Co-infected with trichomonas vaginitis increases the risk of CIN2-3 among HPV16 positive female: a large population-based study

Mei Yang
Xiangyang Central Hospital
Lin Li
Xiangyang Central Hospital
Chunfan Jiang
Xiangyang Central Hospital
Xiaomin Qin
Xiangyang Central Hospital
Min Zhou
Xiangyang Central Hospital
Xiaogang Mao
Xiangyang Central Hospital
Hui Xing (50269@hbuas.edu.cn)
Xiangyang Central Hospital https://orcid.org/0000-0003-0318-1735

Research article

Keywords: Invasive Cervical Cancer; Cervical Intraepithelial Neoplasia; Human Papillomavirus; Vaginal Microenvironment; Trichomonas Vaginitis

Posted Date: April 30th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-22259/v1

Version of Record: A version of this preprint was published on September 1st, 2020. See the published version at https://doi.org/10.1186/s12879-020-05349-0.

Abstract

Background: Evidences suggested that the vaginal microbiome played a functional role in the progression of cervical lesions in female infected by HPV. This study aimed at evaluating the influence of common vaginal infection on the carcinogenicity of hr-HPV.

Methods: From January 15, 2017 to December 31, 2017, 310,545 female aged at least 30 years old had been recruited for cervical cancer screening from 9 clinical research centers in Central China. All the recruited participants received cervical cancer screening and vaginal microenvironment test by a high vaginal swab. Colposcopy-directed biopsy was recommended for female who were infected with HPV 16 and HPV 18 and other positive hr-HPV types through test had undertaken triage using liquid-based cytology, cases with the results ≥ ASCUS among them were referred to colposcopy directly.

Results: Among 310,545 female, 6,067 (1.95%) were tested with positive HPV 16 and HPV 18, 18,297 (5.89%) were tested with other positive hr-HPV genotypes, cervical intraepithelial neoplasia (CIN) 1, CIN 2, CIN 3 and invasive cervical cancer (ICC) were detected in 861 cases, 377 cases, 423 cases and 77 cases, respectively. Candida albicans and Gardnerella were not associated with the progression of cervical lesions. Positive trichomonas vaginitis (TV) was correlated with hr-HPV infection ($p<0.0001$). Co-infection with TV increased the risk of CIN 1 among female infected with hr-HPV (OR 1.18, 95% CI: 1.42-2.31). Coinfection with TV increased the risk of CIN 2-3 among female infected with HPV 16 (OR 1.71, 95% CI: 1.16-2.53).

Conclusions: Co-infection of TV and HPV 16 is an important risk factor for the progression of cervical lesions.

1. Background

Cervical cancer is a common malignant tumor of female reproductive system, and it progresses rapidly with high mortality rate [1]. HPV is a DNA virus, which is widely found in nature. Hr-HPV (e.g. HPV 16, 18, 31, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82) among them are associated with high grade cervical intraepithelial neoplasia (CIN) and invasive cervical cancer (ICC).

Under normal conditions, vaginal flora is composed by more than 20 kinds of microbes [2, 3]. Infection of female reproductive tract refers to a series of infectious inflammation caused by the destruction of the defense system by microorganisms, such as viruses and bacteria [4]. The genital tract infection leads to an imbalance of the vaginal flora, which inhibits or reduces the lactobacillus substantially,which may cause the decline of the clearance of hr- HPV. Candida spp, Gardnerella and TV are the most common vaginal infections. As a part of the human commensal flora, Candida spp always causes systemic and superficial infections [5]. Gardnerella vaginalis is considered as playing a key role in the pathogenesis of bacterial vaginitis (BV) [6], and BV shows correlation with severity of cervical neoplasia in HPV-positive female [7], but the aetiology and pathogenesis of BV are more complex, gardnerella vaginalis is thought

as a potential founder organism. As a sexually transmitted infectious agent, TV is found to cause local inflammation, and it affects the clearance of hr-HPV and contributes to cervical lesion progression in several research, but it is controversial [8]. Some studies have demonstrated an correlation between vaginal infection and the carcinogenicity of hr-HPV, but most of them are small-scale researches and their conclusions are inconsistent [5, 9–16]. This research is with the largest scale and the most comprehensive investigation about the correlation between cervical hr-HPV infection, CIN/ICC and the vaginal microbiome.

Central China is one of the regions with the highest incidence of cervical cancer[17], and the social-economic conditions vary greatly in different districts. In order to study ICC influenced by multiple socioeconomic factors, nine areas in Central China with significantly different economic levels were included in this study. This study aimed at evaluating the influence of common vaginal infection on the carcinogenicity of hr-HPV.

2. Methods

This project had been conducted in Central China from January 15, 2017 to December 31, 2017. Female aged at least 30 years old were recruited to undergo the cervical cancer screening from 9 clinical research centers (Xiangzhou, Fancheng, Xiangcheng, Baokang, Nanzhang, Zaoyang, Yicheng, Gucheng and Laohekou) in Central China via media promotion and government notices. Female who received hysterectomy, were pregnant, without sexual history had been excluded. All the female had not been vaccinated against cervical cancer yet. All the participants received questionnaires, hr-HPV genotyping and vaginal microbiota examination. The screening process was based on the interim clinical guidance of ASCCP in 2015[18], female tested positive for HPV 16 and HPV 18 had been referred to colposcopy directly; female with other positive hr-HPV types through test had undertaken triage using liquid-based cytology, cases with the results ≥ ASCUS among them were referred to colposcopy directly. The protocol was approved by the Ethics Committee of Xiangyang Central Hospital.

2.1 Questionnaires

All the recruited participants were interviewed by a trained interviewer and filled out a questionnaire for the first time. The content of the questionnaire includes age, marital status, ethnicity, the highest level of education, whether in menopause, date of last menstruation, history of past HPV infection, family history of cancer, reproductive history (number of pregnancies and number of births), method of contraception (contraceptive, condom and intrauterine contraceptive device), number of lifetime sex partners and whether in poverty (whether being a poverty alleviation target).

