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# Comparison and Association between Different Types of Vaginitis and Risk Factors among Reproductive Aged Women in Lahore, Pakistan: a Cross-Sectional Study

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# HIGHLIGHTS

- Aerobic vaginitis (AV) is a least explored disease in Pakistan.
- AV is the predominant cause of vaginitis followed by bacterial vaginosis (BV), vulvovaginal candidiasis (VVC) and trichomonas vaginitis (TV).
- AV co-occurs significantly in higher frequency with BV and VVC as compared to TV.
- Patients with history of miscarriage are more prone to AV and BV.
- Unawareness and low socioeconomic conditions predispose women to TV.

**Abstract:** Mixed vaginitis is escalating worldwide and complicates the diagnosis and treatment of vaginitis. Several risk factors have been associated with prevalence of vaginitis. Prevalence and associated risk factors of different types of vaginitis especially, aerobic vaginitis (AV) are less known. A cross-sectional study was conducted to compare the prevalence and association among different types of vaginitis and risk factors in reproductive aged women (n=300) visiting Jinnah Hospital, Lahore for complaints of vaginal discharge. Data

on risk factors was collected through face-to-face interviews. Vaginal swabs were examined using microbiological techniques. Data were analyzed using different tests of descriptive statistics. Prevalence of AV, bacterial vaginosis (BV), trichomonas vaginitis (TV) and vulvovaginal candidiasis (VVC) was 75%, 59.7%, 13.7% and 49.7% respectively. Major pathogens included *Enterococcus faecalis, Escherichia coli, Gardnerella vaginalis, Trichomonas vaginalis* and *Candida albicans*. Overall, 77.67% patients had mixed vaginitis, whereas co-occurrence of AV with BV was the highest. AV and BV were associated with history of miscarriages while TV with unawareness and low socioeconomic conditions. In culmination, AV is the predominant cause of vaginitis, followed by BV, VVC and TV. Miscarriages predispose women to AV and BV, whereas ignorance and poverty are the primary causes of TV.

**Keywords:** cross-sectional study; aerobic vaginitis; bacterial vaginosis; trichomonas vaginitis; vulvovaginal candidiasis; prevalence and risk factors.

#### INTRODUCTION

Vaginitis is one of the most common conditions among reproductive aged women, which is characterized by symptoms of abnormal vaginal discharge, malodor, itching and/or burning sensations [1]. The healthy vaginal tract of reproductive aged women is composed of a complex microbial environment containing different species in variable quantities mainly *Lactobacillus* sp. which maintains a healthy and safe microenvironment [2]. Distortion in this balance leads to urogenital infections, including different types of vaginitis *viz.*, AV, BV, TV and VVC [3].

The AV was defined as a vaginal dysbiosis dominated by aerobic pathogens resulting in foul smelling purulent yellow vaginal discharge, inflammation and epithelial disruption. Major pathogens involved in AV include *Escherichia coli, Enterococcus faecalis, Staphylococcus epidermidis, Staphylococcus aureus,* coagulase-negative *Staphylococci* and *Streptococcus* sp. (including group B streptococci) [4]. BV is the most prevalent vaginitis with 05-50% occurrence rate among reproductive aged women in different parts of the world. BV is characterized by overgrowth of several anaerobic microorganisms mainly *Gardnerella vaginalis* (GV), *Bacteroides* and *Mobiluncus* sp. [5]. Symptoms of BV include creamy gray vaginal discharge, an amine or "fishy" vaginal odor and presence of significant clue cells embedded with anaerobic bacteria [6]. Both AV and BV are treated with antibiotics, which under inappropriate conditions result in treatment failure, mainly due to presence of more resistant strains [7].

*T. vaginalis* is the extracellular parasitic protozoan that causes trichomoniasis, which is characterized by distressing symptoms including diffuse, foul-smelling, yellowish-green vaginal discharge, dyspareunia, dysuria, vulvar itching and abdominal pain [8]. Similarly, VVC is caused by overgrowth of yeasts, mainly *Candida albicans*, resulting in cottage-cheese like vaginal discharge, vulvar erythema and edema with excoriations [9].

