Bacterial Vaginosis Bacterial and Epithelial Cell Adhesion Molecules

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Lactobacilli are member of normal vaginal flora. They maintain low pH of vaginal discharge by metabolising glycogen in vaginal epithelial cell and protects the vagina against the pathogenic microorganisms. However, the vaginal flora can be changed because of birth, taking antibiotics and contraceptive pills. Thus, whereas Lactobacilli population decreases, Gardnerella vaginalis and mixed anaerobic bacteria population increase and this situation causes an infection named Bacterial vaginosis. In this infection, the main diagnostic criteria are clue cells. The clue cell is a squamous epithelial cell which is covered by bacteria. First step of infection progress is adhesion of bacteria to epithelial cell through the adhesion molecules. The most important adhesion molecules of epithelium are cadherins, fibronectins, Toll like receptors and carbohydrates. In bacteria, pilis, lypopolysaccaharide and biofilm have primary importance. In this review, the adhesion molecules are discussed in detail and their roles in formation of clue cell are clarified.

Key Words: Bacterial vaginosis, Clue cell, Cadherin, Pili, Biofilm

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Introduction

Several studies show that Bacterial vaginosis is one of the most common vaginit type in women. This vaginit named bacterial vaginosis is because of anaerobic bacteria causing this vaginit type and although being vaginal discharge there is no inflammation and leucocyte.[1,2] The most important bacteria that cause bacterial vaginosis is *Gardnerella vaginalis*. A lot of anaerobic bacteria such as *Mobiluncus* spp, *Bacteroides* spp., *Ureplasma urealyticum, Peptostreptoccus* spp, *Mycoplasma hominis*, *Fusobacterium*, *Atopobium vaginae, Prevotella bivia, Veillonella* spp., and *Peptoniphilus* spp., also cause this infection.[3,4]

Clue cell which is prior microscobic finding of bacterial vaginosis was first named by Gardner and Dukes.[5] (Figure 1) When *Lactobacilli* population decreases, servico-vaginal epithelial cells are covered by mixed anaerobic bacteria population and these cells become clue cells. *Lactobacillus* spp. maintains the vaginal discharge at 4 - 4.5 pH level by metabolizing glycogen in the vaginal epithelial cells and they protect the vaginal mucosa against the pathogenic microorganisms. In addition, they prevent the growing pathogenic bacteria by producing H_2O_2 and bacteroicin, but absence of *Lactobacillus* spp., mixed anerobic bacteria population increases and this causes bacterial vaginosis.[6] A recent study suggests that bacteriophages can determine increasing *Lactobacilli* population. These phages are first isolated in 1997. Finding the phages in men urogenital system also support that idea and they reported smoking reduces the induction of phages. Accordingly, they suggest phages can cause absence of *Lactobacilli* population by infecting them and the reason for this *G. vaginalis* and mixed anaerobic bacteria population increases.[7] The other important diagnostic criteria are fishy odour, decresased *Lactobacilli* population, vaginal pH > 5 and absence of leucocyte.[3,5]

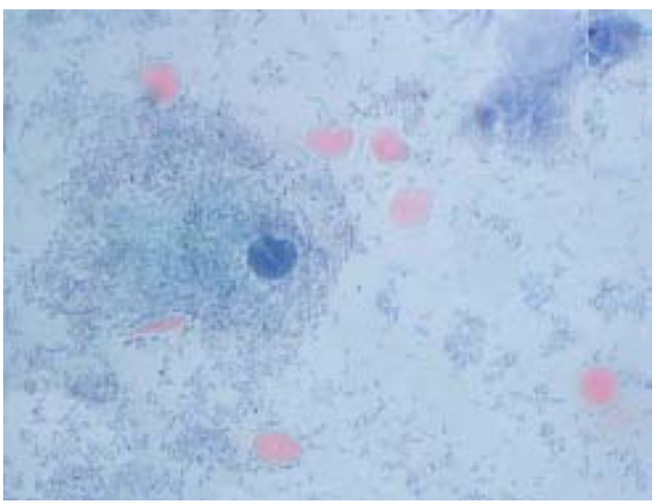

Figure 1: Squomus epithelial cell which is covered by mixed anaerobic bacteria. This cell is named clue cell.

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Bacterial vaginosis is also a serious disorder in pregnant women. Recent studies show that this vaginit causes several complications such as pelvic inflammatory disease (PID), abortus, preterm delivery, low weight delivery, postpartum endometrititis, amnion fluid infection and early membrane rapture.[3,7]

In this review, to understand this infection which cause a lot of serious disorder better, epithelial cell and bacterial adhesion molecules will be discussed in details. These molecules play an important role in formation of clue cell.

