Int J Clin Exp Med 2018;11(11):12026-12036
www.ijcem.com /ISSN:1940-5901/IJCEM0070378

Original Article

Probing the association between HPV-induced cervical lesions and microflora in vagina

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Received December 5, 2017; Accepted August 29, 2018; Epub November 15, 2018; Published November 30, 2018

Abstract: Cervical intraepithelial neoplasia (CIN) and cervical cancer are main threatening contributors for women healthy. Human papilloma virus (HPV) infection is the primary factor for their occurrence and development. Vaginal flora, PH value and cleanliness play crucial role in the progress of cervical lesions. In this research, we found that vaginal micro-ecological imbalance was gradually increased along with the promoted lesion degree. The most serious vaginal micro-ecological imbalance was detected in cervical cancer group ($P<0.01$). Meanwhile, we also studied the micro-ecology characteristics in patients with or without HR-HPV infection before treatments. The results indicated that HPV infection was an important factor, which could influence the dynamic balance of vaginal microbe system ($P<0.01$). In addition, we investigated the relationships between HPV and mycoplasma/chlamydia composition. Mycoplasma and chlamydia infection might be the reason for the infection persistent with high risk HPV ($P<0.05$). Based on the results above, we consider vaginal micro-ecological detection has the potential ability to predict the development of cervical lesion and evaluate the prognosis of lesions, which provide clinical guidance to HPV treatment. The results in this study would provide important information for further research.

Keywords: Cervical intraepithelial neoplasia, cervical cancer, human papilloma virus, micro-ecology, mycoplasma, chlamydia

Introduction

Cervical lesion includes cervicitis, CIN and cervical cancer. Cervical lesion is an HPV continuous infection disease that has been identified as a contributing factor to the development of CIN and cervical cancer [1]. HPV is a group of double-stranded DNA virus. According to the HPV types and the cancer risk level, this virus could be divided into low-risk subgroup and high-risk subgroup. Low-risk subgroup, including HPV6, HPV11, HPV42, HPV43, HPV44, could induce genital warts and other lesions. High-risk subgroup (HR-HPV) contains HPV16, HPV18, HPV31, and so on, which are highly related with CIN and cervical cancer. The detection rates of HR-HPV are gradually increased from normal population, C1N to cervical cancer [2]. CIN could be divided into three grades, including CINI, CINII and CINIII. About 70% of CIN1 patients could be naturally subsided; 20% of CIN1 patients would be continuous infected; 10% of CIN1 patients would develop into higher grades. Of the CIN2 and CIN3 patients, approximately 30-50% patients would evolve into cervical cancer. Meanwhile, portion of CIN1 and CIN2 would become into invasive carcinoma [3]. The evolutional time from CIN to cervical cancer is about 8-10 years. Therefore, it is very important for prognosis detection and treatments of patients with CINs during this period.

Vaginal microecology is composed by vaginal flora, endocrine regulation and anatomical structures. Vagina *Lactobacillus*, *Escherichia coli* and *Gardnerella* are three dominant bacteria in healthy women. Specifically, the prominent *Lactobacillus* accounts for over 95% of the whole vaginal microflora [4]. Vaginal flora plays as a biological barrier, which could keep the highly ordered colonization in vaginal mucosa and secretion. Meanwhile, vaginal flora also plays important roles in maintaining vaginal acidic environment and activating immune system [5]. For example, activation of immune function, interference of cancer cell material metabolism, effective removal of carcinogenic factors, induction of tumor cell apoptosis, and so on. Vaginal microbes, host, and environment maintain the dynamic balance in human body

[6]. The changes of vaginal *Lactobacillus* could lead to dysbacteriosis and pH abnormalities, which result in the invasion of exogenous harmful microorganisms and endogenous pathogens reproduction. Meanwhile, abnormal colony composition could cause the occurrence and development of the vaginal, cervical and even pelvic disease [7]. Moreover, homeostasis damage in vagina would increase the opportunities of HPV infection, which promote the possibility from cervical lesions to cancer. High grade CIN would promote the opportunities for HPV infections, indicating the positive relationships between CIN and HPV infection [8].

