2015). There is a growing recognition of the critical role of platelets in inflammation and immune responses (Thomas & Storey, 2015).

Conclusions and Recommendations

Conclusions

From data that were obtained from the present study, one can be concluded the following facts :-

1-Vitamin D decreased concentration in pregnant women with bacterial vaginosis.

2-There were among physiological parameter with bacterial vaginosis.

3-The *Lactobacillus casei* and *Lactobacillus gasseri* from BV and Non-BV was most common.

4-Antibacterial secretion from *Lactobacillus casei* and *Lactobacillus gasseri* had effective.

Recommendations

1-Study the concentration of Calcium and phosphorus with BV.

2-Study the parathyroid hormone and Thyroid hormone and correlation with *Lactobacillus* .

3-Study the toxicity of *Lactobacillus* spp. CFS on Lab animals .

4-Molucular study of *Lactobacillus* Spp.

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الخلاصة

هناك مدى واسع ومتنوع من العوامل المُمْرضة تتضمن البكتريا والفطريات والفيروسات يمكنها ان تسبب التهاب المهبل وخصوصاً خلال الحمل، صممت الدراسة الحالية لفحص وعزل وتشخيص *Lactobacillus* spp. من النساء المصابات بالتهاب المهبل البكتيري خلال الأشهر الثلاثة الأولى من الحمل وكذلك إيجاد نقص فيتامين د والهرمون جار الدرقية مصحوبة ببعض معلمات الدم.

تضمنت هذه الدراسة عدد من النساء (٩٠)، (٥٠) منهن كانت مصابة بالتهاب المهبل البكتيري خلال الأشهر الثلاثة الأولى من الحمل، وما تبقى (٤٠) امرأة حامل في الأشهر الثلاثة الأولى تم استخدامها كمجموعة سيطرة، كانت اعمار مجموعات الدراسة بين (١٦-٤٥) سنة. تم جمع العينات خلال الفترة من تشرين الثاني ٢٠٢١ ولغاية شباط ٢٠٢٢ في محافظة بابل، مستشفى الامام علي (ع) العام والعيادات الخاصة، تم جمع العينة بواسطة الطبيبة اختصاص الامراض النسائية، وتم جمع عينة دم ومسحة مهبلية عليا لكل امرأة حامل. تم قياس مستوى فيتامين د والهرمون جار الدرقية باستخدام تقنية المقايسة الامتصاصية المناعية للانزيم المرتبط (ELISA) وكذلك تم فحص عد الدم الكامل (CBC) باستخدام جهاز عد الدم الاوتماتيكي، اما بالنسبة للمسحة المهبلية العليا تم زرعها على الوسط الزرعي (MRS) لعزل بكتريا *Lactobacillus* وتشخيصها بإستخدام جهاز الـ (VITIK-2) وتم استخدام الوسط الزرعي (MHA) لفحص فعالية المضادات الحياتية والمضادات البكتيرية.

أظهرت بيانات هذه الدراسة بأن مؤشر كتلة الجسم كان اعلى في مجموعة النساء المصابات بالتهاب المهبل البكتيري مقارنة بمجموعة النساء الاصحاء. اظهر متوسط مستوى فيتامين د في مصل الدم ارتفاعاً معنويا ($P<0.05$) في مجموعة النساء المصابات بالتهاب المهبل البكتيري (١٩,٩١±١١,٢) مقارنة بمجموعة السيطرة (٣٣,٧١±١٤,٩) كلما انخفض مستوى فيتامين د ازداد خطر الإصابة بالتهاب المهبل البكتيري. كان موسط مستوى الهرمون جار الدرقية بارتفاع معنوي ($P=0.21$) في مجموعة النساء المصابات بالتهاب المهبل البكتيري (٣٢,٧±١٣,٦) مقارنة بمجموعة السيطرة (٢٦,٥±١٠,٩).

علاوة على ذلك، اظهر متوسط عدد كريات الدم البيضاء ارتفاع معنوي ($P<0.05$) في مجموعة النساء المصابات بالتهاب المهبل البكتيري (٧,٧٧±٢,٧) مقارنة بمجموعة السيطرة (٦,٤٧±١,٦)، وكذلك بالنسة للصفيحات الدموية أظهرت ارتفاع معنوي ($P<0.05$) في مجموعة النساء المصابات بالتهاب المهبل البكتيري (٢٣٠,٢±٧٧,٦) مقارنة بمجموعة السيطرة (١٩١,٧±٥٣,٤)، ولكن بالنسبة لحجم كريات الدم المظغوط (PCV) لم يكن هنالك فرق معنوي بين مجموعات الدراسة.

تم زرع جميع الـ٩٠ مسحة مهبلية على الوسط الزرعي (MRS agar and broth)، للتحري عن *Lactobacillus* spp. كمؤشر عكسي للاتهاب المهبل البكتيري خلال الأشهر الثلاثة الأولى من الحمل، ٧ (١٤٪) من المسحات المهبلية لمجموعة الدراسة تم تشخيصها كـ *Lactobacillus* بينما ٤٣ (٨٦٪) اظهرت نتيجة سالبة للزرع البكتيري، و ٣٠ (٧٥٪) من المسحات المهبلية لمجموعة السيطرة تم تشخيصها كـ *Lactobacillus* ، بينما ١٠ (٢٥٪)

اظهرت نتيجة سالبة للزرع البكتيري تم التشخيص بجهاز VITIK-2 وتم تشخيص نوعان من بكتريا الـ *Lactobacillus* : *Lactobacillus gasseri* و *Lactobacillus case* .

أظهرت النتائج ان جميع عزلات الـ*Lactobacillus* spp. التي كانت من النساء الاصحاء أظهرت مقاومة اعلى للمضادات الحيوية المستخدمة في الدراسة cefixime وcefepime وNitrofurantoin وAmoxicillin/clavulanic acid وAmoxicillin بنسبة مقاومة (١٠٠٪ و ٨٠٪ و ٦٠٪ و ٦٠٪ و ٣٠٪ على التوالي) في مجموعة النساء الاصحاء و(١٠٠٪ و ٧١٪ و ٧١٪ و ٤٣٪ و٢٨٪ على التوالي) في مجموعة النساء المصابات بالتهاب المهبل البكتيري.

أظهرت هذه الدراسة ان الراشح الخالي من الخلايا (CFS) المستخلص من *Lactobacillus* spp من النساء الاصحاء كان أكثر تأثيراً ضد عزلات البكتريا المرضية من CFS من L. spp. المعزولة من النساء المصابات بالتهاب المهبل البكتيري.

وزارة التعليم العالي والبحث العلمي
جامعة بابل
كلية العلوم للبنات
قسم الاحياء

العلاقة بين الهرمون جار الدرقية وفيتامين د مع العصيات اللبنية المهبلية في الاشهر الثلاثة الاولى من الحمل لدى الحوامل في مدينة الحلة

رسالة مقدمة
الى مجلس كلية العلوم للبنات/ جامعة بابل
كجزء من متطلبات نيل درجة الماجستيرفي علوم الحياة

من قبل الطالبة
زهراء عايد ناهي
بكالوريوس علوم حياة كلية العلوم للبنات/ جامعة بابل (٢٠١٦)

بإشراف
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٢٠٢٢ م ١٤٤٣ هـ