Invasive cervical cancer is often associated with bacterial vaginosis (BV) caused by both non-pathogenic and pathogenic bacteria and other microorganisms. BV goes unnoticed during the clinical course of cervical cancer. The incidence of BV is very high among women from developing countries with poor genital hygiene. Maintenance of normal vaginal ecosystem in healthy vagina is through control of optimal ratio between non-pathogenic and pathogenic organisms. Cervical cancer causes disruption of the normal vaginal and cervical mucosa leading to alteration of ratio between commensals and pathogenic organisms. The natural vaginal microflora is being dominated by lactobacillus species compared to pathogenic aerobic and anaerobic microorganisms. Disruption of vaginal microenvironment leads to change in vaginal flora and associated inflammation. There are very limited literature available on the exact incidence of BV in cervical cancer patients. Increased nitrosamine content in BV could possibly progress to higher DNA damage, change in cytokine profile thus compromise immune defence against human papilloma virus infection. Both radiotherapy and chemotherapy are potential immunosuppressive agent thus could facilitate spread of endogenous bacteria to manifest as poor outcome to treatment secondary to tissue hypoxia and hypoperfusion. This article is a review of current literature associated with BV in cervical cancer.

Keywords: Bacterial vaginosis; Cervical cancer; HPV infection; Treatment; Outcome

Introduction

Cervical cancer is the second common malignancy after breast cancer among women which accounts for more than half a million new cases and quarter of a million deaths annually, and also the second most common virus-related cancer among women in developing countries [1]. A report in 2004 indicated that there were approximately 12,000 cases of invasive cervical cancer with 3,850 deaths among American women [2]. However, prevalence of cervical cancer reported by World Health Organization (WHO) in developing countries remains incomplete and fragmentary. Infection of high-risk human papillomavirus is strongly associated with the development of invasive cervical cancer and its precursor lesions, cervical intraepithelial neoplasia (CIN) [3]. In fact, not all of HPV infection leads to cervical cancer, suggesting that other cofactors could be present in the development of malignancy. Thus, other known risk factors including young age at first intercourse, cigarette smoking, race, multiparity, infection with sexual transmitted disease agent such as Chlamydia trachomatis, herpes simplex virus (HSV) and bacterial vaginosis (BV) [4, 5] were recognized. BV is a common vaginal disorder among women resulted in reduction of naturally occurring lactobacilli growth (normal flora) and is often replaced with a mixed, predominantly anaerobic bacteria consisting of Gardnerella vaginalis, Mobiluncus spp., Atopobium vaginae, Mycoplasma hominis, Bacteroides spp. and Prevotella spp. [6, 7]. This situation may occur when microenvironment of vagina is disturbed and lead to certain clinical presentation including foul smelling discharge, alkaline vaginal pH > 4.5, a positive amine “whiff” test and the presence of clue cells on a wet smear [8]. These are the so-called “Amsel criteria” [9]. Local growth harbor bacterial growth in the vaginal cavity, leading to pelvic inflammatory disease and hydrometra.

BV is known to be associated with some gynecologic and obstetric complications such as postoperative infections, preterm labor during delivery, pelvic inflammation disease (PID), cervicitis, chorioamnionitis and premature rupture of membranes [10]. As abnormal microflora can produce carcinogenic nitrosamines and stimulate the release of cy-
tokines, such as interleukin-1β, it has been suggested that BV may be important in the development of cervical cancer [11]. Carcinogenic nitrosamines increase the probability of DNA damage and an altered cytokine profile may reduce immune’s system ability to eliminate HPV infection. Thus, the changes may create a conducive environment for cancer development [12]. The Centers for Disease Control and Prevention (CDC), which is a part of the principal agency in the United States government for protecting the health and safety of all Americans and for providing essential human services, have included BV on their list of emerging infectious diseases [13].