2.2 HPV genotyping

All the recruited female underwent a gynecological examination. A cervical specimen was taken using a cervical brush, and a high vaginal swab was collected. COBAS4800 (Roche Molecular Systems, Alameda, CA) assay was used for typing HPV DNA. The COBAS 4800 HPV test detected a total of 14 hr-HPV types

simultaneously: HPV-16 individually, HPV-18 individually, and pooled hr-HPV genotypes other than HPV 16 and 18 (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68), in addition to a separate high b-globin control.

2.3 Vaginal microenvironment test

The high vaginal swab was processed for microscopic evaluation of vaginal microenvironment, including presence of Candida albicans, TV, gardnerella, clue cells and miscellaneous bacteria (vaginal bacteria except lactobacilli) density[19]. A vaginal wet mount was prepared for detection of Candida albicans. Candida was diagnosed using the KOH method (10% KOH), and vaginal trichomonas was examined according to conventional methods, the swimming trichomonas was observed under the microscope (x400). Gardnerella, clue cells, and miscellaneous bacteria density(MBD) were observed by Gram staining. The scoring method of miscellaneous bacteria density was listed as follows, no miscellaneous bacteria was scored as □, 1–5 miscellaneous bacteria/OML as □, 6–30 miscellaneous bacteria/OML as □, □30 miscellaneous bacteria/OML as □.

2.4 Thinprep cytologic test

According to manufacturer's instructions, slides for liquid-based cytology were prepared. Bethesda System 2001 terminology was used for reporting the results [20]. The following 5 different categories were reported: NILM (negative for intraepithelial lesion or malignancy); AGC (atypical glandular cells); SIL (squamous intraepithelial lesion): LSIL (low grade squamous intraepithelial lesion) or HSIL (high grade squamous intraepithelial lesion); ASC-US (atypical squamous cells of undetermined significance) or ASC-H (atypical squamous cells not possible exclude HSIL) and SCC (squamous cell carcinoma).

2.5 Colposcopy-directed biopsy and verification of disease status

Female with positive HPV 16 and HPV 18 through test or with other hr-HPV types through test and the result of liquid-based cytology ≥ ASCUS underwent further cervical biopsy and/or endocervical curettage (ECC) by a local trained gynecologist in each hospital. After the cervical tissues were embedded in paraffin, slides were HE stained routinely. Two pathologists made the diagnosis separately. The histological diagnoses of cervical lesions were divided into normal, CIN1, CIN2, CIN3 and ICC.

2.6 Statistical analysis

Histological results (normal, CIN1, CIN2, CIN3 and ICC) were distributed among respective hr-HPV types and then evaluated. Univariate analysis was used for comparing whether the risk of any cervical lesions (tri categorical: CIN1, CIN2-3 and ICC) differed when female having vaginal infection (Candida albicans, Gardnerella and TV) or not. Odds ratios and 95% confidence intervals (CIs) were calculated to study the specific risk among different HPV types. Next, social-demographic and reproductive characteristics were taken into consideration so as to improve the accuracy of the results. These characteristics of the participants were presented as proportions. Correlations between specific vaginal infection and hr-HPV genotype and CIN/ICC were assessed using stepwise logistic regression after adjusting for all potential

risk factors. ORs and 95% CIs were calculated to analyze the correlation between possible risk factors and the pathogenic infection. Data was analyzed with SPSS Software Version 20.0.

3. Results

3.1 The overall prevalence of hr-HPV, Candida albicans, Gardnerella and TV

Among 310,545 participants, 24,364 (7.84%) female were infected with hr-HPV. Candida albicans were detected

in 13,763 (4.43%) female, Gardnerella were detected in 1,050 (0.34%) female, and TV were tested positive in 5,683 (1.83%) female, respectively.

3.2 Distribution of HPV types in CIN and ICC female from Central China

307,071 female received complete screening process finally (1,331 female with HPV 16 and HPV 18 and 1,241 female with other positive hr-HPV were failed to follow up for different reasons, such as changes of the work place, health problems or other personal reasons, 902 samples failed to prepare slides for liquid-based cytology for having too little cells.) 6,067(1.95%) female were tested positive for HPV 16 and HPV 18, including HPV 16 only, HPV 16 + other high risk (OHR), HPV 18 only, HPV 18 + OHR, HPV 16 + 18, and HPV 16 + 18 + OHR types. 4,736 (1.53%) among them, received colposcopy, CIN1, CIN2, CIN3, ICC were detected in 480 (0.15%) cases, 265 (0.086%) cases, 346 (0.11%) cases, 75 (0.024%) cases, respectively. 18,297 (5.89%) female were tested positive only for other hr-HPV genotypes, and undertook triage using liquid-based cytology. 2,350 (0.76%) cases with the results ≥ ASCUS were referred to colposcopy directly, and CIN1, CIN2, CIN3 and ICC were detected in 381 (0.12%) cases, 112 (0.036%) cases, 77 (0.025%) cases, 2 cases, respectively (Fig. 1).

3.3 Correlation between Candida Albicans, Gardnerella, TV and the progression of cervical lesions stratified by different hr-HPV types

Distribution of potential risk factors among CIN/ICC and normal participants is shown in S-Table 1. Univariate analysis of the correlation between different vaginal infection (Candida albicans, Gardnerella, TV micro-environment) and the histological results (three categories: CIN1, CIN2-3 and ICC) among different hr-HPV types is displayed in Fig. 2 and S-Table 2. In HPV 16 infection groups, when co-infected with TV, female had a significantly higher incidence of CIN 1 (OR = 1.301, 95% CI = 1.003–1.685), CIN 2–3 (OR = 2.066, 95% CI = 1.625–2.627) and ICC (OR = 2.546, 95% CI = 1.248–5.191). In HPV 18 positive groups, co-infection of TV didn't increase the risk of CIN1, CIN2-3 or ICC. In all the different hr-HPV types,

no significant correlation was found between Candida albicans, Gardnerella co-infection and any cervical lesions.

