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Microbiome & Endometriosis

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ABSTRACT

Endometriosis is a chronic disease of the female reproductive system, which is characterised by the presence of endometrial tissue outside the uterus especially in the pelvic cavity that includes ovaries, fallopian tubes and it may occur extra genitally in some rare cases. 176 million women all over the world including 26 million in India suffer from endometriosis and high frequency was observed between the age group of 26-30 years, resulting in increased primary infertility. Various studies surveying the female reproductive tract have confirmed the existence of microbiota starting in the vagina, cervix, uterus, fallopian tubes and ovaries. These microbes play a crucial role at different stages ofthe reproduction, in forming a physical barrier against pathogen and the stimulation of host defence mechanisms. Direct and indirect mechanisms are observed including the production of biochemically active compounds that directly kill or inhibit pathogens. Women suffering from endometriosis lead an impaired quality of life and continue to deal with endometriosis-associated symptoms even after diagnosis and treatment of the disease. In endometriosis condition, adhesions are observed in the fallopian tubes and ovaries that block tubal motility and damage the oocyte-pickup. Progesterone resistance is familiar in endometriosis which changes the implantation window period causing the loss of implantation markers and finally leading to infertility. Malignant tumours of ovaries have also been identified and known to arise from endometriosis. Microbiome can be used as a novel diagnostic tool for endometriosis as there is a variation observed in the composition and distribution along the female reproductive tract of healthy women and endometriosis patients. A non-invasive diagnosis is achieved through this, aiming at early diagnosis and alternative treatment for endometriosis.

KEYWORDS: *Endometriosis, infertility, microbiome, early diagnosis.*

I. INTRODUCTION

Endometriosis is a chronic inflammatory disease of the female reproductive system which is characterised by the presence of endometrial tissue outside the uterus especially in the pelvic cavity that includes ovaries, fallopian tubes and it may occur extra genitally in some rare cases (Figure 1). The most common symptoms include recurrent painful menstruation (dysmenorrhoea), chronic lower abdominal pain and infertility. Endometriosis is classified into three different phenotypes as peritoneal (or) superficial, ovarian and deep infiltrating endometriosis [1]. Other phenotype is adenomyosis, where the endometrium is seen within the myometrium.

In endometriosis, the endometrial tissues migrate from the uterus to other pelvic organs.[2]. When there is presence of endometriotic deposits over the peritoneum, it is defined as peritoneal (or) superficial endometriosis. Involvement of the ovaries leads to the formation of endometrioma. The deep infiltrating type of endometriosis refers to the presence of endometriotic implants greater than 5 mm beneath the peritoneum, is a major indicator of the severe pain, women with this disorder experience [3,4].

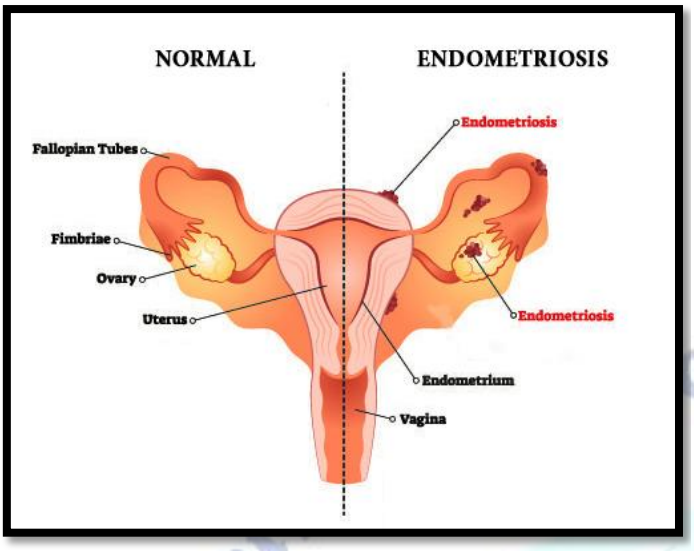


Figure 1: Comparison of healthy and endometriosis condition

A. Epidemiology

176 million women all over the world including 26 million in India suffer from endometriosis. High frequency was observed between the age group of 26-30 years, resulting in increased primary infertility (Figure 2) [5]. It is known to affect 10% to 15% of women in reproductive age and 70% of them have chronic pelvic pain and 48% suffer from infertility. The recurrence rate was estimated as 21.5% at 2 years and 40-50% at 5 years [6]. About 25 to 50% of infertile women suffer from endometriosis, and 30 to 50% of women with endometriosis are infertile.

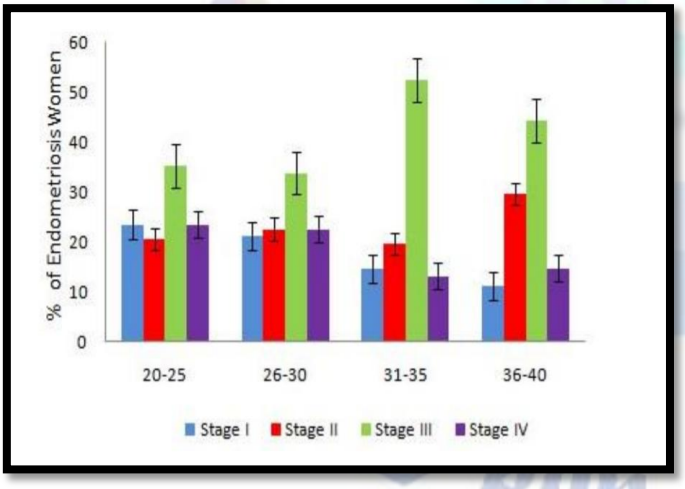


Figure 2: Percentage of endometriosis women against the various age groups (showing various stages)

B. Stages, Symptoms and Diagnosis

The American Society for Reproductive Medicine (ASRM) criteria is used for classifying the severity of endometriosis. It is based upon the recordings of laparoscopic findings and comparisons of the efficacy of therapeutic interventions. The ASRM stages endometriosis from Stage I (minimal) to Stage IV (severe) based on the location and size of the lesions seen during laparoscopy. Higher the score, greater is the severity of endometriosis (Table 1) [7]. (Figure 3-5)

Newer classification system has also been introduced such as the Endometriosis Fertility Index (EFI) which combines the scoring system of the ASRM during laparoscopy and from history which can predict the fertility outcome called endometriosis fertility index. Patients are given a score ranging from 0 to 10. Patients with scores between 0 and 3 had a 10% probability of conception those with scores between 9–10 had a 75% chance of conceiving [8].