Incidence and Prevalence of BV in Cervical Cancer

So far, the association of BV and development of cervical cancer is still remain unclear and conflicting. Some studies show the association of bacterial vaginal infection with cervical cancer [14] and other studies show no association at all [15]. However, the possibility still exist that BV is in some way associated with the development of CIN, as a co-factor to human papillomaviruses (HPV). Therefore, BV was taken into consideration in series of studies on CIN [10, 16]. In previous studies, the prevalence of BV in patients varies; 32-64% from STD clinics, 12-25% from gynecology outpatients clinics, 10-26% from antenatal clinics [17-20] in their reports indicate the similarity of BV prevalence in both, 18% of women with squamous intraepithelial lesion (SIL) and 12% of women without SIL. However, a higher rate of BV (33%) was found among women with high-grade SIL [20]. Besides, study in Japan by Mikamo et al [21] indicates that 50% of Gardnerella vaginalis and 80% of BV including other co-existing bacterial species was detected in cervical cancer patients. The different prevalence of BV among these studies might be due to that different technique, diagnostic criteria and clinician’s opinions in each study (Table 1) [15, 22-32]. For example, the prevalence obtained using Nugent’s criteria was consistently higher as opposed to studies using clinical “Amsel criteria” [7].

Pathogenesis

The pathogenesis of BV in cervical cancer is complex. BV

<table>
<thead>
<tr>
<th>Year of publication</th>
<th>Authors</th>
<th>Country</th>
<th>Age range (Years)</th>
<th>HPV Prev (%)</th>
<th>BV Diagnosis</th>
<th>BV Prev (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1995</td>
<td>Peters et al</td>
<td>Netherlands</td>
<td>20-66</td>
<td>71.1</td>
<td>Amsel</td>
<td>20.0</td>
</tr>
<tr>
<td>1997</td>
<td>Sikstrom et al</td>
<td>Sweden</td>
<td>-</td>
<td>6.8</td>
<td>Amsel</td>
<td>13.0</td>
</tr>
<tr>
<td>2001</td>
<td>Castle et al</td>
<td>Costa Rica</td>
<td>-</td>
<td>59.6</td>
<td>Nugent</td>
<td>37.8</td>
</tr>
<tr>
<td>2003</td>
<td>Mao et al</td>
<td>USA</td>
<td>18-24</td>
<td>22.8</td>
<td>Amsel</td>
<td>3.0</td>
</tr>
<tr>
<td>2003</td>
<td>Boyle et al</td>
<td>UK</td>
<td>16-58</td>
<td>21.1</td>
<td>Amsel</td>
<td>30.9</td>
</tr>
<tr>
<td>2004</td>
<td>da Silva et al</td>
<td>Brazil</td>
<td>15-35</td>
<td>50.0</td>
<td>Amsel</td>
<td>34.6</td>
</tr>
<tr>
<td>2005</td>
<td>Watts et al</td>
<td>USA</td>
<td>-</td>
<td>56.1</td>
<td>Nugent</td>
<td>43.7</td>
</tr>
<tr>
<td>2005</td>
<td>Samoff et al</td>
<td>USA</td>
<td>13-19</td>
<td>53.5</td>
<td>Nugent</td>
<td>47.2</td>
</tr>
<tr>
<td>2008</td>
<td>Figueiredo et al</td>
<td>Brazil</td>
<td>-</td>
<td>84.2</td>
<td>Clue cells</td>
<td>17.0</td>
</tr>
<tr>
<td>2009</td>
<td>Verteramo et al</td>
<td>Italy</td>
<td>17-58</td>
<td>31.0</td>
<td>Amsel</td>
<td>6.3</td>
</tr>
<tr>
<td>2009</td>
<td>Nam et al</td>
<td>South-Korea</td>
<td>-</td>
<td>69.1</td>
<td>Amsel</td>
<td>11.0</td>
</tr>
<tr>
<td>2009</td>
<td>Rahkola et al</td>
<td>Finland</td>
<td>18-69</td>
<td>53.3</td>
<td>Clue cells</td>
<td>15.2</td>
</tr>
</tbody>
</table>

Reproduce from Gillet et al [7]. Association between BV and CIN: systematic review and meta-analysis. BMC Infectious Disease 2011;1-9.
is not attributable to a single causative organism but it’s due to immense overgrowth of composite of flora including *G. vaginalis, Peptostreptococci, Bacteroides spp., Mobiluncus spp., Mycoplasma* and *Ureaplasma urealyticum. Fusobacterium* and *Atopobium vaginae* are also commonly found in BV. Inflammation plays a little role since this disease is due to the disruption of the vaginal microenvironment rather than a true tissue’s infective state. This overgrowth shifts the balance from Lactobacillus-predominated vaginal ecosystem to microenvironment with anaerobic bacteria dominance. This conclusion is corroborated by experimental studies conducted in humans and animal models that demonstrated upon vaginal inoculation with a single vaginosis-causing bacterial species, BV will rarely occur.