Epithelial Cell Adhesion Molecules

Human vagina and ectoservics are covered by stratified squomus epithelium. Stratification of this epithelium and tight junctions protect the epithelial cells against viral and bacterial microorganisms. Several adhesion molecules participate in tight junctions among epithelial cells and these molecules will be discussed in details.[8]

Cadherins

Cadherins are cell-cell adhesion molecules that belong to transmembrane protein family. They constitute about 10-15 subfamilies. E- cadherin (uvomorulin) is expressed in vaginal epithelial cells and it is a member of Type I cadherins. These proteins comprise three domains. There is an extracellular domain named amino-terminal domain, a transmembrane domain in cell membrane and carboxy-terminal domain in the cytosol (Figure 2). [9] Amino-terminal domain is responsible for cell-cell adhesion. In this domain histidin, valin and alanin are involved in adhesion by making dimmers.[10] Ca^{+2} is an essential molecule for adhesion because amino-terminal domain is activated by binding these molecules. In the absence of Ca^{+2} molecules E-cadherins are in active.[9,11] Even the subfamily of cadherins have same structure, they can adhere different cells by changing aminoacid sequences in amino-terminal domains.[11]

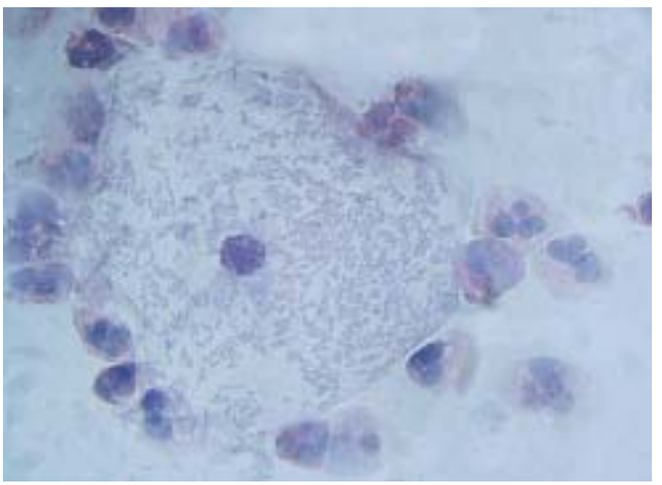

Figure 2: False clue cell. Lactobacillus spp. morphotype no gram negative or gram variable rods.

The carboxy- terminal domain binds catenins in cytosol. Catenins are intracellular binding proteins. These intracellular proteins bind actin myoflaments and they compose adhesion zone together.[9]

Adhesion zone are abundant in basal and parabasal cells. Therefore, tight junctions are like barriers which protect the stratified epithelium. However, some researchers suggest that as squomus epithelial cells mature, cadherins expression decrease because of pyknotic nucleus. Thus, they are exfoliaded.[9,12] Recent studies show that E-cadherin expression increases during inflammatory prosess whereas, in neoplastic prosses it decreases.[13]

A research used in immunocytochemistry shows that *G. vaginalis* and *Bacterioides* spp. cause altered expression in E-cadherin. In this study, *G. vaginalis* makes cadherin and catenin expression increased but while cadherin expression undergoes down regulation because of *Bacteroides* spp., catenin expression undergoes upregulation. They suggested that immune response and increased cytokine molecules can cause these changes.[14] Also it is suggested that cadherins can involve in formation of clue cell by recognising bacteria.

Carbohydrates

On the epithelial cell membrane surface, there are many carbohydrates attached to the perihebral and integral proteins. These carbohydrates serve as a receptor for many pathogenic bacteria. Bacteria adhere to epithelial cell by recognizing these molecules and they cause infection. Studies show that carbohydrates can involve in adhesion of *G. vaginalis* to epithelial cell. In a study, after treatment of clue cell with sodium-meta-periodate which destroys the C-C bond between hdyroxl groups of carbohydrates, adhesion of *G. vaginalis* to epithelial cell was inhibited. Thus, they suggested carbohydrates play a part in adhesion. However, findings were insufficient for determining which molecules is necessary for adhesion.[15] On the other hand, after a couple of years N-acytl-galactoseamine and D-galactoseamine receptors were found on *G. vaginalis* cell wall so it was suggested carbohydrates of epithelial cell can involve in adhesion by recognizing these receptors. Also they suggested that other mechanisms can take part in adhesion of *G. vaginalis* to epithelial cells.[16]