In most of the cases, etiological agents of AV, BV, TV and VVC occur in consortium resulting in dreadful conditions known as mixed vaginitis. In principle, mixed vaginitis is defined as the condition that involves  $\geq 2$  types of vaginitis [3]. Mixed infections are among the most significant factors complicating the diagnosis and treatment of vaginitis. Inaccurate diagnosis or failure to recognize pathogens may lead to recurrent infection [10].

Numerous risk factors *viz.*, marital status, multiple partners, unawareness, low socioeconomic status and personal hygiene status have been associated with vaginitis [11]. In Pakistan, vaginitis is one of the most common problems for which women seek medical care. Overall, 23.8% prevalence of reproductive tract infections (RTIs) have been reported in Pakistan, which result in substantial economic impact in terms of financial costs, fertility loss and valuable pregnancies [12]. Literature is available about prevalence and association of BV, TV and VVC with various risk factors. However, there is scarcity of information about AV and its associated risk factors. Therefore, an effort was made to compare the prevalence of different types of vaginitis and to assess the relationship between different types of vaginitis and predisposing factors among married reproductive aged women with complaints of vaginal discharge attending Jinnah Hospital, Lahore, Pakistan.

## MATERIALS AND METHODS

#### Study design and ethical consideration

The present study was carried out in accordance with the ethical principles for human subjects, as described by the Helsinki Declaration. Written Informed consent was obtained from all participants and Board of Advanced studies and Research (BASR), University of the Punjab, Pakistan, approved all the study procedures.

#### Inclusion and exclusion criteria

Participants with clinical symptoms of vaginitis as examined by clinicians were included in the study. Those having antibiotic, antifungal (oral or topical application to the vaginal area in the preceding week), under post pelvic radiotherapy or infected with any UTIs were excluded.

### **Data collection**

The sample size was calculated using the single proportion sample size determination formula, which resulted in a total required sample size of 292 [13]. Data (n=300) was collected using a questionnaire that was designed to collect clinical symptoms, socio-demographic characteristics, routine hygienic practices and contraceptive usage through face-to-face interview.

## Sample collection from patients

After an initial physical assessment, vaginal samples (n= 3/patient) were collected from the posterior and lateral fornix of each subject using a cotton-tipped swab by trained nurses during a speculum (Cusco's) examination with a patient in lithotomy position. The patients were diagnosed for a specific type of vaginitis and etiological agents were cultured from vaginitis positive samples.

### Diagnosis of vaginitis and screening of vaginitis-related pathogens

AV was diagnosed following the criteria of Donders and coauthors [14]. BV was detected according to the scoring scheme of Nugent and coauthors [15]. After Gram-staining, oxidase and catalase tests, the pathogenic strains were identified using API 20 commercial kit with Vitek 2 automated system and Ramel RapID<sup>TM</sup> ONE system [16]. *T. vaginalis* was cultured in Diamond media and trichomoniasis was declared positive following the examination of live *T. vaginalis* under light microscope. For diagnosis of VVC, direct smear examination was done by Gram-staining and 10% potassium hydroxide (KOH) preparation [17].

## Data analysis

Data were analyzed using SPSS 25 (SPSS Inc., Chicago, Illinois, USA). Chi-square test (goodness of fit for equal distribution of all types of infection) was used to evaluate the differences among frequencies of mixed vaginitis and homogeneity test was made to find association of different types of vaginitis. Fisher's chi-square analysis was employed to test associations between clinical symptoms and occurrence rate of vaginitis. Bivariate logistic regression analysis was used to indicate predisposing factors, which have significant influence on the prevalence of vaginitis.

## RESULTS

AV and BV were diagnosed based on the presence of parabasal cells, leukocytes, background flora and lactobacillary grades. Out of 300 patients, 224 (74.67%) patients had a score of  $\geq$  07 score, 51 (17%) had an intermediate score of 4-6 and the remaining 25 (8.33%) scored  $\leq$ 3. The patients scoring high and intermediate were defined as vaginitis-positive while those with low score were considered as low or normal cases. The typical clinical finding (symptoms) of TV was found in 127 (42.33%) patients. In the laboratory, infection was diagnosed in 72 (24.0%) patients by wet mount microscopy, 60 (20%) by Giemsa staining and 41 (13.67%) by culture-based method. VVC was diagnosed in 149 (49.67%) patients by 10% KOH test. The types of vaginitis were decided based on the isolation of dominant etiological agents (Figure 1).