The factors that lead to anaerobes bacterial overgrowth remain uncertain. Increased availability of the substrates, raised in pH and loss of *Lactobacillus* spp. were implicated for BV because the vagina of normal women were predominantly inhabited by *Lactobacillus* spp. that produced hydrogen peroxide which play an important role in suppressing the overgrowth of anaerobes either directly or by producing a hydrogen-halide complex catalyzed by naturally-occurring cervical peroxidase. Following the anaerobic bacteria overgrowth, there will be an elevated polyamine production by the anaerobes, enhanced by the decarboxylases which broke down the vaginal peptides into polyamines. These polyamines along with vaginal organic acids (acetic and succinic acids) are cytotoxic to the vaginal cells, leading to the vaginal cell’s exfoliation manifesting as vaginal discharge which is malodorous and volatile due to its high amine content.

It has been hypothesized that BV results in cervical cancer through an increase in vaginal nitrosamines and altered cytokine profiles [12]. The presence of raise nitrosamines in vagina will lead to higher probability of DNA damage and change in cytokine profiles will cause altered response of immune system to clear up HPV infection. Therefore, BV will interact with HPV infection with the consequence of higher risk of developing cervical cancer among those with BV and HPV co-infection than those with a mono-infection. However, other infections for instance Candida was not associated with the development of CIN or cervical carcinoma [33]. Nevertheless, the causal relationship between BV-HPV interaction and cervical cancer development may not be entirely infallible since the causation may be bidirectional due to reverse causality. Owing to the relative immunodeficiency state in advanced cancer stage, BV thrives and therefore seems to be antecedent factor of cervical carcinoma whilst in fact the opposite is true. It’s equally coherent to postulate that BV-HPV infection is a bona-fide cause of cervical carcinoma. Unless there’s a properly executed and methodologically sound cohort study, the temporal order between BV-HPV and cervical carcinoma cannot be fully ascertained.

Besides that, BV is diagnosed by a multitude of diagnostic criteria. The most common ones used are *Amsel* and *Nugent* diagnostic criteria (Table 2) [34]. However, several studies for example two studies conducted in Netherlands, have used a unique diagnostic system called KOPAC to diagnose BV which was subsequently termed as vaginal dysbacteriosis, a completely different concept since its diagnosis is based on microscopic appearance rather than clinical *Amsel*

### Table 2. *Amsel* and *Nugent* Diagnostic Criteria

<table>
<thead>
<tr>
<th>Amsel criteria</th>
<th>Nugent Score criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amsel criteria is a criteria for clinical diagnosis of bacterial vaginosis. The diagnosis is made if three of the following four criteria are positive:</td>
<td>The Nugent Score is a Gram stain scoring system for pap tests to diagnose bacterial vaginosis. The Nugent score is calculated by assessing for the presence of:</td>
</tr>
<tr>
<td>1) Homogeneous vaginal discharge (color and amount may vary).</td>
<td>1) large Gram positive rods (<em>Lactobacillus</em> morphotypes; decrease in <em>Lactobacillus</em> scored as 0 to 4).</td>
</tr>
<tr>
<td>2) Amine (fishy) odor when potassium hydroxide solution is added to vaginal secretions (commonly called the “whiff test”).</td>
<td>2) small Gram-variable rods (<em>Gardnerella vaginalis</em> morphotypes; score as 0 to 4).</td>
</tr>
<tr>
<td>3) Presence of clue cells (greater than 20%) on microscopy.</td>
<td>3) curved Gram-variable rods (<em>Mobiluncus</em> spp. Morphotypes; score as 0 to 2) and can range from 0 to 10. A score of 7 to 10 is consistent with bacterial vaginosis.</td>
</tr>
<tr>
<td>4) Vaginal pH greater than 4.5.</td>
<td></td>
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</table>
or Nugent criteria. Therefore, the heterogeneity of diagnostic criteria further hampered the unifying conclusion on the causative potential of BV as cervical carcinoma’s etiology.