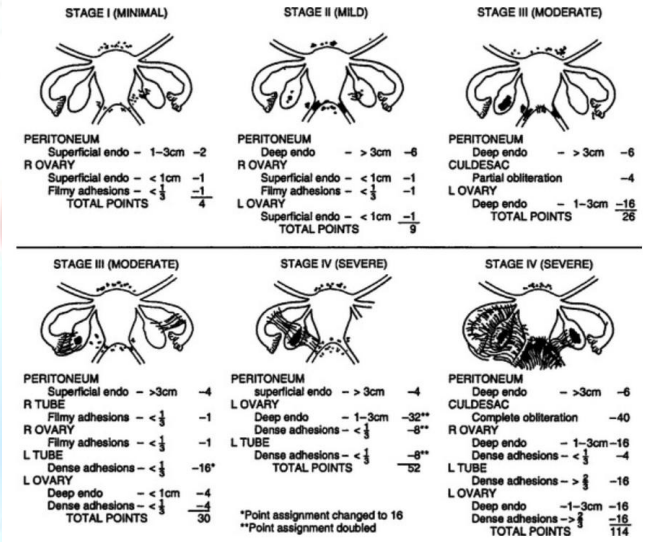
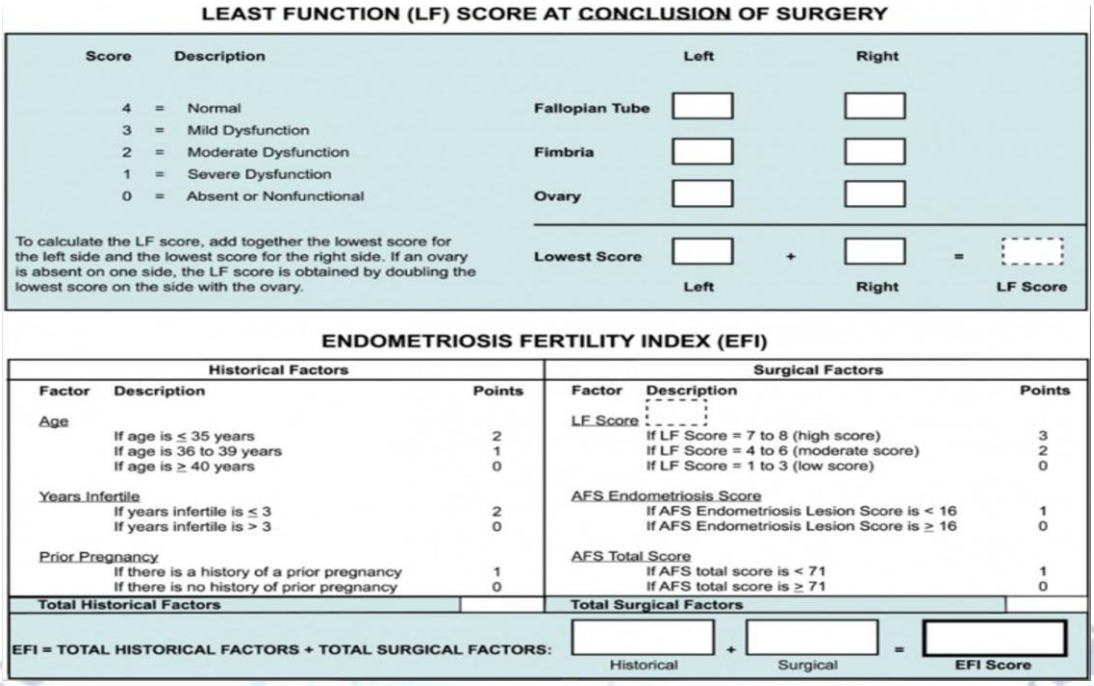


Figure 3: ASRM classification of Endometriosis

THE REVISED AMERICAN SOCIETY FOR REPRODUCTIVE MEDICINE CRITERIA FOR

Table 1 : ASRM classification of endometriosis

STAGES	POINT SCORE
I (MINIMAL)	1-5
II (MILD)	6-15
III (MODERATE)	16-40
IV (SEVERE)	> 40



LEAST FUNCTION (LF) SCORE AT CONCLUSION OF SURGERY

Score		Description		Left	Right	
4	=	Normal	Fallopian Tube			
3	=	Mild Dysfunction				
2	=	Moderate Dysfunction	Fimbria			
1	=	Severe Dysfunction				
0	=	Absent or Nonfunctional	Ovary			
			Lowest Score		+	=
				Left	Right	LF Score

To calculate the LF score, add together the lowest score for the left side and the lowest score for the right side. If an ovary is absent on one side, the LF score is obtained by doubling the lowest score on the side with the ovary.

ENDOMETRIOSIS FERTILITY INDEX (EFI)

Historical Factors			Surgical Factors		
Factor	Description	Points	Factor	Description	Points
Age			LF Score		
	If age is ≤ 35 years	2		If LF Score = 7 to 8 (high score)	3
	If age is 36 to 39 years	1		If LF Score = 4 to 6 (moderate score)	2
	If age is ≥ 40 years	0		If LF Score = 1 to 3 (low score)	0
Years Infertile			AFS Endometriosis Score		
	If years infertile is ≤ 3	2		If AFS Endometriosis Lesion Score is < 16	1
	If years infertile is > 3	0		If AFS Endometriosis Lesion Score is ≥ 16	0
Prior Pregnancy			AFS Total Score		
	If there is a history of a prior pregnancy	1		If AFS total score is < 71	1
	If there is no history of prior pregnancy	0		If AFS total score is ≥ 71	0
Total Historical Factors			Total Surgical Factors		

EFI = TOTAL HISTORICAL FACTORS + TOTAL SURGICAL FACTORS: Historical + Surgical = EFI Score

Figure 4: Endometriosis Fertility Index

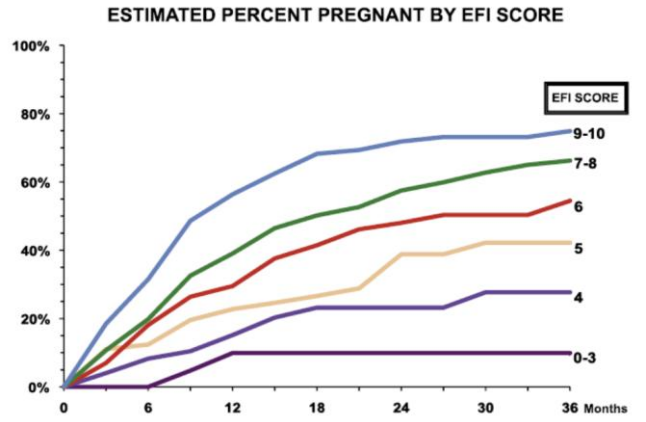


Figure 5: EFI Graph

The most common symptoms of endometriosis include cyclic menstrual pain, chronic pelvic pain, dyspareunia, abnormally heavy or prolonged menstruation, painful micturition and difficult bowel movements but can be asymptomatic in some cases [9].The pelvic pain in women starts before the onset of menses and it worsens upon menstrual flow.

The first diagnostic evaluations done are physical examination and pelvic ultrasound [10]. Ovarian endometriomas (also known as chocolate cysts) are observed, which are large, haemorrhagic cysts that develop on the ovary due to endometrial tissue deposition through retrograde menstruation. The gold standard for diagnosing endometriosis is by laparoscopy with biopsy to reveal the histological presence of the endometrial tissues [9]. Pelvic magnetic resonance imaging (MRI) has given high-resolution images with excellent tissue characterization in patients with deeply infiltrating pelvic endometriosis [11].

II. EFFECTS OF ENDOMETRIOSIS

Women suffering from endometriosis have an impaired quality of life and continue to have endometriosis-associated pelvic pain even after diagnosis and treatment of the disease. This leads to a significant impact on both the physical and mental health of the patient affecting education, work and social wellbeing [12].

In a study involving 78 women diagnosed with endometriosis, 49.3% women had impaired work ability, 15% had problems in their sexual relationships, 8.5% had reduced learning capacity and 7.7% suffered from a broken relationship due to the symptoms related to endometriosis [13]. Similarly, in another study with 107 women, who had undergone previous surgery for endometriosis, work was known to be affected in 66% [14].

A. Infertility

In the recent years, infertility occurrence has increased so much that it is now currently documented as a worldwide health issue and WHO states that it is a disease [15]. One in seven couples are affected by infertility referring to the inability to conceive after 1 year of regular unprotected intercourse [16].

Although it is controversial that endometriosis causes infertility or a decrease in fecundity, there is reasonable evidence of association between

endometriosis and infertility. In normal couples, the fecundity rate ranges between 15% to 20% per month and tends to decrease with the ascending age of the female partner. However, in cases of untreated women suffering from endometriosis, fecundity rate is reduced to 2% to 10% [17,18]. Infertile women are 6 to 8 times more prone to have endometriosis than fertile women [19]. Furthermore, there is a lower live birth-rate observed among women with endometriosis [20].