Toll Like Receptors

Toll gene was first described in drosphila and it is required for dorsa-ventral polarity in developing embryo. Some genetic researches show that a protein which homologue of the drosophila toll gene was identified in human so it was named toll like receptor (TLR). There are 10 members in TLR family.[17]

TLR4 is expressed in servicovaginal epithelium. It activates several signal pathways by recognizing bacterial ly-

popolysaccaharide and it creates immune response. Hence, TLR is important for immunity.[18] It has been demonstrated that TLR4 recognize lypopolysaccaharide on *G. vaginalis* cell wall and it is responsible for increasing cytokine in epithelial cell.[19] It is possible that during the formation of clue cell, TLR4 binds lypopolysaccaharide on *G.vaginalis* cell wall.

Fibronectin

Fibronectin is a extracellular matrix protein. It involves in bacterial adhesion by binding epithelial cells. These proteins are high molecular weight glycoproteins.[20]

Fibronectin is found soluable form in the body fluid and insoluable form in the extracellular matrix.[21]

These glycoproteins bind basal cells trough integrins.[22] Basal cells have mitotic activity and they are first cell line of stratified epithelium. *Lactobacillus* spp. adheres to vaginal epithelial cells by binding fibronectin. With this way, they cause false clue cell.[6] (Figure 3)

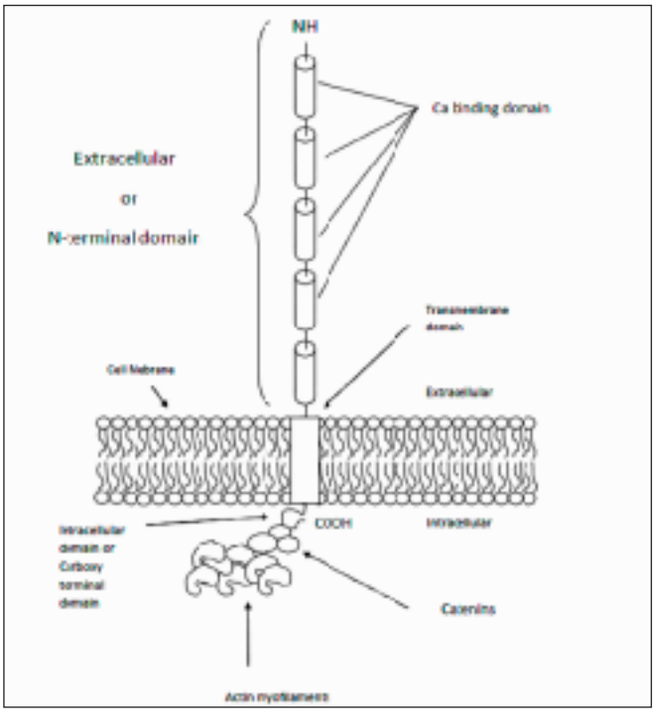

Figure 3: Cadherin structure. Ca+2 is important in adhesion. Catenins are bound actin myofilaments.

Bacterial Adhesion Molecules

Lypopolisaccharide

The cell envelope of Gram negative bacteria comprise three layers. These layers are cell membrane, periplasmic zone and outer membrane. Cell membrane has similar structure of biological membranes and it consists of lipid and proteins. In periplasmic space, there is a peptidoglycan layer. This layer is also named murein. Murein layer made of glycan strands consisting N-aceytlglucoseamine and N-acetylmuramicaside. In Gram positive bacteria, murein layer is thicker. Outer membrane takes part in adhesion. Peptidoglycan layer and outer membrane form cell wall together.[23,24] Outer membrane contains protein, phospholipid and as well as variable amounts of lypopolisaccaharide (LPS). LPS is highly antigenic and it plays an important role in pathogenesis of Gram negative bacteria. This molecule is a chain and it contains carbohydrate. LPS extends towards to outside from the outer membrane with carbohydrate chains. Head of this chain is named O - specific polysaccharide or O- antigens.[25,26] Lipid A is lipid part of lypopolisaccaharide. It is a ligand for TLR4 and creates an immune response in host cell by recognizing this receptor. Also, O antigens binds epithelial cell and involve in immune response.[27]