Cervical cancer maintains a high incidence in China [9]. *Lactobacillus* negative rates in cervical cancer group and CIN group were significantly higher than that in normal group and chronic cervicitis group. Moreover, the number of *Lactobacillus* colonies in cervical cancer group and CIN group was lower than that in normal group and chronic heroitis group, which indicated that *Lactobacillus* was related with HPV infection, precancerous lesions and cervical cancer [10]. *Lactobacillus* shows excellent adhesion to cervical epithelial cells. In addition, the saccharides and phospholipids in its surface could promote antigen molecules expression in cervical cancer cells surface. Overexpressed antigen molecules in cancer cells would help immune cells to recognizing themselves, which accelerate the apoptosis and death of cervical cancer cells [11].

Recently, vaginal micro-ecology had been gradually understood. The combination of microbiology and gynecological infectious could be helpful to establish standardized clinical evaluation system. Cervical lesions occurrence is a series of complex biological process, which could be regulated by multi-steps and multi-factors. Therefore, HPV infection, CIN and cervical cancer could be the potential induction factors. Relationships of these factors would enable us to explore the potential mechanism of CIN and carcinogenesis. Meanwhile, it would also provide comprehensive information for CIN, cervical cancer and HPV infection.

Method and materials

Patients

432 female with HPV infection in Renmin Hospital of Wuhan University from December 2014 to October 2016 have been recruited in this study. Meanwhile, 100 healthy female were selected as control group. Patient enrollment conditions are as follows: married or sexual life, menstrual regularity; pregnancy, lactation and menopausal cases have been excluded out of this study. Before 24 h of samples collection, all members are forbidden for ban bath, sexual intercourse, vaginal examination, vaginal lavage and other vaginal medical. Mean age of all 532 members was 42.22±8.76. All members have reviewed and signed the study's informed consent. This research has been approved by the ethics committee of Wuhan University. In experimental group, 432 female have been further divided into three subgroups (CINI: 136 female; CINII & CINIII: 263 female; Cervical cancer: 33 female) based on their pathological diagnosis [12].

Vaginal secretions collection and diagnostic criteria

Vagina was opened with a diffuser, which was not coated with a lubricant. A sterile cotton swab was rotated for 10-15 seconds in the upper 1/3 of vaginal, which was near the dome. The secretions on the swab were sent for further study. Vaginal microbiological diagnostic criteria were listed as following: (1) Gram staining with micro-mirror test [13]: 1) Colony density: observation under 10×100 times oil mirror, the average number of bacteria per field was divided into I-IV level. The average number of bacteria per field from 1 to 9 was considerate as level I; the average number of bacteria per field from 10 to 99 was considerate as level II; the average number of bacteria per field over 100 or bacteria full field of vision was considerate as level III; Bacteria was gathered into clusters; Densely covered mucosal epithelium was considerate as level IV. 2) Bacterial diversity: Bacteria were divided into I-IV level according to the number of identified bacteria. Distinguishing 1 to 3 types bacteria was regarded as level I; Distinguishing 4 to 6 types bacteria was regarded as level II; Distinguishing 7 to 10 types bacteria was regarded as level III; Distinguishing over 11 types bacteria was regarded as level IV. 3) Dominant bacteria: The most common microorganism was defined as dominant bacterium. 4) H_2O_2:H_2O_2 content of vaginal secretions represented vaginal microbial function situation. When the content of

Table 1. Age distribution of different groups

Group	No.	$\bar{x} \pm s$	F	P
CINI	136	41.81±7.97	14.24	<0.0001
CINII & CINIII	263	42.22±9.13		
Cervical cancer	33	50.70±11.06*		

*: there are significant difference between cervical cancer group and other two groups.

$H_2O_2 \geq 2$ umol/L, it was judged as H_2O_2 positive. H_2O_2 and other four important indicators, including the neuraminidase (SNa), Leukocyte Esterase (LE), β-glucuronidase (GUS) and coagulase (GADP), were examined by ELISA kit for aerobic vaginitis/bacterial vaginosis (ABV, Beijing Zhongsheng Jinyu diagnostic technology Limited by Share Ltd, China). The detailed operation steps were followed by protocol provided by manufacture. Meanwhile, we employed the HPV genotypes test kit (21 kinds-HPV typing test kit, Hybribio, China) to judge HPV genotypes. Amplification products were detected by reverse dot blot hybridization with coating type-specific probe membrane. alkaline phosphatase qualitative testing was used to identify 21 HPV genotypes (HPV 6, HPV l1, HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV42, HPV43, HPV44, HPV45, HPV51, HPV52, HPV53, HPV56, HPV58, HPV59, HPV66, HPV68 and HPV81). Furthermore, we also performed mycoplasma and chlamydia examination with commercial kits (Mycoplasma Stain Assay Kit, Beyotime, China) (Chlamydia test kit, ASI, USA). The detailed steps were based on the instruction provided by provider.