3.4 Multivariate analysis of the risk of CIN/ICC by TV stratified by hr-HPV infection status

Among the overall participants, 5,683 (1.83%) participants were infected with TV, among them, 1,952 (0.63%) female were co-infected with hr-HPV. Among the co-infection population, 1,643 cases had normal cytology or histologic diagnosis, 171 cases had CIN 1, 60 cases had CIN 2, 67 cases had CIN3 and 11 cases had ICC (Table 1).

Table 1
Histologic diagnosis distribution in 1952 hr-HPV and TV co-infection population.

HPV genotype	Normal#	CIN1	CIN2	CIN3	Cancer	Total
	n (%)	n (%)	n (%)	n (%)	n (%)	n
16	1127(61.48)	234(12.77)	161(8.78)	260(14.18)	51(2.78)	1833
Co-infection	35(71.69)	68(13.85)	23(4.68)	42(8.55)	6(1.22)	491
16 + OHR	492(63.48)	121 (15.61)	75 (9.68)	75 (9.68)	12 (1.55)	775
Co-infection	132(69.84)	25(13.23)	15(7.94)	15(7.94)	2(1.06)	189
16 + 18	24(55.81)	10(23.26)	5(11.63)	4(9.3)	0(0)	43
Co-infection	5(45.45)	3(27.27)	1(9.09)	1(9.09)	1(9.09)	11
16 + 18 + OHR	30(63.83)	6(12.77)	9(19.15)	1(2.13)	1(2.13)	47
Co-infection	3(50)	1(16.67)	1(16.67)	1(16.67)	0(0)	6
18	401(77.12)	82(15.77)	16(3.08)	10(1.92)	11(2.12)	520
Co-infection	87(82.86)	13(12.38)	3(2.86)	1(0.95)	1(0.95)	105
18 + OHR	169(73.48)	51(22.17)	6(2.6)	4(1.74)	0(0)	230
Co-infection	15(46.87)	14(43.75)	2(6.25)	1(3.13)	0(0)	32
OHR	16916(96.95)	357(2.05)	105(0.60)	69(0.39)	2(0.01)	17449
Co-infection	1049(93.83)	47(4.20%)	15(1.34)	6(0.54)	1(0.09)	1118
#: female who have normal histologic or cytology results. OHR: other high risk type.						

Multivariate logistic regression analyses were used for analyzing the risk of CIN 1, CIN 2–3 and ICC by TV stratified by different hr-HPV infection status. After adjusting the age, education, number of pregnancies, number of births, menopause, candida albicans, gardnerella and miscellaneous bacteria density, method

of contraception, lifetime sex partners and poverty, it was found that among total female infected with hr-HPV, TV increased the risk of CIN1 (OR 1.18, 95% CI: 1.42–2.31), but it did not increase the risk of CIN2-3 and ICC. For different HPV types, TV increased the risk of CIN2-3 among the female infected with HPV16 (single HPV16 or simultaneous infection of HPV 16 and other high-risk HPV types) (OR 1.71, 95% CI: 1.16–2.53). For there were only 77 ICC female in this study, no efficient results were found among HPV 16 positive ICC female. No positive correlation were found between TV and CIN 1/CIN 2–3/ICC among none HPV16 positive female in this study (Table 2).

Table 2
Multivariate logistic regression analyses of the risk of CIN1/CIN2-3/ICC by TV stratified by hr-HPV infection status

	CIN1			**CIN2-3**			**ICC**		
	n	OR	*p*	n	OR	*p*	n	OR	*p*
Total	861	1.18(1.42–2.31)	< .0001	800	1.17(0.88–1.55)	0.2735	77	0.99(0.36–2.75)	0.9898
16	371	0.69(0.48–1.01)	0.0501	590	1.71(1.16–2.53)	0.0069	64	-	-
18	149	1.96(0.85–4.51)	0.112	55	2.18(0.75–6.34)	0.1544	12	-	-
OHR	535	0.73(0.49–1.08)	0.1147	344	0.90(0.56–1.45)	0.6704	15	0.36(0.12–1.06)	0.0633

Adjusted for age, the highest level of education, number of pregnancies, number of births, menopause, miscellaneous bacteria density, poverty, method of contraception, lifetime sex partners, gardnerella and candida spp.

3.5 Potential Risk Factors of hr-HPV mono-infection or co-infection with TV

To find the risk factors of hr-HPV infection, multivariate logistic regression analyses were utilized. The results are shown in Fig. 3, it was found that age ≥ 60 ($p < 0.001$), number of pregnancies ≥ 1 ($p < 0.001$), postmenopause ($p < 0.01$), poverty ($p < 0.01$), personal HPV history ($p < 0.01$), miscellaneous bacteria density ($p < 0.0001$), trichomonas ($p < 0.0001$), were positively associated with hr-HPV infection significantly; Education($p = 0.0154$), was not associated with hr-HPV infection; Number of birth, and candida infection were negatively correlated with hr-HPV infection ($p < 0.0001$).

Baseline features of the participants with hr-HPV and TV co-infection are shown in Table 3, primary education, intrauterine contraceptive device as a method of contraception and female with multiple sex partners were more likely to be infected with both TV and hr-HPV.