Figure 1. Culture-based prevalence of microbial community in vaginitis-positive patients in Lahore, Pakistan.

The prevalence of AV, BV, TV and VVC among women was 75% (224/300), 59.67% (179/300), 13.67% (41/300), and 49.67% (149/300), respectively. Overall, 22.33% (67/300) patients had single infection and 77.67% (233/300) had mixed vaginitis. Out of 233 mixed vaginitis, 173 (74.25%) had a double vaginal infection and 60 (25.75%), a triple infection (Figure 2).



Figure 2. Distribution of different types of vaginitis among patients. None of the patients was diagnosed with solo TV and VVC.

The patients with high scores ( $\geq$ 7) on diagnostic criteria were further subjected for isolation of pathogens. Total 557 pathogens were identified based on morphological and biochemical characteristics. Among these isolates, 178 (31.95%) were Gram-positive including *E. faecalis, E. avium, B. subtilis, S. aureus* and *Streptococcus* sp., while 102 (18.31%) were Gram-negative; *E. coli* and *Pseudomonas*. Additionally, 87 (15.62%) were Gram-variable (*G. vaginalis*). *T. vaginalis* was isolated from 41 (13.7%) and confirmed by the typical movement of TV in microscopic examination of swab culture observed under a light microscope while *C. albicans* was isolated from 149 (26.75%) patients.

Regarding the prevalence of microbial vaginitis, 224 (75%) had AV. Of total 224 AV patients, 52 (23.21%) had single AV infection and 172 (76.79%) had concurrent infections. Out of 172 mixed infections, combination of AV and BV was found in 115 (66.86%), AV and TV in 18 (10.47%) and AV and VVC in 95 (55.23%) cases. Among 300 patients, 179 (59.67%) had BV, 15 (8.38%) of which had single BV infection, 164 (91.62%) had

mixed infection; the combined infection of BV with VVC was diagnosed in 86 (52.44%) patients. TV was confirmed in 41 of 300 patients but solo TV infection could not be diagnosed. TV with BV was found in 07 (17.07%) while TV with VVC in 12 (29.27%) cases. Of the 149 (49.67%) patients with VVC, none of the patients was diagnosed with single VVC infection (Table 1).

Diagnosis	Туре	Frequency (%)	
	Single	52/224 (23.21)	
	Mixed	172/224 (76.79)	
	AV+BV	64 (37.21)	
AV	AV+TV	6 (3.49)	
n= 224	AV+VVC	46 (26.74)	
	AV+BV+VVC	44 (25.58)	
	AV+TV+VVC	5 (2.91)	
	AV+BV+TV	7 (4.07)	
	AV+BV+TV+VVC	0	
	Single	15/179 (8.38)	
	Mixed	164/179 (91 62)	
	BV+AV	64 (39 02)	
BV	BV+TV	7 (4 27)	
n=179	BV+VVC	38 (23 17)	
	BV+AV+VVC	44 (26 83)	
	BV+TV+AV	7 (4.27)	
	BV+ TV+VVC	4 (2.44)	
	BV+AV+TV+VVC	0	
	Single	_	
		0	
	Mixed	41 (100)	
	TV+AV	6 (14.63)	
τν	TV+BV	7 (17.07)	
n= 41	TV+VVC	12 (29.27)	
	TV+AV+VVC	5 (12.20)	
	TV+BV+VVC	4 (9.76)	
	TV+AV+BV	7 (17.07)	
	TV+BV+AV+VVC	0	
	Single	0	
	Mixed	149 (100)	
VVC	VVC+AV	46 (30.87)	
n= 149	VVC+BV	38 (25.50)	
	VVC+TV	12 (8.05)	
	VVC+BV+AV	44 (29.53)	
	VVC+1V+BV V//C+T//+A//	4 (2.68) 5 (3.35)	
	VVC+TV+AV+BV	0	

**Table 1.** Frequency of different types of vaginitis occurring individually or in combination with other types of vaginitis

Vaginitis was considered a single or mixed infection, based on isolation of dominant etiological agents (*E. faecalis*, *G. vaginalis*, *T. vaginalis* and *C. albicans*) from vaginal swabs. For instance, AV (single) means isolation of *E. faecalis* only from a sample. AV (mixed) indicates isolation of two or more etiological agents (*e.g.*, AV+BV indicates isolation of both *E. faecalis* and *G. vaginalis*). Similarly, mixing with other types of vaginitis was calculated.