Statistical analysis

SPSS 11.5 software (SPSS Inc., Champaign, IL) was used for data analysis. We employed X^2 test method to test data difference. P<0.05 was regarded as significant difference, and P<0.01 was regarded as extremely significant difference.

Results

Micro-ecology characteristics of people with HPV infection before treatments

In this study, we choose 432 female with HPV infection as experimental group and 100 healthy female as control group. 432 female were further divided into CINI subgroup (136 female), CINII & CINIII subgroup (263 female) and cervical cancer subgroup (33 female) according to the lesion degree. **Table 1** showed the age distribution in different experimental subgroups. The mean age in different subgroup was compared using variance analysis, the mean age differences between subgroups met statistically significant (F=14.24 P<0.0001). Cervical cancer subgroup owned the highest mean age (50.70±11.06). We also employed the SNK-q method to compare the mean age differences between any two subgroups. The results indicate that there are statistical difference between cervical cancer subgroup and other two subgroups (P<0.05). However, it could not satisfy statistical difference between CINI subgroup and CINII & CINIII subgroup (P>0.05). This outcome suggested that cervical cancer was more likely occurred in older women. Moreover, we also study the detailed microecology characteristics of all members before treatments. Dominant bacteria composition in different subgroups is analysis by chi-square test. Dominant bacteria differences between subgroups met statistically significant (X^2=80.20, P<0.0001) (**Figure 1A**). Dysbacteriosis ratio was gradually increased along with promoted lesion degree. Bacterial diversity differences between subgroups meet statistically significant (X^2=47.57, P<0.0001) (**Figure 1B**). Level II ratio and level IV ratio of bacterial diversity had the highest values in cervical cancer subgroup, which suggested that it would be related with cancer formation. Colony density differences between subgroups met statistically significant (X^2=27.93, P<0.0001) (**Figure 1C**). Level I ratio and level IV ratio of colony density were increased accompanied with the promoted lesion degree. However, Level II ratio and level III ratio were decreased. Furthermore, we also invested the other indicators of micro-ecology in vagina, including H_2O_2, SNa, LE, GUS and GADP (**Figure 1D-H**). Chi-square test indicated that there were differences of H_2O_2, SNa and GADP between different subgroups (P<0.05). The highest values of H_2O_2 positive and SNa positive could be retrieved in CINII & CINIII subgroup. Meanwhile, the highest values of GADP could be detected in cervical cancer subgroup.

Micro-ecology characteristics of people with or without HR-HPV infection before treatments

In order to study the micro-ecology characteristics between no HPV infection and HR-HPV infection, we investigated the detailed informa-