Table 3
Baseline features of the participants with hr-HPV and TV coinfection

	OR(95%CI)	P
education		< .0001
Primary	ref	
Middle and high	0.424(0.377–0.476)	
Graduate	0.540(0.458–0.636)	
Marital status		0.1745
Married	0.729(0.462–1.150)	
Divorced or widowed	ref	
Method of contraception		< .0001
Non-contraception	0.236(0.207–0.270)	
Contraceptive	0.335(0.280–0.401)	
Condom	0.244(0.208–0.286)	
Intrauterine contraceptive device	ref	
Lifetime sex partners		< .0001
1	0.009(0.008–0.010)	
≥ 2	ref	

4. Discussions

According to the report "Global Cancer Statistics 2018", cervical cancer ranks the fourth in the incidence and mortality of female tumors worldwide, and the second in the incidence of female tumors in developing countries [1]. HPV infection is a necessary but insufficient condition for cervical cancer [21], the number of cervical cancer patients is far lower than the number of HPV infection female[22]. With the development of microbiome, more and more studies have shown that the composition and changes of cervical-vaginal microenvironment are closely related to HPV invasion, persistent infection and the occurrence and development of cervical cancer [23, 24].

In our study, the prevalence of hr-HPV in this region was lower compared with the hr-HPV prevalence from other parts of China (from 9.03–16.8%) [17]. It was found that Candida spp was not associated with the progression of cervical lesions in this study, which was consistent with previous studies [5, 13, 14]. Gardnerella vaginalis was not the risk factors of CIN or ICC in our study, either. Gardnerella vaginalis was considered to possess characteristics which are important for the pathogenesis of BV[6, 25–27], including

production of sialidase [27] and vaginolysin, [26, 28]. Although some research found that BV played a functional role in female with CIN or ICC[2, 3], the correlation between Gardnerella and the progression of cervical lesions had yet to be described. In this study, Gardnerella has had a low prevalence and was only detected in 1,050 (0.34%) female, and co-infected with Gardnerella didn't increase the risk of CIN or ICC. In the vagina of female without BV, Gardnerella vaginalis was also detected, although it had a lower abundance and prevalence [29, 30]. There were enormous substantial genetic diversity existing within Gardnerella vaginalis[16, 31] and virulence potential differentiate greatly between various genetic types/clades[15], this may correlate with the result in our study.

Several researches had studied the relationship between TV, HPV and CIN/ICC, but their conclusions were inconsistent. Gweneth's study found that trichomonas vaginitis was associated with hr-HPV infection (specifically type 16) significantly, but no correlation between trichomonas and cervical lesions [10]. Ishita Ghosh's study found that the higher risk of cervical cancer observed in the female co-infected with HPV and TV was without any enhanced risk of CIN[5]. But both of them were small-scale studies. Rui-Mei Feng's pooled analysis found that current TV-positive female had an increased risk for hr-HPV infection compared with currently TV negative female. Both past and current TV-positive female had a decreased risk for CIN 2+[9], but hr-HPV was detected by Hybrid Capture 2 and HPV genotype can't be differentiated, TV was diagnosed by thinprep cytologic test in that research, and this method had a lower detection rate.

In this study, 5,683 among the overall participants were TV positive and 1,952 (34.35%) among these, were co-infected with hr-HPV. Co-infection with TV increased the risk of CIN 1 among female with hr-HPV, and increased the risk of CIN 2–3 among female with HPV 16. It was expected that TV was often associated with more severe inflammatory reaction and HPV persistence, and was likely closely related to the invasion, persistent infection of HPV 16. HPV including hr-HPV infections are mostly temporary, and the virus can be cleared through host immune responses spontaneously, only a small number of infections persisted and progressed to cervical cancer [32]. HPV 16 was found to be the most prevalent hr-HPV type with the highest risk among all hr-HPVs to progress to CIN2-3 and ICC [33]. Studies found that the outcome of HPV infection was closely related to the local micro-environmental of the cervix [23]. A lot of research have discovered that TV led to an imbalance of the vaginal flora, which inhibited or reduced the lactobacillus substantially [5], and caused severe inflammation. Evidences were found that the continuous activation of inflammatory transcription factors can lead to cervical tissue damage, and thus improved the susceptibility and carcinogenic ability of HPVI6[10, 34]. Meanwhile, the severity of cervical lesions was correlated with the abundance and diversity of cervicovaginal flora positively and correlated with the number of lactobacilli negatively [23, 35]. Unlike CIN 1, CIN 2–3 had been considered no longer reversible and required a more definite clinical treatment, the composition and characteristics of the cervical and vaginal flora were very different [36]. [2, 3]. There may be some complex host immune response to TV and HPV 16 co-infection, but the hypothesis needed further research for validation. There were only 72 ICC in this study, so the correlation between TV, HPV and ICC were inconclusive.

Both TV and genital tract HPV infection are sexually transmitted disease (STD). In the districts with lower economic levels, many people went out to the developed areas to work and couples usually work in different places, and sex act extramarital is common[17]. Risk factors including primary education, intrauterine contraceptive device as a method of contraception and female with multiple sex partners significantly increased the odds of hr-HPV and TV co-infection. Compared with female with only 1 sexual partner, it was found that female with 2 or more lifetime sex partners had a higher risk of HPV and TV co-infection. Meanwhile, it was found that female who chose intrauterine contraceptive device as a method of contraception had a higher risk of co-infection, as compared with female who chose contraceptive or condom. Female who had primary education also were found to have higher risk of co-infection compared with higher educated female. As TV is a STD, and it was found that 2 or more lifetime sex partners and choosing intrauterine contraceptive device instead of condom were associated with co-infection, it was expected that the co-infection is likely closely related to the risky sex behaviors. Sexual interactions with multiple hosts were expected to contribute to the co-infection, because each host may be infected with one kind of special pathogen species [37, 38]. The transmission and clinical progression of the sexually transmitted infectious diseases may be changed by sexual interactions [39, 40]. Risk factors, including number of pregnancies ≥ 1, post-menopause, poverty, personal HPV history and miscellaneous bacteria density were associated with HPV infection significantly, but they were not related to co-infection significantly.