The occurrence of AV with either BV, TV and VVC was also compared (Table 2). AV occurred significantly in higher frequency with BV as compared to TV. Similarly, the occurrence of AV with VVC was

also more frequent than TV but co-occurrence of AV with BV and VVC was not significantly different (p=0.168). BV occurred in different frequencies with AV, TV and VVC. The occurrence of BV with other vaginal infections was compared and higher association was found between BV with AV (p=0.000) followed by TV (p=0.000) and VVC (p=0.041). TV showed no significant association with any specific vaginal infection. VVC co-occurs more frequently with BV and AV as compared to TV (p=0.000).

Null hypothesis (H <sub>0</sub> )	Diagnosis	Frequency (%) <sup>*</sup>	Chi-square	<i>p</i> <0.05
H <sub>0</sub> = AV occurs equally frequently with BV, TV and VVC	AV n=224	BV=115 (66.86) TV=18 (10.47) VVC=95 (55.23)	69.03	0.000
	BV n=179	AV=115 (70.12) TV=18 (10.98) VVC=86 (52.44)	69.92	0.000
	TV n=41	AV=18 (43.90) BV=18 (43.90) VVC=21 (51.22)	0.316	0.854
	VVC n=149	AV=95 (63.75) BV=86 (57.71) TV=21 (14.09)	57.66	0.000
H <sub>0</sub> = Pairwise comparison of occurrence of different types of vaginitis	AV	AV+BV vs. AV+TV AV+BV vs. AV+VVC AV+TV vs. AV+VVC	70.74 01.91 52.47	0.000 0.168 0.000
	BV	BV+AV vs. BV+TV BV+AV vs.BV + VVC BV+TV vs. BV+VVC	70.74 04.18 44.46	0.000 0.041 0.000
	VVC	VVC+AV vs. VVC+BV VVC+BV vs. VVC+TV VVC+AV vs. VVC+TV	03.79 37.33 60.19	0.052 0.000 0.000

Table 2.	Comparison	and assoc	iations an	nong different	types c	of vaginitis
				- 3		

\*Frequency (%) of mixed vaginitis in different combinations.

Clinical symptoms varied among the patients depending upon the type of vaginitis. Patients with yellowgreen colored discharge were significantly more prone to AV (179; 79.9%) than patients with white discharge 45 (20.1%) (p=0.000). The prevalence of AV was more in patients who showed a genital malodor 224 (100%) and itching 129 (57.6%) (p=0.000). Among 179 (59.7%) of BV-positive women, BV was significantly higher among women with yellow-green colored vaginal discharge (p=0.005). Other clinical signs (odor and itching) were not significantly associated with BV. Change in color of vaginal discharge (p=0.006), malodor (p=0.004) and vulvovaginal itching (p=0.027), showed significant association with TV. On the other hand, of the 149 (49.7%) VVC-positive women, only vaginal itching was found significantly (p=0.001) associated with VVC (Table 3). Shazadi, K.; et al.

<b>Table 3.</b> Association of clinical	symptoms with different t	ypes of vaginitis
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Factor	Outcome	N <sup>†</sup> (300)	Types of vaginitis				
			AV	BV	TV	VVC	
			n (%)‡	n (%)	n (%)	n (%)	
	White	121	45 (20.1)	81 (45.3)	16 (39)	57 (38.3)	
Color	Yellow-green/grey	179	179 (79.9)	98 (54.7)	25 (61)	92 (61.7)	
	$oldsymbol{ ho}^{\#}$ (chi square)		0.000	0.041	0.006	0.482	
Odor	Foul-smelling	294	224 (100)	174 (97.2)	37 (90.2)	145 (97.3)	
	No	6	0	5 (2.8)	4 (9.8)	4 (2.7)	
	$oldsymbol{ ho}$ (chi square)		0.000	0.407	0.004	0.446	
Itching	Yes	132	129 (57.6)	73 (40.8)	16 (39)	80 (53.7)	
	No	168	95 (42.4)	106 (59.2)	25 (61)	69 (46.3)	
	$oldsymbol{ ho}$ (chi square)		0.000	0.193	0.027	0.001	

<sup>†</sup>Total number of patients included in the study (N= 300); <sup>‡</sup>n= Patients confirmed with specific type of vaginitis; <sup>#</sup>*p*= Fisher's test.