Few mechanisms that have been believed to cause infertility due to endometriosis includes distorted pelvic anatomy, altered peritoneal function, abnormalities in oocyte and embryo and altered hormonal functions in the endometrium (Figure 6).

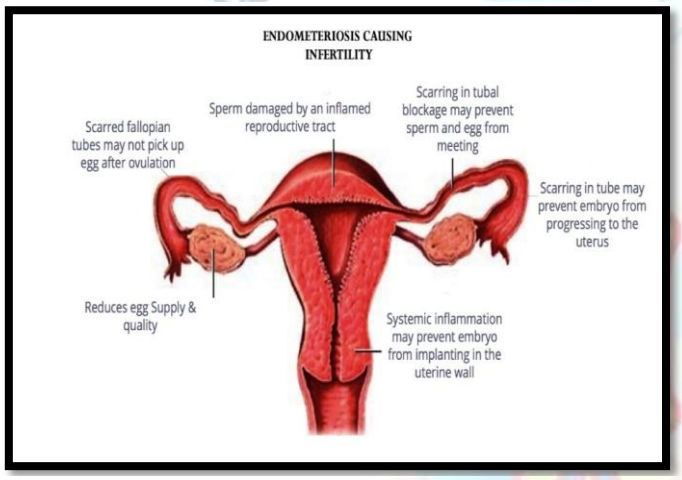


Figure 6: Endometriosis causing infertility by various mechanisms

Altered Peritoneal Function

Women suffering from endometriosis have shown to have an increased volume of peritoneal fluid accompanied by increased concentrations of prostaglandins, proteases and cytokines including inflammatory cytokines such as IL-1, IL-6 and TNF-α, and angiogenic cytokines such as IL-8 and VEGF produced by macrophages [21,22].

These alterations may have an adverse effect upon the proper functioning of oocyte, sperm, embryo and fallopian tube. Additionally, in the peritoneal fluid of endometriotic patients have an ovum capture inhibitor (OCI) which is thought to be responsible for fimbrial failure of ovum capture [23].

Distorted Pelvic Anatomy

In the fallopian tubes, ovaries and Douglas pouch, formation of flimsy or dense adhesions are observed by laparoscopy in endometriosis. These adhesions block tubal motility and damage the oocyte-pickup by the fimbria [24].

Hormonal Changes

Increased activity of NK cells, IgG and IgA antibodies and lymphocytes are observed in the endometrium of women with endometriosis. These irregularities may alter endometrial receptivity and embryo implantation [25]. Due to an increased number of activated macrophages and their secretory products, they behave abnormally. There is an association between endometriosis and luteinized unruptured follicle syndrome that leads to infertility [26].

Progesterone resistance (decreased responsiveness of target tissue to bioavailable progesterone) is the hallmark in endometriosis which changes the implantation window period, further causing the loss of implantation markers and finally leading to infertility [27,28].

Oocyte And Embryo Quality

In women with endometriosis, abnormalities in oocyte and embryo quality have been described. Embryos derived from these women cleave slowly compared to embryos derived from women suffering from tubal infertility. These observations suggests that infertility in endometriosis patients may be related to poor oocyte and embryo quality and thus poor implantation [29].

B. Ovarian Carcinoma

Endometriosis and its relationship with epithelial ovarian cancer has been a debated often, although their association has been studied vastly. Malignant tumours like clear cell carcinoma and endometroid carcinoma are known to arise from endometriosis (Figure 7) [30].

It has also been established that malignant transformation occurred in cases of benign ovarian endometrioma [31] and the risk of endometriosis associated carcinoma is higher in older women (about 13%, in women more than 50 years of age) [32].

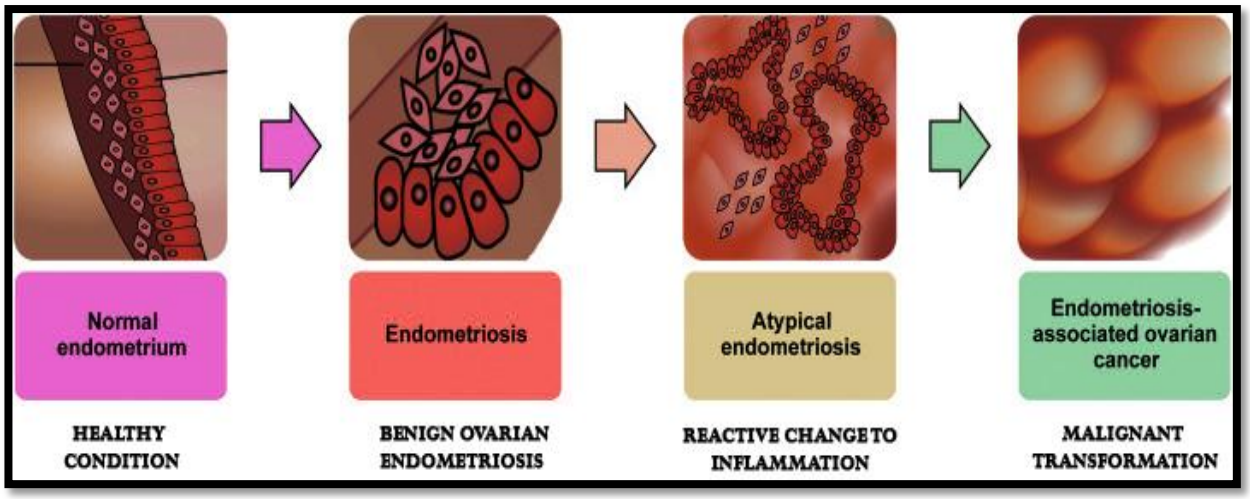


Figure 7: Malignant transformation to ovarian cancer from endometriosis condition

Endometriosis-associated carcinoma are found to be clear cell carcinoma (35% in 390 cases), endometrioid carcinoma (27% in 648 cases), serous carcinoma (5% in 1372 cases), and mucinous carcinoma (4% in 614 cases). This study was conducted by Somigliana et al, leading to the following conclusions: Malignancyoccurs as some endometriotic cells might undergo somatic mutations and endometriosis and ovarian cancer might represent two distinct biological entities of a different set of causative molecular events [33].

C. Perimenopausal and Postmenopausal Effects

Clinical characteristics of perimenopausal and postmenopausal women with endometriosis were investigated to study any relation with other gynaecologic conditions. It was found that endometrioma and uterine leiomyoma were the most common condition among women with perimenopausal endometriosis compared to the postmenopausal group and adenomyosis was found to be higher in postmenopausal patients. In both the groups, endometriosis-associated ovarian cancer and uterine cancer were similar (Table 2) [34].

Table 2: Coexistence of endometriosis with various benign and malignant conditions [34]

Gynaecological conditions	Group 1 perimenopausal women with endometriosis (45–54 years)	Group 2 postmenopausal women with endometriosis (55–80 years)
1. Endometrioma	125(68%)	5(10.8%)
2. Ovarian or Para ovarian cyst	21(11.4%)	7(15.2%)
3. Uterine Leiomyoma	82(44.5%)	8(17.4%)
4. Adenomyosis	29(16%)	15(32.6%)
5. Uterine leiomyoma and adenomyosis	21(11.4%)	15(32.6%)
6. Other benign gynaecologic conditions	13 (7%)	3 (6.5%)
7. Endometriosis associated ovarian cancer	8 (4.3%)	3 (6.5%)
8. Uterine cancer	6 (3.2%)	6 (13.1%)
9. Bowel cancer	3 (1.65)	0
10. Other cancers	2 (1%)	1 (2.1%)
11. DED (Dry eye disease)	0	24 (52.1%)
Total	184/1100(16.7%)	46/1100(4.2%)

D. Irritable Bowel Syndrome (IBS)

Irritable bowel syndrome (IBS) (spastic bowel or **nervous colon)**, is a functional gastrointestinal disorder that leads to symptoms of abdominal pain and inconsistency of bowel movements. Bowel and bladder problems are common in endometriosis patients and comparison of endometriosis patients to controls was done and reported that 22% had symptoms of IBS [35]. Women with endometriosis are 3.5 times more likely to have an IBS diagnosis compared to control [36] and endometriosis implants on the bowel have been reported in women diagnosed with endometriosis [37]. 6076 subjects with endometriosis were studied and found that 15% had IBS [38].