As it was known that, this research had the largest number of female who received COBAS human papillomavirus primary testing for cervical cancer screening up to now worldwide, and it was an multisite investigation with the largest scale about the correlation between cervical hr-HPV infection, CIN/ICC and vaginal microenvironment. The detection methods of hr-HPV and vaginal microenvironment in this study were highly sensitive and recognized internationally.

Of note, there were some limitations to our study. Because our study was a cross-sectional analysis of current hr-HPV and TV infections, it was not clear whether there was a persistent HPV infection among these female with HPV or not, this study will continue to following up on female with hr-HPV female and attempting to analyze the time and rates of HPV clearance. In addition, there are some participants who failed to be followed up for a variety of reasons in the screening process.

In one word, from this large population-based study, it was found that co-infection of TV and HPV 16 is an important risk factor for the progression of cervical lesions.

Declarations

Ethics approval and consent to participate

This study received the verification of Medicine Ethics Committee Xiangyang Central Hospital(Approved Code: 2017-004). The project “Biological Sample Bank Construction Project of Xiangyang City ‘Two Cancers Screening’” hosted by Xiangyang Central Hospital involves the collection of samples and related

indicators detection of human cervical exfoliated cells and vaginal secretions. The related experiments of this project will strictly abide by ICH-GCP, declaration of Helsinki, International Ethical Guidelines for Biomedical Research Involving Human Subjects. Respect for the right of informed consent of the patient. All the experiments meet routine medical and make protection scheme for patients.All the members of Medicine Ethics Committee approved this project. Informed consent to participate in this study had been obtained from participants.

Consent to publish

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

Competing interests

All authors declare that they have no competing interests. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Funding

This work was supported by the National Natural Science Foundation of China(No.81972449). Health Commission Foundation of Hubei, China (No. WJ2019M069);

Authors' Contributions

Designed project: Hui Xing, Lin Li. Collected samples: Xiaomin Qin, Min Zhou, Xiaogang Mao. Analysed data: Mei Yang, Chunfan Jiang. Generated figures and tables: Mei Yang, Chunfan Jiang. Wrote manuscript: Mei Yang. All authors critically reviewed the manuscript.

Acknowledgements

The authors thank the clinic staff of the hospitals which take part in the cervical cancer screening project in Central China in 2017.

References

[1] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018. 68(6): 394-424.

[2] Mitra A, MacIntyre DA, Lee YS, et al. Cervical intraepithelial neoplasia disease progression is associated with increased vaginal microbiome diversity. Sci Rep. 2015. 5: 16865.

[3] Piyathilake CJ, Ollberding NJ, Kumar R, Macaluso M, Alvarez RD, Morrow CD. Cervical Microbiota Associated with Higher Grade Cervical Intraepithelial Neoplasia in Women Infected with High-Risk Human Papillomaviruses. Cancer Prev Res (Phila). 2016. 9(5): 357-66.

[4] Frati ER, Fasoli E, Martinelli M, et al. Sexually Transmitted Infections: A Novel Screening Strategy for Improving Women's Health in Vulnerable Populations. Int J Mol Sci. 2017. 18(6).

[5] Ghosh I, Muwonge R, Mittal S, et al. Association between high risk human papillomavirus infection and co-infection with Candida spp. and Trichomonas vaginalis in women with cervical premalignant and malignant lesions. J Clin Virol. 2017. 87: 43-48.

[6] Muzny CA, Schwebke JR. Gardnerella vaginalis: Still a Prime Suspect in the Pathogenesis of Bacterial Vaginosis. Curr Infect Dis Rep. 2013. 15(2): 130-5.

[7] das Neves J, Nunes R, Machado A, Sarmento B. Polymer-based nanocarriers for vaginal drug delivery. Adv Drug Deliv Rev. 2015. 92: 53-70.

[8] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. IARC monographs on the evaluation of carcinogenic risks to humans. Ingested nitrate and nitrite, and cyanobacterial peptide toxins. IARC Monogr Eval Carcinog Risks Hum. 2010. 94: v-vii, 1-412.

[9] Feng RM, Z Wang M, Smith JS, et al. Risk of high-risk human papillomavirus infection and cervical precancerous lesions with past or current trichomonas infection: a pooled analysis of 25,054 women in rural China. J Clin Virol. 2018. 99-100: 84-90.

[10] Lazenby GB, Taylor PT, Badman BS, et al. An association between Trichomonas vaginalis and high-risk human papillomavirus in rural Tanzanian women undergoing cervical cancer screening. Clin Ther. 2014. 36(1): 38-45.

[11] Noël JC, Fayt I, Romero Munoz MR, Simon P, Engohan-Aloghe C. High prevalence of high-risk human papillomavirus infection among women with Trichomonas vaginalis infection on monolayer cytology. Arch Gynecol Obstet. 2010. 282(5): 503-5.

[12] Verteramo R, Pierangeli A, Mancini E, et al. Human Papillomaviruses and genital co-infections in gynaecological outpatients. BMC Infect Dis. 2009. 9: 16.

[13] Menon S, Broeck DV, Rossi R, Ogbe E, Harmon S, Mabeya H. Associations Between Vaginal Infections and Potential High-risk and High-risk Human Papillomavirus Genotypes in Female Sex Workers in Western Kenya. Clin Ther. 2016. 38(12): 2567-2577.

[14] Kiseki H, Tsukahara Y, Tajima N, Tanaka A, Horimoto A, Hashimura N. Influence of co-infection complicated with human papillomavirus on cervical intraepithelial neoplasia development in patients with atypical squamous cells of undetermined significance. J Infect Chemother. 2017. 23(12): 814-819.

[15] Shipitsyna E, Krysanova A, Khayrullina G, et al. Quantitation of all Four Gardnerella vaginalis Clades Detects Abnormal Vaginal Microbiota Characteristic of Bacterial Vaginosis More Accurately than Putative G. vaginalis Sialidase A Gene Count. Mol Diagn Ther. 2019. 23(1): 139-147.