Pili

To infect a cell, adhesion is an important step for pathogens and pili prior adhesion molecule for bacteria. Pili is a fibrous molecule.[28] Gram (-) bacteria and gram (+) bacteria have different pili type. In this review, we discuss pili of gram (-) bacteria, for the organisms involving in formation of clue cell have this pili type. Presence of pili was demonstrated with electron microscopy in *G. vaginalis* and *Bacteroides* spp.[29,30] Studies show that *G. vaginalis* has Type I pili.[31] At first, it was suggested that *G. vaginalis* can adhere vaginal epithelial cell trough pili. After couple of years, this idea was confirmed and adhesion of *G. vaginalis* to epithelial cell was demonstrated with electron microscopy.[32] Besides, type I pili is recognized by TLR4 and it causes increasing of inflammatory cytokine.[33]

In addition to type I pili, gram (-) bacteria have curly pili, Type IV pili and trimeric ototransporter adhesions. Also, it is suggested that gram (-) bacteria have a lot of different pili types. Curly pili contains abundant starch, so they are very sticky. Trimeric ototransporter adhesions can adhere host cell or extracellularmatrix protein. Adhesion mechanism of Type IV is still unknown.[33]

Biofilm

Biofilm is a complex aggregation of microorganisms embedded within a extracellular polysaccharide (extracellular polymeric material) on a solid surface. Extracellular polysaccharide (EPS) consists of abundant carbohydrate. Besides this molecule, aminoacids and water molecules are contained too. Furthermore, it was demonstrated that the major EPS of *E. coli* include colanic acid.[34,35] Bacterial biofilms are associated with several recalcitrant infections such as endocarditis, periondantitis, pelvic inflammatory disease and bacterial vaginosis. Moreover, it can form on catheters, silicons, contact lenses, intra uterine device and surgical instruments.[36]

Bacterial adhesion is first step of biofilm formation.

Bacteria adhere to solid surface trough the pili.[35] Also, pili ensure conjugation of bacteria. During the conjugation bacteria undergo adaptation. Some of these adaptation are UV resistance, increased rates of genetic exchange and increased secondary metabolite production.[37] EPS is produced after getting signal named "quorum sensing". This signal means that bacteria reach enough population to form a biofilm. Acylhomoserin lactones are one of the quorum sensing molecules.[35] Activation of these molecules is still unknown.

The last step of biofilm formation is detachment of bacteria. Detachment signal is also mystery. However, it is suggested that insufficient food might be one of these signals.[38] Biofilm involve in antibiotic resistance. It is believed that EPS prevents to reach antimicrobial agents to the bacteria.[38]

It was stated that in bacterial vaginosis, *G. vaginalis* strains which is attached to squomus epithelial cell, can form biofilm.[3,39] Studies show that during the infection, in this biofilm predominant organism is *G. vaginalis*. Its concentration reaches up to 10^{11} bacteria per millimeter.[3] Another study shows that after standard therapy with metranidazole, bacterial biofilm was determined and it is suggested that biofilm formation is cause of relapsing bacterial vaginosis.[39]

Bakteriyel Vaginosis: Bakteri ve Epitel Hücre Adezyon Molekülleri

İnsan vajinal florasında yaşayan laktobasiller epitel hücrelerin glikojenini kullanarak ortam pH'sını 4-4,5 gibi asidik bir değerde tutmakta ve vajinal florayı çeşitli mikroorganizmalara karşı korumaktadırlar. Fakat doğum, antibiyotik ve doğum kontrol haplarının kullanımı gibi çeşitli nedenlerle floranın dengesi bozulmakta ve laktobasillerin sayısı azalmaktadır. Bu durumda da ortama başta *Gardnerella vaginalis* olmak üzere çeşitli anaerobik bakteriler hakim olmakta ve bakteryel vajinozis dediğimiz enfeksiyon meydana gelmektedir. Bu enfeksiyonun en önemli tanı bulgusu da "clue cell" adını verdiğimiz üzeri bakterilerle kaplı skuamoz epitel hücrelerinin görülmesidir. Enfeksiyon oluşumunun ilk basamağını ise adezyon molekülleri ile bakterilerin epitel hücreye yapışması oluşturmaktadır. Bu basamakta da çeşitli adezyon molekülleri görev almaktadır. Epitel hücre zarındaki adezyon moleküllerinden en önemlileri kaderinler, fibronektinler, Toll benzeri reseptörler ve karbonhidratlardır. Bakterilerde en önemli adezyon molekülleri ise pili, bakteri hücre duvarı yapısında bulunan lipopolisakkarit ve biyofilmdir. Bu derlemede adezyonda görev alan bu moleküllerin yapısına detaylı olarak değinilecek ve clue cell oluşum mekanizmasındaki rolleri açıklanacaktır.

Anahtar Kelimeler: Bakteryel vajinozis, Clue cell, Cadherin, Pili, Biofilm

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