**Relation to Host Immunity**

Patients with CIN and BV exhibited raised level of interleukin (IL) 1, IL-6, IL-8, IL-10 and nitric oxide (NO) in both endocervical (EC) and vaginal secretions. Apart from that, bacteria in BV made negligible amount of lactic acid, while producing massive amount of immunomodulatory substances such as proteases, sialydases, succinate and possesses other inflammatory-inducing components for instance lipoteichoic acid (LA), peptidoglycans (PG) and lipopolysaccharides (LPS). These proinflammatory cytokines produced in response to the above substances and components promote the growth of neoplastic cells both *in vitro* and *in vivo*. Besides that, IL-6 and IL-8 have proangiogenic properties which encouraged the tumorigenesis of solid tumor such as non-small cell carcinoma of the lung and glioblastoma. Therefore, the elevated level of IL-6 and IL-8 may play a role in tumor angiogenesis and driver of neoplastic growth. Nevertheless, the inflammatory events might also be incited by other factors such as personal hygiene and sexual activities, and therefore these two factors were also cofounders for cervical carcinoma. Therefore, the purpose of establishing BV as the main driver for the activation of inflammatory cascades with ensuant development of cervical carcinoma remains elusive.

The role of infectious agents, particularly BV in upregulating integrin expression should also be elucidated. It has been recognized that integrin expression was regulated by cytokines such as transforming growth factor beta (TGF-beta). The signaling pathway of TGF-beta was hypothesized to be activated by various microbial components acting through Toll-like Receptor (TLR). Upon activation, TGF-beta will stimulate the expression of integrin which plays a major role in cervical carcinoma pathogenesis by recruiting and phosphorylating focal adhesion kinase (FAK), which is in turn activating Src and c-Jun N terminal kinase (JNK) together with P-I3K phosphorylation. This will culminate in the activation of protein Kinase B/AKB, Rac and extracellular signal regulated kinase (ERK). Since these events regulate the cell cycle, upregulation of beta 6 integrin will promote tumorigenesis through perpetuating the proliferation and survival of cancer cells.

**Difference Between Commensals and Invasive Bacterial Infection**

In female genital tract the differences between commensals and pathogens which may lead to invasive bacterial infection is complex. The essence of the argument lies in the fact that pathogens are not only defined by the microorganism’s type and its intrinsic virulence but also by the intricacies of the microfloral ecosystem in the vagina which is dependent on the relative dominance of bacterial species in the vaginal tract. According to the conventional views, a pathogen is defined as a microbe which possesses genetically determined factors that when expressed will result in diseases in susceptible individuals. However, BV does not follow the traditional route of definition for pathogens since it does not conform to Koch’s postulates; no single agent was noted to reliably cause the disease when inoculated into a healthy woman and no animal model can be sufficiently developed due to constant adaptation of the bacterial species when it comes to life. Therefore, the traditional orthodoxy of monomicrobial approach proposed by Koch is utterly challenged.
therapy is administered using cisplatinum 40 mg/m² every ing a course of radiotherapy, concurrent systemic chemo -intracavitary brachytherapy is delivered in various fraction-
tion (usually ~50 Gy) is delivered using high energy photon
to the tumor volume and including lymphatic region. Then
radiotherapy includes a combination of external beam
radiotherapy a homogenous dose of radia-
the radiotherapy followed by intracavitary brachytherapy. Dur-
radiotherapy in spread of urinary tract infection. Thus, cli-
clinical conditions. Further researches on BV focus
worldwide requires a compulsory screening and treatment in
The association of BV in cervical cancer among women
References
2. US Cancer Statistics Working Group. United States
4. Castellsague X, Bosch FX, Munoz N. Environmental
cancers. A prospective study from India showed 75% bacte-
G. vaginalis, which have been isolated from high vaginal swab (HVS) (Fig. 1).
There was no change in the bacterial count post radiotherapy

by the polymicrobial nature of BV.
The main factor that will influence the shift from com-
mensal-predominant ecosystem to BV is pH. Most of the
main organisms associated with BV, for instance G. vagina-
alis, anaerobes and M. hominis proliferate in high vaginal pH
(pH > 4.5). Factors associated with this are changes in hor-
monal level especially during period nearly menstruation, semen, race and other differences in physiologic response.