[16] Cornejo OE, Hickey RJ, Suzuki H, Forney LJ. Focusing the diversity of Gardnerella vaginalis through the lens of ecotypes. Evol Appl. 2018. 11(3): 312-324.

[17] Li L, Ding L, Lyu YJ, et al. [Interaction between vaginal micro-environment alterations and HPV16 infection in cervical intraepithelial neoplasia]. Zhonghua Liu Xing Bing Xue Za Zhi. 2018. 39(11): 1486-1490.

[18] Huh WK, Ault KA, Chelmow D, et al. Use of primary high-risk human papillomavirus testing for cervical cancer screening: interim clinical guidance. Gynecol Oncol. 2015. 136(2): 178-82.

[19] Yu F, Tang YT, Hu ZQ, Lin XN. Analysis of the Vaginal Microecological Status and Genital Tract Infection Characteristics of 751 Pregnant Women. Med Sci Monit. 2018. 24: 5338-5345.

[20] Solomon D, Davey D, Kurman R, et al. The 2001 Bethesda System: terminology for reporting results of cervical cytology. JAMA. 2002. 287(16): 2114-9.

[21] de Sanjosé S, Diaz M, Castellsagué X, et al. Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. Lancet Infect Dis. 2007. 7(7): 453-9.

[22] Monnier-Benoit S, Mauny F, Riethmuller D, et al. Immunohistochemical analysis of CD4+ and CD8+ T-cell subsets in high risk human papillomavirus-associated pre-malignant and malignant lesions of the uterine cervix. Gynecol Oncol. 2006. 102(1): 22-31.

[23] Huang X, Li C, Li F, Zhao J, Wan X, Wang K. Cervicovaginal microbiota composition correlates with the acquisition of high-risk human papillomavirus types. Int J Cancer. 2018. 143(3): 621-634.

[24] Mitra A, MacIntyre DA, Marchesi JR, Lee YS, Bennett PR, Kyrgiou M. The vaginal microbiota, human papillomavirus infection and cervical intraepithelial neoplasia: what do we know and where are we going next. Microbiome. 2016. 4(1): 58.

[25] Schwebke JR, Muzny CA, Josey WE. Role of Gardnerella vaginalis in the pathogenesis of bacterial vaginosis: a conceptual model. J Infect Dis. 2014. 210(3): 338-43.

[26] Swidsinski A, Mendling W, Loening-Baucke V, et al. Adherent biofilms in bacterial vaginosis. Obstet Gynecol. 2005. 106(5 Pt 1): 1013-23.

[27] Lewis WG, Robinson LS, Gilbert NM, Perry JC, Lewis AL. Degradation, foraging, and depletion of mucus sialoglycans by the vagina-adapted Actinobacterium Gardnerella vaginalis. J Biol Chem. 2013. 288(17): 12067-79.

[28] Gelber SE, Aguilar JL, Lewis KL, Ratner AJ. Functional and phylogenetic characterization of Vaginolysin, the human-specific cytolysin from Gardnerella vaginalis. J Bacteriol. 2008. 190(11): 3896-903.

[29] Schwebke JR, Flynn MS, Rivers CA. Prevalence of Gardnerella vaginalis among women with lactobacillus-predominant vaginal flora. Sex Transm Infect. 2014. 90(1): 61-3.

[30] Yang L, Hao Y, Hu J, et al. Differential effects of depot medroxyprogesterone acetate administration on vaginal microbiome in Hispanic White and Black women. Emerg Microbes Infect. 2019. 8(1): 197-210.

[31] Hinderfeld AS, Phukan N, Bär AK, Roberton AM, Simoes-Barbosa A. Cooperative Interactions between Trichomonas vaginalis and Associated Bacteria Enhance Paracellular Permeability of the Cervicovaginal Epithelium by Dysregulating Tight Junctions. Infect Immun. 2019. 87(5).

[32] Borgdorff H, Gautam R, Armstrong SD, et al. Cervicovaginal microbiome dysbiosis is associated with proteome changes related to alterations of the cervicovaginal mucosal barrier. Mucosal Immunol. 2016. 9(3): 621-33.

[33] Vintermyr OK, Andersland MS, Bjørge T, et al. Human papillomavirus type specific risk of progression and remission during long-term follow-up of equivocal and low-grade HPV-positive cervical smears. Int J Cancer. 2018. 143(4): 851-860.

[34] Doerflinger SY, Throop AL, Herbst-Kralovetz MM. Bacteria in the vaginal microbiome alter the innate immune response and barrier properties of the human vaginal epithelia in a species-specific manner. J Infect Dis. 2014. 209(12): 1989-99.

[35] Zhang C, Liu Y, Gao W, et al. The direct and indirect association of cervical microbiota with the risk of cervical intraepithelial neoplasia. Cancer Med. 2018. 7(5): 2172-2179.

[36] Łaniewski P, Barnes D, Goulder A, et al. Linking cervicovaginal immune signatures, HPV and microbiota composition in cervical carcinogenesis in non-Hispanic and Hispanic women. Sci Rep. 2018. 8(1): 7593.

[37] Ye X, Liu J, Yi Z. Trends in the Epidemiology of Sexually Transmitted Disease, Acquired Immune Deficiency Syndrome (AIDS), Gonorrhea, and Syphilis, in the 31 Provinces of Mainland China. Med Sci Monit. 2019. 25: 5657-5665.

[38] Silverman RA, Katz DA, Levin C, et al. Sexually Transmitted Disease Partner Services Costs, Other Resources, and Strategies Across Jurisdictions to Address Unique Epidemic Characteristics and Increased

Incidence. Sex Transm Dis. 2019. 46(8): 493-501.

[39] Pedersen AB, Fenton A. Emphasizing the ecology in parasite community ecology. Trends Ecol Evol. 2007. 22(3): 133-9.