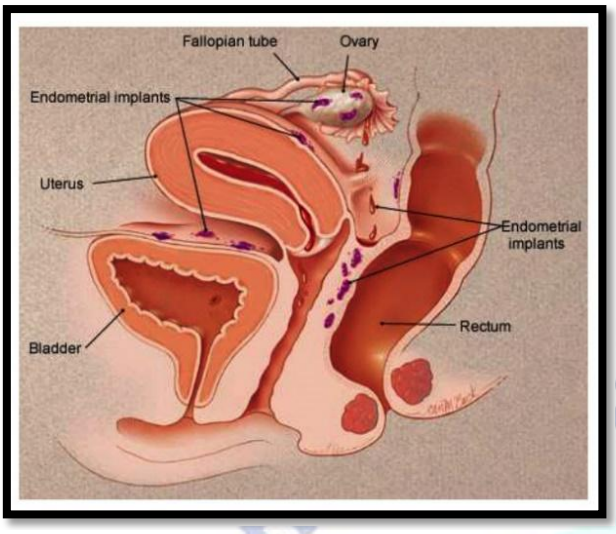


Figure 8: Migration of endometrial implants in the bowel region

The relationship between gastrointestinal complaints and histologic findings in women with endometriosis was examined and reported that 18% of women with endometriosis had bowel lesions [39]. The endometriosis deposits located in close proximity to the terminal large bowel are inflammatory in nature of leading to local prostaglandin release and this explains the altered bowel function (Figure 8). These are observed mainly in deep infiltrative endometriosis [36].

III. MICROBES INFLUENCING ENDOMETRIOSIS AT MOLECULAR LEVEL

The exact pathogenesis of endometriosis has been controversial and many hypothesis have been studied regarding the role of microbes influencing the disease in a molecular level. Studies have proposed that microbiota play a crucial role in a fertile endometrium due to its influence on the uterine immunity [40]. In all aspects of reproductive success, especially during the time of conception and the implantation period, it has been shown that local and systemic immunity is significantly influenced by microbiota [41,42].

Retrograde menstruation theory is the oldest, that explains the cause of endometriosis. According to this theory, during the normal process of menstruation, a reflux of endometrial tissue and cells occurs through the fallopian tubes and to the ovaries, where it enters into the peritoneal cavity and implants abnormally on to pelvis (Figure 9) [43]. Larger volume of retrograde menstrual fluid found in the pelvis of patients with endometriosis as compared with healthy women may increase the risk of endometriotic implantation [44].

As a result of retrograde menstruation process, it gives rise to a hypothesis that the development of endometriosis could be due to microbial dysbiosis and numerous studies have been conducted to characterize the microbiome of endometriosis patients in order to identify the role of microbes in relation to the disease.

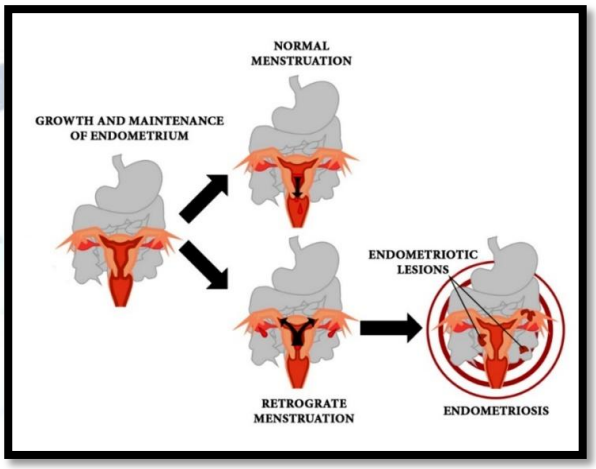


Figure 9: Retrograde menstruation theory

A. Bacterial Contamination Hypothesis

This hypothesis states that women with endometriosis had a substantial increase in *Escherichia coli* (*E. coli)* in menstrual blood and endotoxin levels in menstrual and peritoneal fluid [45]. Endotoxin refers to the lipopolysaccharide (LPS) that is found in the cell wall of gram-negative bacteria and asa pyrogen, it induces various biological reactions when it enters the bloodstream. These initiate the secretion of many secondary inflammatory mediators like cytokines, chemokines, and growth factors by mature or activated macrophages [46].

It was found that gram-negative bacteria infect the uterine wall after ascending migration from the vagina to contaminate the menstrual blood leading to accumulation of endotoxin in the menstrual or peritoneal fluid and this initiates the onset of pelvic inflammation (Figure 10). In this study, the stimulatory effect of E. coli–derived LPS on the secretion of various macromolecules by macrophages that are known to be involved in endometriosis was assessed along with the growth promoting effect of LPS on endometrial cells and the role of Toll-like receptor 4 (TLR4) which is a receptor recognizing LPS.

Women with regular ovulatory cycles were recruited for this study and peritoneal fluid (PF) and menstrual fluid (MF) was collected from women with endometriosis and women without endometriosis (control). Anti- TL4 antibodies,

polymyxin B and a potent LPS antagonist were used to treat the endometriosis patients and compared with the non- treated patients.

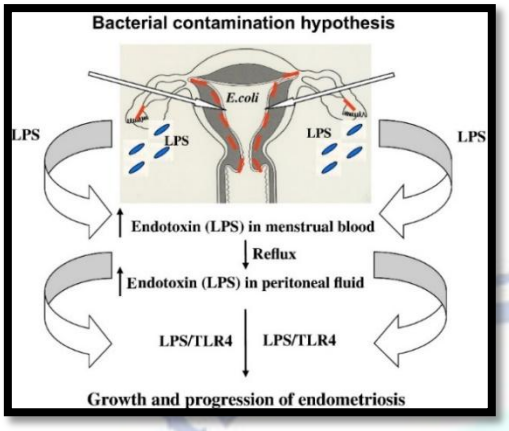


Figure 10: Accumulation of endotoxin in the menstrual blood leading to progression of endometriosis [45]

The highlight points in the interpreted results were: (1) A higher colony formation of E. coli was observed in the menstrual blood of women with endometriosis than that of control women (2) RT-qPCR analysis showed a substantial increase of TLR4 gene expression in women with endometriosis than in those without endometriosis (3) Increased endometrial cell growth in response to LPS and retraction of these LPS-mediated effects by anti-TLR4 antibody, promoting TLR4-mediated growth of endometriosis.

The accumulation of bacterial endotoxin in the pelvis may be due to the translocation of *E. coli* or endotoxin from the gut through enterocytes and then entering into the pelvis [47,48] or contamination of menstrual blood by E. coli after its migration from the vagina to the uterine cavity (Figure 11). The study suggested that targeting TLR4 could be a potential therapeutic approach to reduce pelvic inflammation and growth of endometriosis.