Association of vaginitis with socio-demographic parameters was also determined and results are presented in Table 4. The estimates of the demographic factors such as education, economic status, family size, residential area, were not significantly associated with occurrence of AV and BV. The unawareness about associated risk factors was linked to an increase in the likelihood of infection. For instance, it was found that uneducated patients were 1.27 times (OR=1.27, 95% CI: 1.13-6.52; p=0.026), while patient with lower economic status were 3.0 times (OR=3.0, 95% CI: 1.32-6.60; p=0.008) more susceptible to TV. Other factors such as family size and residential area had no significant impact on incidence of TV. Similarly, none of the socio-demographic factors was found to be significantly associated with VVC.

The association of vaginitis with obstetric and hygiene conditions was also computed in this study. Patients with a history of miscarriage were about 3.9 times more likely to be infected with AV (OR=3.9, 95% CI: 2.10-7.18; p = 0.000) and had a 30% higher risk of BV (OR= 0.7, 95% CI: 0.42-1.12; p = 0.013). Other obstetric and hygiene factors such as use of contraceptives and sharing of clothes were not found significantly associated with AV and BV. In contrast, TV and VVC were not significantly associated with any of the hygiene practices and history of poor obstetric outcomes.

Diagnosis	Statistics	Education		Economic status		History of miscarriage	
		Educated (N=139)	Uneducated (N=161)	Employed (N=138)	Unemployed (N=162)	No (N=153)	Yes (N=147)
AV	n (%)†	102 (45.5)	122 (54.5)	103 (46)	121 (54)	96 (42.9)	128 (57.1 )
	OR (95% CI)‡	Reference	1.2 (0.61-2.26)	Reference	0.6 (0.29-1.13)	Reference	3.9 (2.10-7.18)
	( <i>p</i> )#		0.634		0.106		0.000
BV	n (%)	76 (42.5)	103 (57.5)	80 (44.7)	99 (55.3)	97 (54.2)	82 (45.8)
	OR (95% CI)	Reference	1.5 (0.89-2.69)	Reference	1.1 (0.70-1.97)	Reference	0.7 (0.42-1.12)
	( <i>p</i> )		0.114		0.541		0.013
TV	n (%)	12 (29.3)	29 (70.7)	28 (68.3)	13 (31.7)	18 (43.9)	23 (56.1)
	OR (95% CI)	Reference	2.7 (1.13-6.52)	Reference	3.0 (1.32-6.60)	Reference	0.6 (0.28 -1.27)
	( <i>p</i> )		0.026		0.008		0.182
VVC	n (%)	70(47)	79	71 (47.7)	78 (52.3)	82 (55)	67 (45)
	OR (95% CI)	Reference	0.9 (0.54-1.60)	Reference	0.8 (0.48-1.34)	Reference	0.7 (0.45 -1.17)
	( <i>p</i> )		0.813		0.416		0.195

#### **Table 4.** Association of socio-demographic factors, hygiene and poor obstetric practices with vaginitis among reproductive aged women

<sup>†</sup>n= Patients confirmed with vaginitis, <sup>‡</sup>OR= Odd Ratio and Confidence Interval (CI), <sup>#</sup>*p* value of Hosmer & Lemeshow

#### DISCUSSION

Most of the vaginal infections are the consequence of the invasion of pathogenic bacteria (AV & BV), protozoan (TV) and fungi (VVC). The incidence rate of AV is not known in Pakistan. In current study, AV was recorded as the most frequent infection, accounting for 75% among symptomatic women of total vaginitis cases. The overall incidence of BV, TV and VVC was 59.7%, 13.7% and 49.7%, respectively. Previously, Sami [18] reported the incidence rate of BV, TV and VVC as 30.7%, 7.2% and 10%, respectively in Quetta, Pakistan. The vaginal hygiene practices, geographical distribution, and systematic differences in different studies could account for the variability in magnitude of prevalence [19].