[40] Sternberg ED, Lefèvre T, Rawstern AH, de Roode JC. A virulent parasite can provide protection against a lethal parasitoid. Infect Genet Evol. 2011. 11(2): 399-406.

Figures

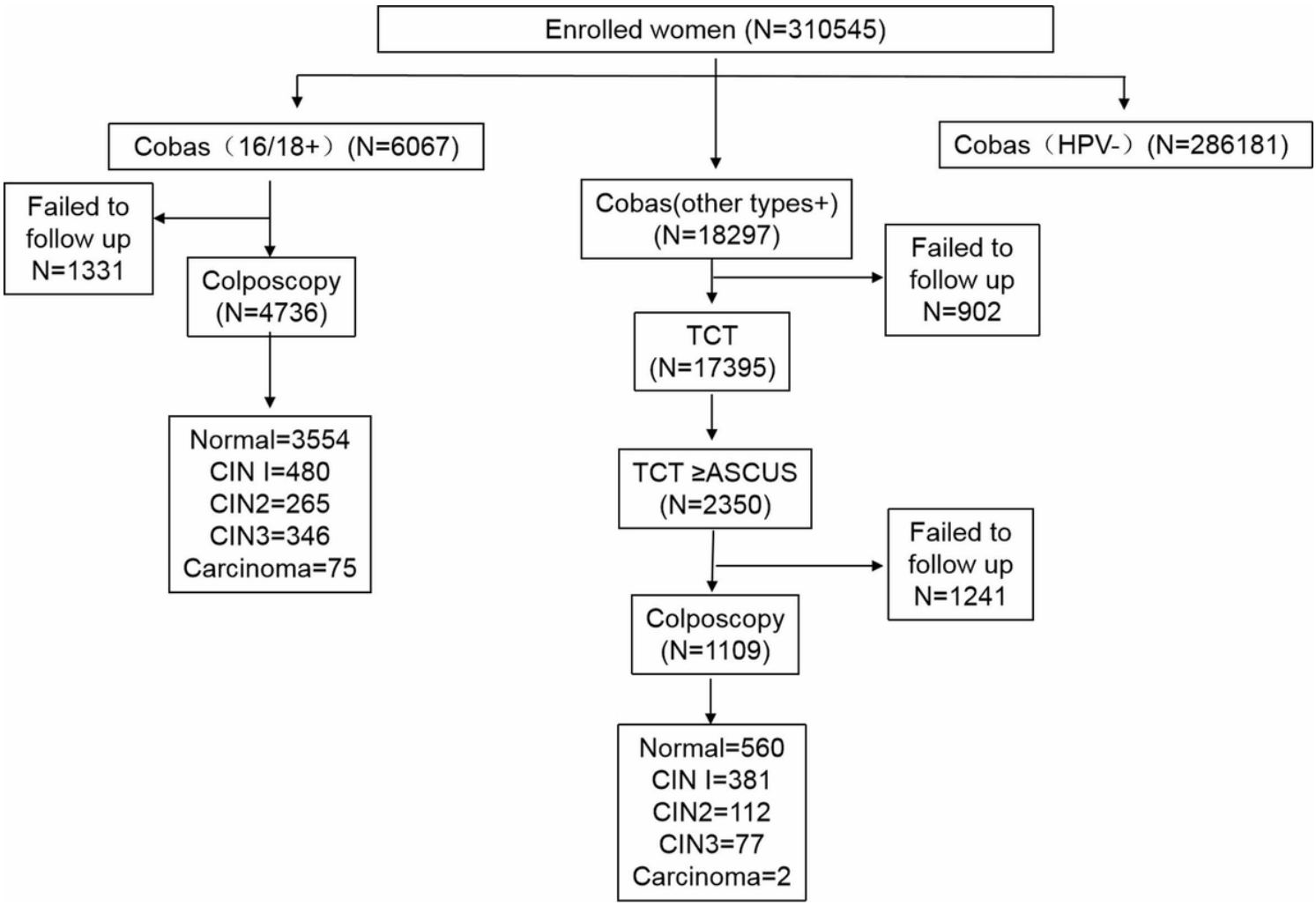

Figure 1

Flowchart of screening profile. Among 310545 women, 6067 were tested positive for HPV 16/18, CIN1, CIN2, CIN3, ICC were detected in 480, 265, 346, 75 cases, respectively. 18297 were tested positive for other hr-HPV genotypes, CIN1, CIN2, CIN3, ICC were detected in 381, 112, 77, 2 cases, respectively.