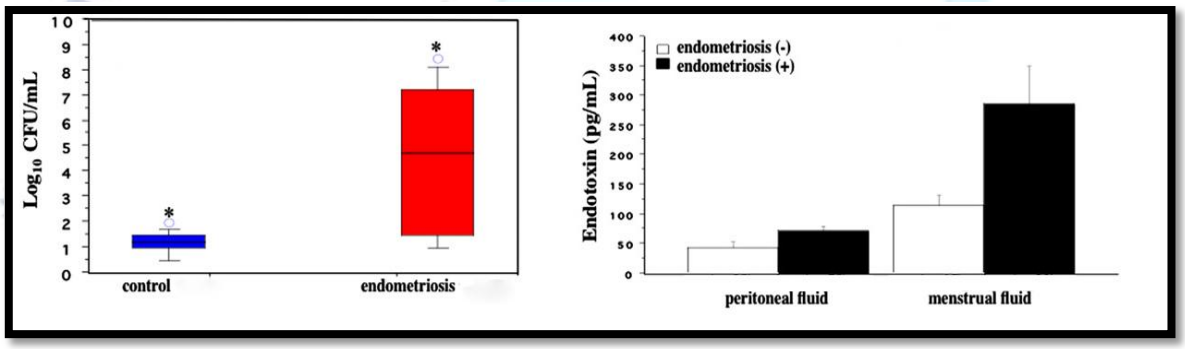


Figure 11: Significantly higher endotoxin levels are observed in endometriosis condition [45]

B. *Mycoplasma Genitalium* – ROLE IN ENDOMETRIOSIS

Mollicutes are the smallest self-replicating free-living microorganisms, found in genital disorders and also found in healthy individuals and some were considered to be infectious agents of the human urogenital tract [49]. *Mycoplasma genitalium* belongs to the class mollicutes and it specifically causes urethritis in both men and women and cervicitis and pelvic inflammation in women [50].

*M. genitalium has the ability to*adhere to the epithelium of the oviduct and to infect the upper reproductive tract [51] and its prevalence was 14.1% fallopian tubes of women who have experienced ectopic pregnancy [52] and 8.4% prevalence in peritoneal fluid samples [53]. Mollicutes found in the peritoneal cavity follows the bacterial contamination hypothesis, that as a result of retrograde menstruation, delivery of microorganisms throughout fallopian tubes up to the ovaries can occur.

Interleukin-1β levels were prominently higher in endometriosis patients with *M. genitalium* colonization and also IFN-γ levels were increased in the study group colonized by *M. genitalium*, thus supporting the hypothesis that the microorganism was the trigger for the increased production of cytokines in women with endometriosis [53].

Functional alterations in immune system cells like macrophages, natural killer cells and cytotoxic lymphocytes are observed in women with endometriosis. Thus, the peritoneal microenvironment becomes immunotolerant, allowing development of endometriosis after retrograde menstruation. Additionally, it could also lead to endometriosis progression rather than prevention [54].

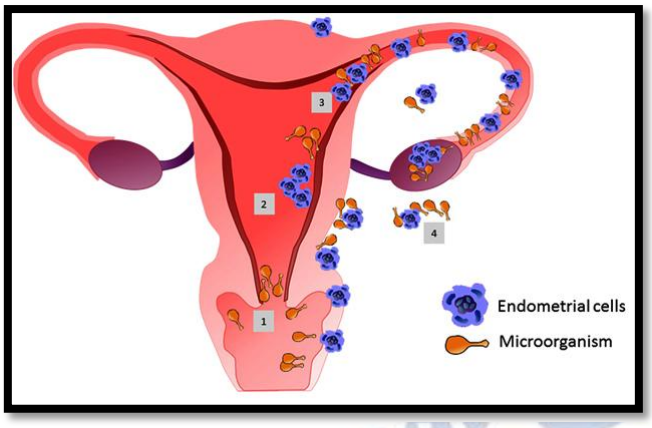


Figure 12: Micro-organisms influencing the immune response leading to progression of endometriosis [53]

Figure 12 shows events in retrograde menstruation and how micro-organisms influence on the immune response: (1) Micro-organisms could colonize and persist in the lower genital tract, leading to infections. (2) These microbes may ascend to the upper reproductive tract and infect the uterine cavity. Toll-like receptors recognize pathogens, associated these molecular patterns that are involved in the inflammatory response and innate immunity. (3) Due to retrograde menstruation endometrial cells and micro-organisms could be carried to the fallopian tubes, ovaries, and peritoneal cavity. *Mycoplasma genitalium* are able to infect these regions (4) In the peritoneal cavity, endometrial cells can attach to surfaces and invade nearby structures. Activated macrophages found to be abundant in the peritoneal fluid of endometriosis patients. This can promote the secretion of proinflammatory cytokines including interleukin 1β, IL-8, IL-6 and tumor necrosis factor α [53].

IV. MICROBIOTA COMPOSITION AND DISTRIBUTION ALONG THE FEMALE REPRODUCTIVE TRACT

A microbiota continuum along the female reproductive tract indicates that it is a non- sterile environment and is composed of diverse microbial communities. It is crucial that we understand the complex interactions between the host and the microbiome so as to foresee and understand a variety of diseases. Microbiota play an important role in development of endometriosis by affecting the host's immunological and biochemical functions. From the effects of the endometriosis, we now know that the peritoneal cavity of endometriosis patients is an inflammatory environment and thus having elevated levels of immune cells in the peritoneal fluid. This results in abnormal immune responses in the endometrium and peritoneal cavity which may further lead to decreased fertility [19].

Inflammatory diseases have been linked to changes in the microbiome across the human body [55]. For instance, due to the presence of a pathogenic organism like *Helicobacter pylori*, which is known to cause stomach ulcers, gastritis, and gastric cancer [56]. The chronic inflammation in endometriosis condition may have direct effects on the micro-organisms related to organs in the reproductive tract. There are diverse uterine and cervical bacterial communities present which has been demonstrated by recent research [57,58]. Now studies are aimed at examining how these diseases may cause deviations in these communities and how these can be used as indicators for the diseases.

A. The Endobiota Study: Comparison of Vaginal, Cervical and Gut Microbiota

A study was done to compare gut, vaginal and cervical microbiota between women with and without endometriosis [59]. Fourteen women with endometriosis and another fourteen healthy controls were included in the study and women in the endometriosis group had moderate to severe endometriosis. Cervical, vaginal and stool samples were obtained from all participants. The V3 and V4 regions of the 16S rRNA gene were amplified by polymerase chain reaction (PCR) for analysis. In a set of 84 samples, 327 different bacterial genera were identified (Figure 13).

The results obtained were: (1) For vaginal samples, at genus level, the complete absence of Gemella and *Atopobium* in the endometriosis group was observed. (2) In cervical samples, Atopobium and *Sneathia* were completely absent, while *Alloprevotella* at genus level was significantly increased in the endometriosis group. (3) In stool samples, *Sneathia*, *Barnesella* and *Gardnerella* we re found to be significantly decreased in the endometriosis group. (4) In gut microbiota, more women having *Escherichia* and *Shigella* dominant in the endometriosis group were observed (Table 3).