The mucosal surfaces of the female genital tract are coated with cervical mucus and cervical vaginal secretion (CVS) that are colonized with a dynamic microbial community. The mucosal surfaces act as a barrier against invading microorganisms. However, some pathogens *viz.*, *C. albicans*, *T. vaginalis* and *G. vaginalis* produce proteases and/or glycosidases. These enzymes can degrade mucins and host defense components within the mucus [20]. In current study, the strains of genera *Enterobacteriaceae*, *Staphylococcus*, *Streptococcus*, *Bacillus*, *Pseudomonas* and *Gardnerella* were cultured from patients with severe AV and BV while *E. faecalis* and *G. vaginalis* (GV) were the dominant pathogens among AV and BV patients, respectively. These findings are consistent with those of Tansarli and coauthors [21], and Aroutcheva and coauthors [22].

Mixed infections are among the most significant factors complicating the diagnosis and treatment of vaginitis. Previously, it has been reported that mixed vaginal infections are escalating worldwide [23]. Inaccurate diagnosis or failure to recognize mixed infections may lead to recurrence of infection and highlights the importance of culture-based diagnosis [10]. In this study, 77.7% cases were of polymicrobial etiology, with the most frequent combination being that of AV with BV (21.33%). Our findings are in agreement with Liang and coauthors [24] who reported that AV occurs more frequently with BV as compared to TV. Similarly, Rivers and coauthors [25] reported higher co-occurrence of AV with VVC and BV followed by TV. The BV with VVC was found in 12.4% cases in their study and TV and VVC were observed only in cases of mixed vaginitis. Mixed infections might be due to trophic mutualism and complex interaction of aerobic and anaerobic opportunistic pathogens [3]. Secondly, the majority of the participants of the current study belonged to rural communities where poor hygiene conditions and lack of proper sanitary conditions, might have contributed to co-occurrence of BV with VVC. The contrasting prevalence rates of mixed infection in different studies may also be associated with several risk factors *viz.*, educational background, economic status, study population and methods used for diagnosis [26].

The information of patients related to clinical symptoms, socio-demographic, hygiene and obstetric characteristics was correlated with occurrence of different types of vaginitis. Copious vaginal discharge, which may be white, yellow, red or black in color is considered abnormal and associated with vaginitis [27]. Regarding color and odor of vaginal discharge in current study, AV, BV and TV were found associated with yellow green discharge while malodor was associated with AV and TV. It seems difficult to diagnose a typical infection by color or odor of vaginal discharge alone. It is therefore recommended that initially abnormal vaginal discharge should be investigated and treated as a mixed infection.

In this study, vaginal itching was found associated with AV, TV and VVC (Single/mixed). Patients with AV usually do not complain of genital itching [3]. Therefore, vaginal itching in AV patients could be due to co infection with TV or VVC in current study. Previously, Abdul-aziz and coauthors [28] as well as Squire and coauthors [29] also reported the association of genital itching with TV and VVC. In essence, clinical symptoms cannot be used as an independent marker in identifying positive cases of specific type of vaginitis and a culture-based technique is recommended for accurate diagnosis and targeted treatment.

Demographic factors evaluated as potential risk factors for vaginitis included, awareness, family size, socioeconomic conditions and area of residence. AV, BV and VVC did not show association with sociodemographic factors. However, the patients who were uneducated (70.7%) and lived under low socioeconomic conditions (68.3%) were found to be 1.27 and 3.0 times more prone to TV, respectively. Previously, TV has been linked with increased age, intravenous drug use, incarceration and commercial sex work [30]. Consistent with our findings, Kadhum [31] reported a proportion of 50.76% among uneducated and 63.07% among women who lived under low socio-economic conditions.

Among obstetric factors and hygiene practices, AV and BV have been associated with severe complications such as preterm delivery and late miscarriage [32]. In this study, the presence of AV and BV was found to be 3.9 times and 30% higher in patients who had history of miscarriages. In consistent to

Torondel and coauthors [33], and Abdul-aziz and coauthors [28], we could not observe association of TV and VVC with obstetric outcomes and personal hygiene.

# CONCLUSION

It is concluded that vaginitis usually exists in the form of mixed vaginitis. Overall, risk factors including miscarriage, low awareness and socioeconomic conditions predispose women to vaginitis. The high prevalence rate in Pakistan necessitates future studies on controlling and monitoring potential risk factors.

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