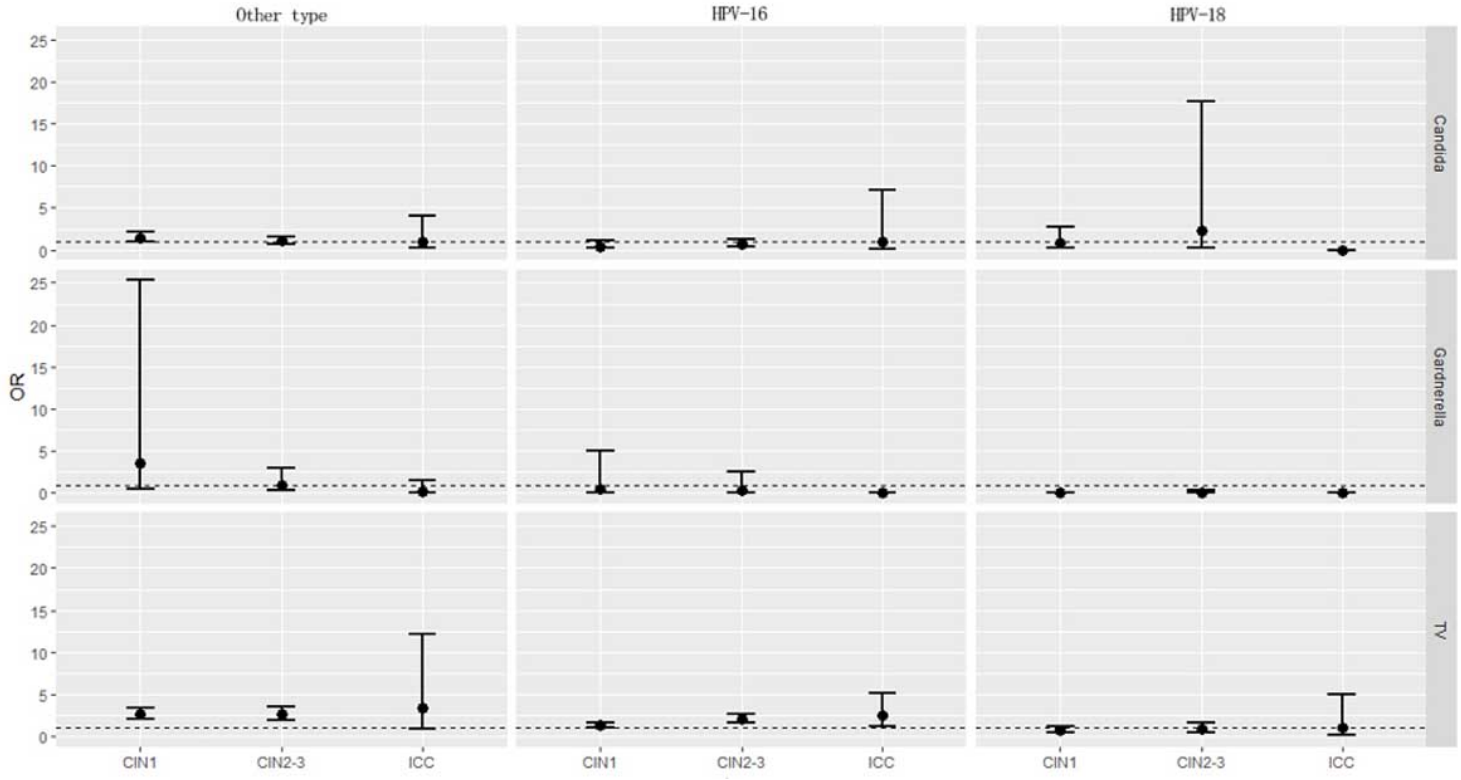

Figure 2

Univariate analyses of Candida albicans, Gardnerella, TV for the risk of CIN1/CIN2-3/ICC in different hr-HPV types.

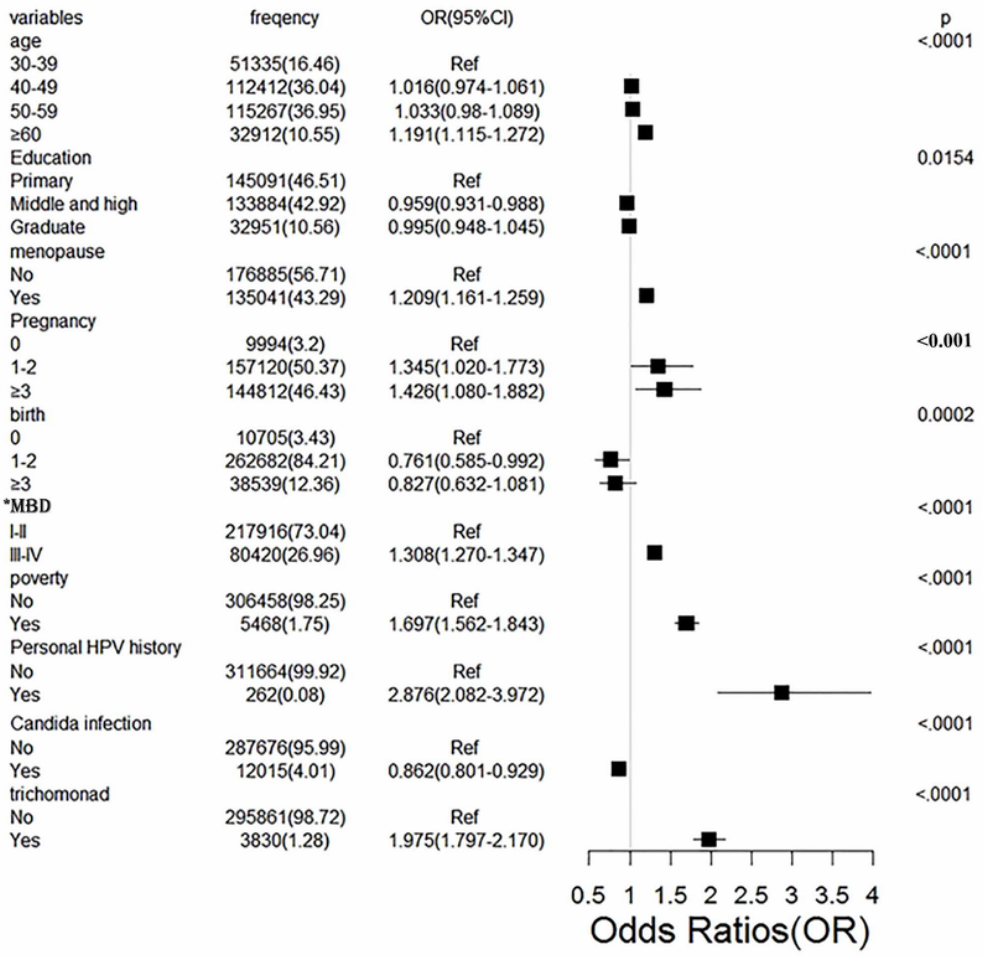

Figure 3

Multivariate analysis of hr-HPV infection. Age, highest level of education, number of pregnancies and number of births were set as rank variables. * MBD means miscellaneous bacteria density.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryBMC.pdf