Table 3: Variation of microorganisms found in the endometriosis group

NICHE	DECREASED	INCREASED
Vagina	Gemella* Atopobium*	
Cervix	Atopobium* Sneathia	Alloprevotella
Stool	Gardnerella, Sneathia, Barnesella	

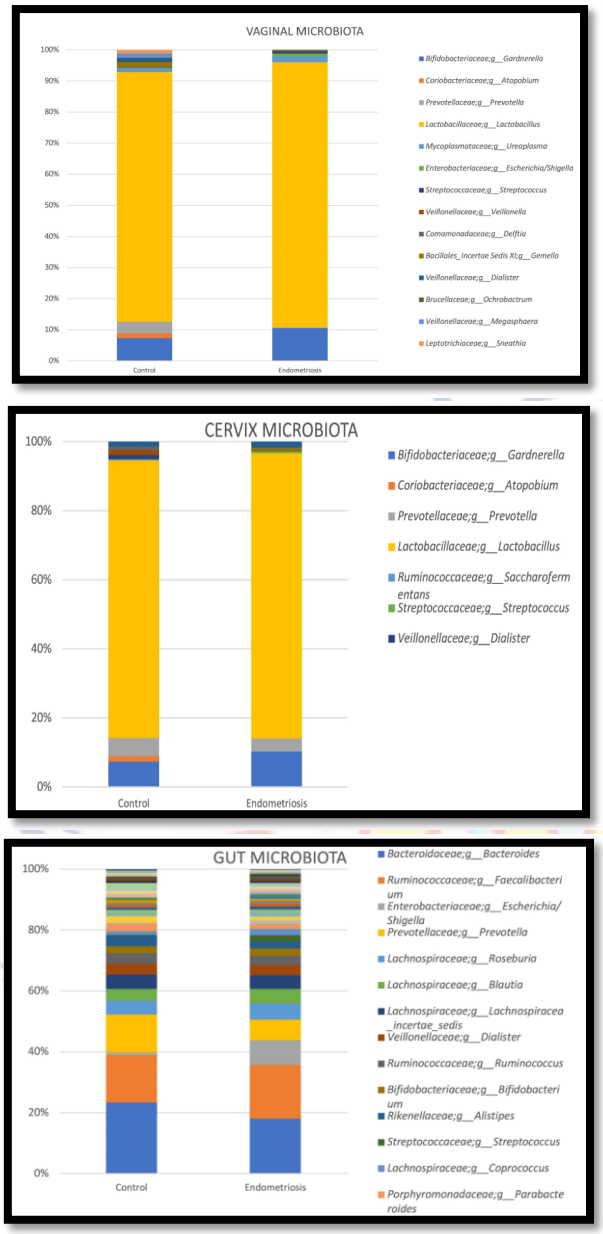


Figure 13: Comparison of vaginal, cervical and gut microbiota in control and endometriosis

Two women in the endometriosis group who had *Escherichia* or *Shigella* dominant gut microbiome underwent segmental colon resection as part of surgical treatment of deep infiltrating endometriosis. In severe endometriosis condition, the colon or rectum may be involved in approximately 25% of cases [60].Surgical removal of the affected tissue is the standard treatment selected in this case. This showed gut microbiome analysis could be an additional tool to predict the possibility of bowel resection.

B. Altered the Uterine and Cervical Bacterial Community in Endometriosis [61]

This study aimed at identifying how endometriosis alters the uterine and cervical bacterial community and to identify indicator taxa for diagnosis within the cervix that might possibly help clinicians to diagnose active endometriosis using a cervical swab and thereby avoiding unnecessary laparoscopic surgery. A hypothesis was assumed that endometriosis patients would have altered uterine and cervical bacterial communities and a distinct effect would be seen in patients suffering from advanced endometriosis.

10 pre-menopausal women undergoing laparoscopic surgery for pelvic pain with suspicion or known endometriosis were enrolled for the experimental group while 9 control women were scheduled for a laparoscopy (or laparotomy or hysterectomy) for benign uterine or ovarian conditions were enrolled as controls. DNA was extracted from the cervical swabs and uterine washes and 16S rRNA gene amplification and sequencingwas performed.

The study conducted showed that the cervical bacterial communities of the patients were *Lactobacillus* species in majority which is consistent with other studies performed for characterizing the cervical and vaginal bacterial communities [58]. It was observed that a stage III endometriosis patient had a prominently different bacterial community than all of the other patients and the cervix of this patient showed depletion in the typically predominant *Lactobacillus* sp.and *Firmicutes* and *Bacteroidetes* levels were increased.

C. Endometriosis Induces Gut Microbiota Alterationsin Mice [62]

The studies involving relationship between gut microbiota and endometriosis are rare. Altered intestinal microflora profiles in endometriosis rhesus monkeys were detected by isolating and culturing bacteria in differential and selective agars [63] but this did not characterize the original complex composition of the gut microbiota. To understand the changes occurring in gut microbiota during the development of murine endometriosis, this study was performed in murine endometriosis model by 16S ribosomal-RNA gene sequencing.

Endometriosis models were induced by intraperitoneal injection of endometrial tissues to mimic endometriosis formation in humans. The mice were divided into endometriosis and mock groups for the study. Faecal sample collection and body weight measurement was done and the mice were sacrificed finally to evaluate the results (Figure 14).

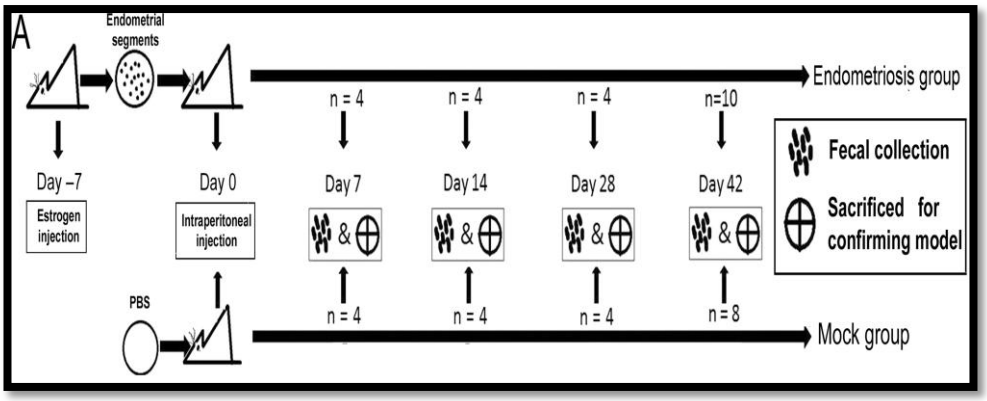


Figure 14: Flow diagram of this study [62]

In this study, quite a few changes to the gut microbiota in different taxon levels were discovered in endometriosis mice. Enrichment of *Firmicutes* and *Actinobacteria* was observed in the endometriosis group, whereas *Bacteroidetes* levels were high in the mock group. It was observed that at the class level, the endometriosis group had an abundance of unidentified *Actinobacteria* and *Betaproteobacteria* and in themock group, more *Bacteroidia* were involved. *Firmicutes*/*Bacteroidetes* ratio whichis considered as a crucial feature of dysbiosis, was compared between the endometriosis and mock groups at 42 days. It was observed that the endometriosis group showed nearly twofold higher in the ratio than the mock group.

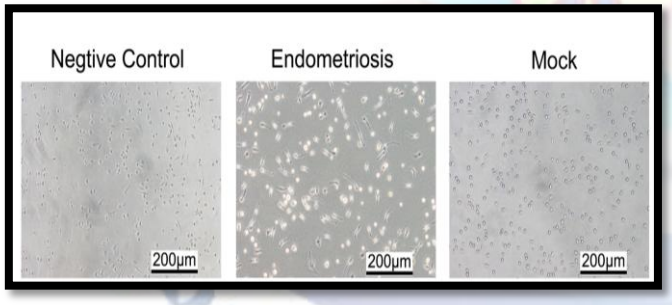


Figure 15: Morphology of peritoneal macrophages

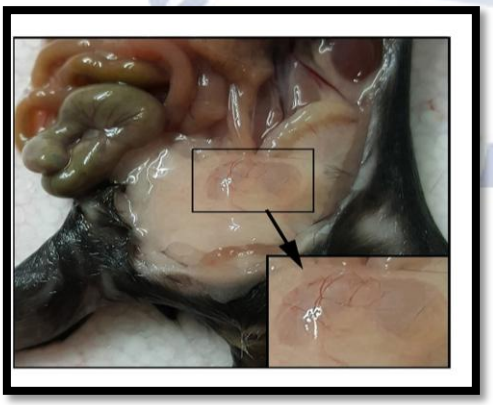


Figure 16: Ectopic endometrial foci in peritoneal cavity of endometriosis mice

The morphology of peritoneal macrophages in endometriosis mice was abnormal as observed under a light microscope when compared with those in the negative control and mock groups (Figure 15) and typical ectopic endometrial foci was found in the peritoneal cavity of endometriosis mice was observed (Figure 16).