Implication to Treatment
The mainstay of treatment in carcinoma of the cervix is
radical radiotherapy and concurrent chemo-radiotherapy.
The radiotherapy includes a combination of external beam
radiotherapy followed by intracavitary brachytherapy. Dur-
ing external beam radiotherapy a homogenous dose of radia-
tion (usually ~50 Gy) is delivered using high energy photon
to the tumor volume and including lymphatic region. Then
intracavitary brachytherapy is delivered in various fraction-
ation (8 Gy in 3#HDR or 30 Gy in 1#LDR) schedules using
three-dimensional (3D) conformal treatment planning. Dur-
ing a course of radiotherapy, concurrent systemic chemo-
therapy is administered using cisplatinum 40 mg/m² every
week for 5 weeks. Addition of chemotherapy helps to im-
prove radiosensitivity of tumor and sterilize micrometasta-
ases outside the radiation zone. Exposure of radiation therapy
and chemotherapy to the bone marrow and lymphatic region
could lead to immunosuppression and depression of leuco-
cytes count. Therefore in presence of bacterial infection in
the vaginal canal or endocervix could lead to spread of in-
fec tion to the pelvic tissue causing pelvic inflammatory dis-
erases. Infection in the pelvic region result in inflammation,
tissue edema and vascular compromise. Vascular obstruction
could result in hypovascularity of tumor and increasing
hormou hypoxia. Hypoxic tumors are radioresistant thus
the outcome of radiotherapy treatment could be suboptimal
leading to local recurrence. The intensity of chemotherapy
also affects on peripheral blood lymphocyte (PBL) popula-
tion by lowering its level and this will increase in oppor-
tunistic infections in patient treated for cancer. Brunvard et
al [35] reported decreased in CD4+ count and concurrent
pneumocystis pneumonia in two women treated with multi-
agent chemotherapy and radiation therapy for breast cancer.
Few clinical trials have been studied to evaluate pattern of
vaginal bacterial growth during radiotherapy. Gilstrap et al
[36] evaluated the vaginal and rectal aerobic bacterial flora
among 12 gynecologic cancer patients undergoing pelvic ra-
adiotherapy. They isolated 59% and 27% Gram positive cocci
(GPC) and Gram negative bacilli (GNB) respectively from
the vaginal isolates. For example of GPC was Staphylococ-
cus aureus, and for GNB was Klebsiella pneumonia, which
have been isolated from high vaginal swab (HVS) (Fig. 1).
There was no change in the bacterial count post radiotherapy

Similar study by Gordon et al [37] also did not find any
change in the microbial flora before, during and after pelvic
radiotherapy. However, in individual patients over 50% mi-
croorganisms found in initial culture were no longer present
at the completion of radiotherapy.
A study compared the bacteriologic outcome of three
groups of patients on water (22), povidone-iodine (22) and
lactic acid vaginal douche. The microbiologic evaluation
was preformed on day-0, day-20 Gy and day-40 Gy of treat-
ment. Klebsiella spp., Staphylococcus spp., Pseudomonas
pyocynaе and Streptococcus fеcаlis were grown most fre-
quently. The maximum suppression of bacterial growth was
encountered in lactic acid vaginal douche group compared
to povidone-iodine or water douche only [38]. Bialas et al
[39] from Nottingham General Hospital evaluated urinary
tract infection before and during pelvic radiotherapy among
172 patients without urinary instrumentation. They observed
17% bacterial infection at the outset and additional 17%
during pelvic radiotherapy thus emphasize the influence of
radiotherapy in spread of urinary tract infection. Thus, cli-
ricians use empiric antibiotics for prevention of pelvic in-
fec tion during a course of pelvic radiotherapy in carcinoma
cervix. A prospective study from India showed 75% bacte-
rial infection rate and resistance to ciprofloxacillin (38%),
levofloxacillin (32%) and cephalosporine (22%) respec-
tively [40]. Therefore, authors recommend use of antibiotics
based on microbiologic profile rather than on empiric basis
to avoid antibiotic resistance.

Conclusion
The association of BV in cervical cancer among women
worldwide requires a compulsory screening and treatment in
different clinical conditions. Further researches on BV focus
pathogenesis and treatment might be important to expand our
knowledge on BV and its association with cervical cancer.

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