V. MICROBES AS A NOVEL DIAGNOSTIC TOOL

It has now become evident that the microbiome that inhabit our human body plays a vital role in regulating various functions like digestion and immune responses [55], building resistance to cancers [64] and also modulating environmental conditions in the human body like pH [65]. Dysbiosis can result in a numerous of diseases on the other hand, a variety of diseases can alter the indigenous microbiota present in specific niches.

It is important that we understand the complex interactions between the microbes and the host to foresee and study a variety of diseases. The female reproductive tract, once believed to be sterile have now shown to host a diverse set of microorganisms [66].

The development of staging models to clinically classify endometriosis, does not provide information on how pathogenesis takes place and how disease development occurs at each stage affecting the physiological systems. It is also unclear how the formation of endometriosis lesions alters the microbiota residing the uterus, cervix, or vagina [61].

Based on the extensive literature available, it is now evident that an individual's microbiome is more than just presence of bacteria in the body. A symbiotic host-microbe relationship is created to regulate the immune system and maintain healthy tissue physiology. Higher fluctuation in any

microbial community leaves the host susceptible to pathogen entry and as well as over- colonization of certain bacteria. This can lead to serious damage to cellular layers, tissues and multiple system impairments, including reproductive efficiency.

A. Microbiome Variation in Endometriosis

The study conducted by [59] showed that vaginal, cervical and gut microbiota composition was found to be similar in between women with stage 3–4 endometriosis and controls in overall but few differences were found in between bacterial groups: (1) Absence of a particular genus *Atopobium in vaginal and cervical microbiota* (2) Increased presenceof*Gardnerella* in cervical microbiota (3) Womenwith endometriosis were found to have *Escherichia* and *Shigella* dominant gut microbiota.

In the vagina and cervix, there is complete absence of *Atopobium* along with the increased presence of *Gardnerella, Escherichia/Shigella* in the cervix.Microbiome of patients with endometriosis could be a relevant discovery done by this study, if corroborated by future research as well. *Atopobium* is a gynaecological pathogen associated with various diseases like bacterial vaginosis, endometrial cancer etc [64,67].

The same study compared the uterine microbiome between women suffering from endometrial cancer and with benign pathologies and has reported high presence of *Atopobium vaginae* in women with endometrial cancer and less in women with benign pathologies [64]. Although the association is unclear, may be the absence of Atopobium is related to the occurrence of endometriosis, which is also a benign gynaecological pathology.

Similarly, the study conducted by [61] showed that a stage III endometriosis patients had a different bacterial community and the cervix of this patient showed depletion in the predominant *Lactobacillus* sp. and *Firmicutes* and *Bacteroidetes* levels were increased. This is possibly due to a significant progression in the disease that is observed in the transition from stage II (mild, superficial lesions) to stage III due to a high inflammatory abdominal environment. This could explain why stage III endometriosis patient had a different bacterial community.

B. Endometriosis Influences Gut Microbiome

Gut microbiota alterations induced by endometriosis, as studied by [62] showed various alterations in the gut microbiome between the endometriosis and control groups. The ratio of *Firmicutes/Bacteroidetes* is an important indicator in assessing the microbial composition in the gut and an elevated ratio was observed in in mice with endometriosis which essentially indicates that endometriosis induced the dysbiosis.

Bifidobacterium (phylum: Acitonobacteria) contributed to the increased level of Acitono bacteria in endometriosis group and *Bifidobacterium* is a commonly used probiotic that plays a significant role in strengthening of the intestinal barrier, modulation of the immune response and pathogen defence by production of antimicrobial compounds or through competition with pathogens for mucosal binding sites [68]. In summary, this study has shown that endometriosis induced changes in gut microbiome in mice and this offers a novel pathway to study the mechanisms involved in the development of various stages in endometriosis.

Gut microbiota can be inherited by environmental factors and influenced by the same [69,70]. Disturbance of homeostasis takes place due to alterations in the microbial communities by the production of their metabolites [71]. A high-fat diet of the mother leads to changes in the gut microbiome of offspring that can lead to behavioural alterations. This can be re-established by reintroduction of a specific commensal bacterial strain [72].

On the contrary, an altered gut microbiota due to endometriosis can be considered as an important factor, as the same microbiome can influence endometriosis by environmental and genetic pathways (Figure 17).

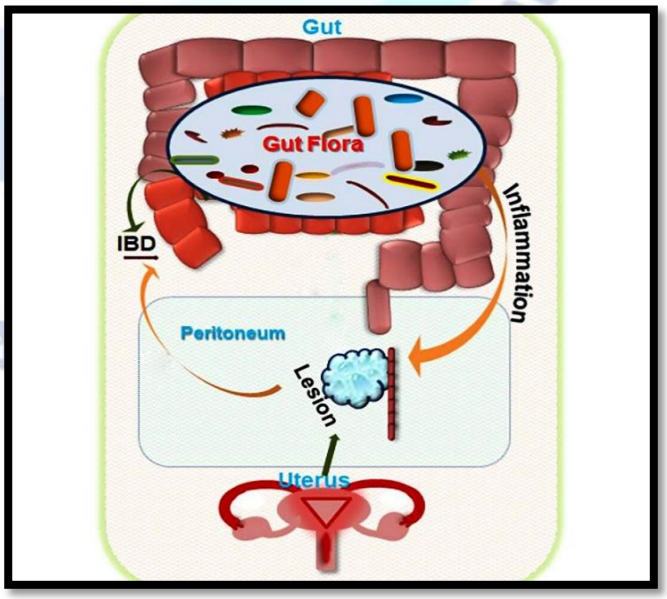


Figure 17: Endometriosis and gut microbiome

It is highly crucial that we understand the microbial community structures in different diseases as this can lead to the discovery of biomarkers for the development of microbial intervention strategies into modern day medicine. The variation of specific microorganisms that were found in endometriosis condition may prove to be potential biomarkers for early detection of the condition.

In the field of individualized medicine, microbiome-associated diseases are considered to be preventable or treatable as it becomes the means to maintain or restore the healthy state of a microbiome. This can be accomplished by the use of unique gut-derived or reproductive-tract derived commensal microorganisms as treatment options to generate a balanced microbiome. Thus, microbiome will prove to be a novel diagnostic tool in order to assess the development of the disease and in treatment as well.

VI. CHALLENGES AND FUTURE STRATEGIES

Microbiota or microbial communities in the human body that influence human health and disease has become the focus of interest due to the various advances in technology and computational biology that has occurred in the past few years. Diagnostic profiling and manipulation of the microbiome in various regions of the body has been gaining acceptance. There is a crucial need for accurate and efficient testing in diagnostic applications as the concept of microbiota profiling grows from research to clinical trials.

A. Challenges

The quality of cohorts that are used in research for studying the links between host microbial communities and the occurrence of diseases is of utmost importance as it is the first step towards potential clinical use. Contaminations during sampling (e.g., From surrounding environment or from a non-target body site of the sampling person) can obscure the analysis as they might lead to false conclusions due to analysis of exogenous micro-organisms not relevant to the study.

The sequencing results are also known to be affected by the protocols used for extraction of nucleic acid. Thus, establishment of high throughput and efficient extraction protocols is necessary from highly heterogeneous starting materials for implementation in clinical diagnostics. Analysis and data interpretation using various bioinformatics tools have given rise to extraordinary opportunities but on a condition that standardization and benchmarking work is carried out efficiently [73].

Microbiome adverse events (MAEs) collectively refer to the ability of microbiome to alter a treatment and a treatment to alter the microbiome. Mitigating MAEs is necessary in order to avoid any negative effects of the treatment. As our microbiomes are closely connected to our basic physiology, diet, and lifestyle and these indirect relationships could help predict MAEs (Figure 18). In fact, many studies have shown that age, health and specific diseases can be predicted from one's microbiome data [74].

Events where treatment directly or indirectly alters the microbiome can have their own negative effects and similarly adverse phenomena mediated by the microbiome exists. One of the examples is chemical modifications of food and drugs that occurs by enzymes in certain bacteria. Many medical interventions are known to have harmful effects on the microbiome and the best-known example is the effect of long-term use of antibiotics [75].

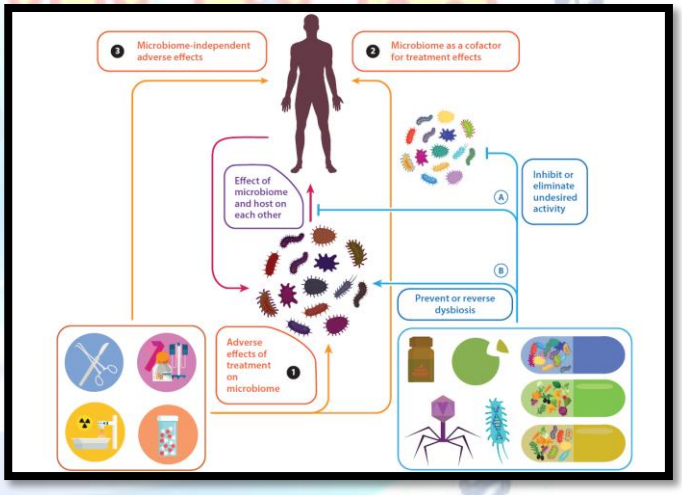


Figure 18: Microbiome adverse effects and their prevention

B. Future Strategies

Understanding the microbiome allows us to use it as an evaluative tool. The relationship between the microbiome and biological processes like diseases within the host helps us to look at microbiome as a robust, cost-effective diagnostic tool to quickly identify abnormalities and to rectify them earlier than expected. Microbiome could also serve as a classification tool using samples collected and assessing its composition as well as to gain knowledge about origin and history of the provided sample.

Bacteria have definite metabolic requirements and thus their metabolites may be used as

biomarkers for indirectly detecting and characterizing the microbiome. Future studies will be required to define relationships between metabolites and microbiome, also to validate these metabolic signatures.

In addition to the use of microbiomes as a means of diagnosis, its use may further be extended as an interventional tool. If a particular microbiome is well understood, there is a possibility to alter the microbiome to attain a desired physiological effect in the host and thus it may be used as a control-device to indirectly intervene in other processes within a microbiome-host system [76].

One of the therapeutic approaches is in the microbiome-based treatment, microbiota becomes the part of the treatment itself, called as faecal microbiota therapy (FMT) or faecal transfer. FMT refers to the administration of a solution of faecal matter from a donor into the intestinal tract of the patient in order to directly alter the patient's microbial composition to produce a health benefit [77]. FMT has been used to treat *Clostridium difficle* infection successfully and there has been an average 87–90% cure rate for more than 500 cases that have been reported [78,79].

In the case of endometriosis, if the disease-associated microbiome is extensively studied, it is possible to identify and alter the specific organisms required to treat the condition. In totality, based on various studies performed, it indicates that the microbiome field is progressively improving. Microbiome markers specific for endometriosis can be used for diagnosis of the disease then analysis of the patient's microbiota may help in predicting the various treatment options.

A personalised treatment strategy can be formulated based on the patient's microbiome profile and it can be administered even as probiotics, prebiotics or specific antibiotics to enhance or deplete the growth of target microorganisms. In the end, it is important that we monitor the treatment- established microbiome if there is any further dysbiosis is occurring.

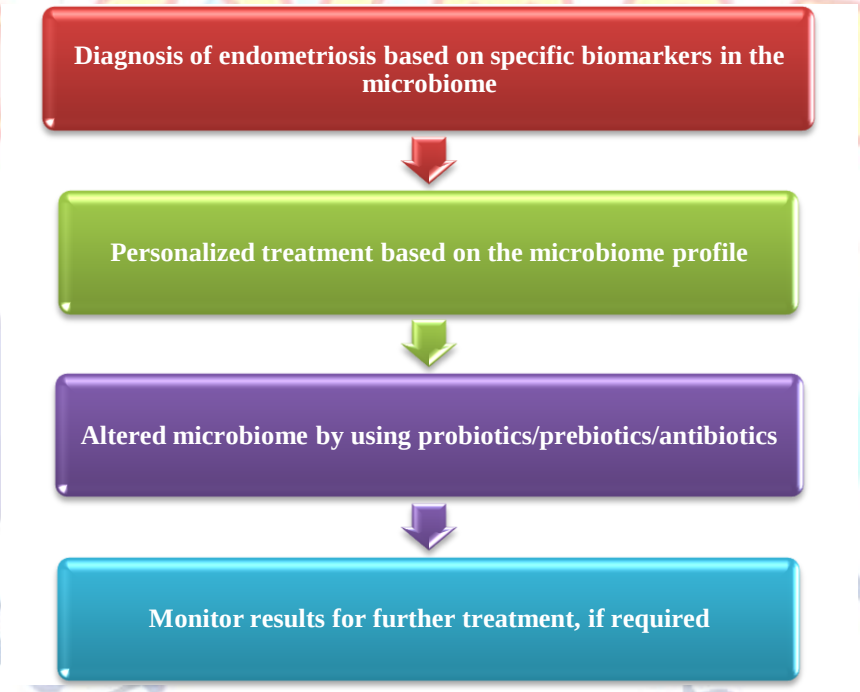


Figure 19: Personalized treatment strategy

VII. CONCLUSION

Endometriosis is a chronic inflammatory disease that is characterized by the presence of endometrial tissue in locations outside the uterus. It affects 10% of reproductive-aged women and 50% of women who suffer from infertility. Various studies surveying the female reproductive tract have confirmed the existence of microbiota starting in the vagina, cervix, uterus, fallopian tubes and ovaries. These microbes play a crucial role at different stages ofthe reproduction, in forming a physical barrier against pathogen and the stimulation of host defence mechanisms. The endogenous female reproductive microbiome uses various direct and indirect mechanisms that kill or inhibit pathogens. Among the theories that have been proposed regarding the pathogenesis of endometriosis, the most widely accepted theory is the implantation theory, where the endometrial

tissue spreads to other pelvic regions by retrograde menstruation.Women suffering from endometriosis lead an impaired quality of life and continue to deal with endometriosis-associated symptoms and complications like infertility, ovarian carcinoma and bowel diseases.Microbes have been known to influence endometriosis at the molecular level which led to the inference that the development of endometriosis could be due to microbial dysbiosis. Women with endometriosis were found to have a substantial increase in *Escherichia coli* in menstrual blood leading to increased endotoxin levels in menstrual and peritoneal fluid which induced further advancement of the disease. Microbes also influence the body's the immune response leading to progression of endometriosis. So, it is crucial that we understand the complex interactions that take place between the host and the microbiome. Comparison of microbiota in cervix, vagina and gut between control and endometriosis women showed significant variation in specific organisms which may prove to be potential biomarkers for early detection of the condition. The relationship between the microbiome and biological processes like diseases within the host helps us to look at microbiome as a robust, cost-effective diagnostic tool to quickly identify abnormalities and analysis of the patient's microbiota may also help in predicting the various personalised treatment strategies.

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