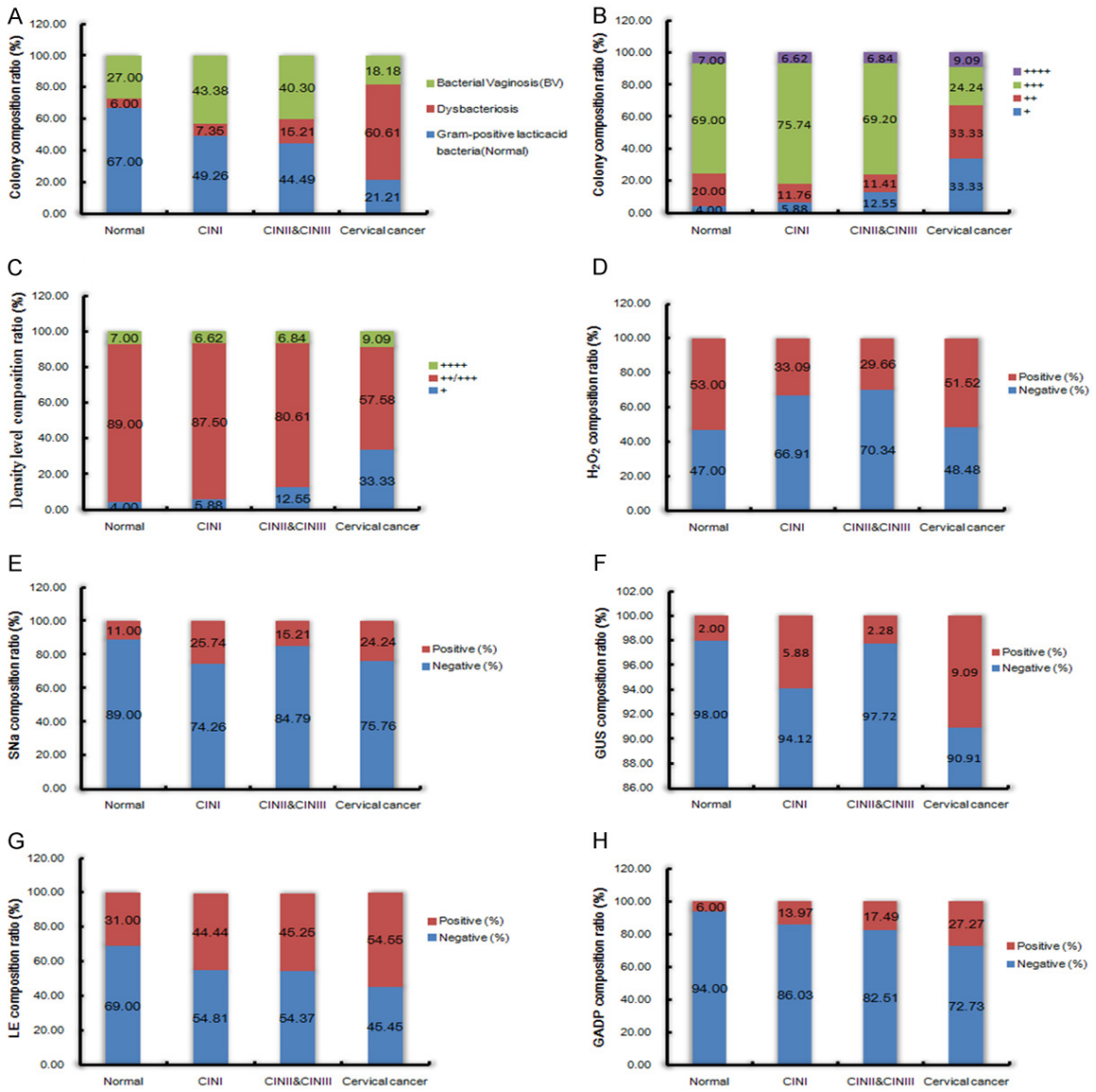

Figure 1. Characteristics of microecology in different subgroups before treatments. A. Dominant bacteria analysis of Normal, CINI, CINII & CINIII, and Cervical cancer. Green color represents bacterial vaginosis (BV); red color represents dysbacteriosis; blue color means Gram-positive lacticacid bacteria (Normal). B. Bacterial diversity analysis of Normal, CINI, CINII & CINIII, and Cervical cancer. C. Colony density analysis of Normal, CINI, CINII & CINIII, and Cervical cancer. D. H_2O_2 analysis of Normal, CINI, CINII & CINIII, and Cervical cancer. E. SNa analysis of Normal, CINI, CINII & CINIII, and Cervical cancer. F. GUS analysis of Normal, CINI, CINII & CINIII, and Cervical cancer. G. LE analysis of Normal, CINI, CINII & CINIII, and Cervical cancer. H. GADP analysis of Normal, CINI, CINII & CINIII, and Cervical cancer.

tion of those two groups, respectively. In the group without HPV infection, 154 female were divided into four subgroups (Normal: 74 female; CINI: 28 female; CINII & CINIII: 49 female; Cervical cancer: 3 female). Dominant bacteria composition in different subgroups was analysis by chi-square test. Dominant bacteria differences between subgroups met statistically significant (X^2=14.00, P<0.0297) (**Figure 2A**). Bacterial diversity differences between subgroups also met statistically significant (X^2= 17.70, P<0.0389) (**Figure 2B**). Level II ratio had the highest values in cervical cancer subgroup. Colony density differences between subgroups could not meet statistically significant (X^2=2.24, P<0.8966) (**Figure 2C**). Level II ratio and level III ratio had the highest values in cervical cancer subgroup. Furthermore, we also invested the

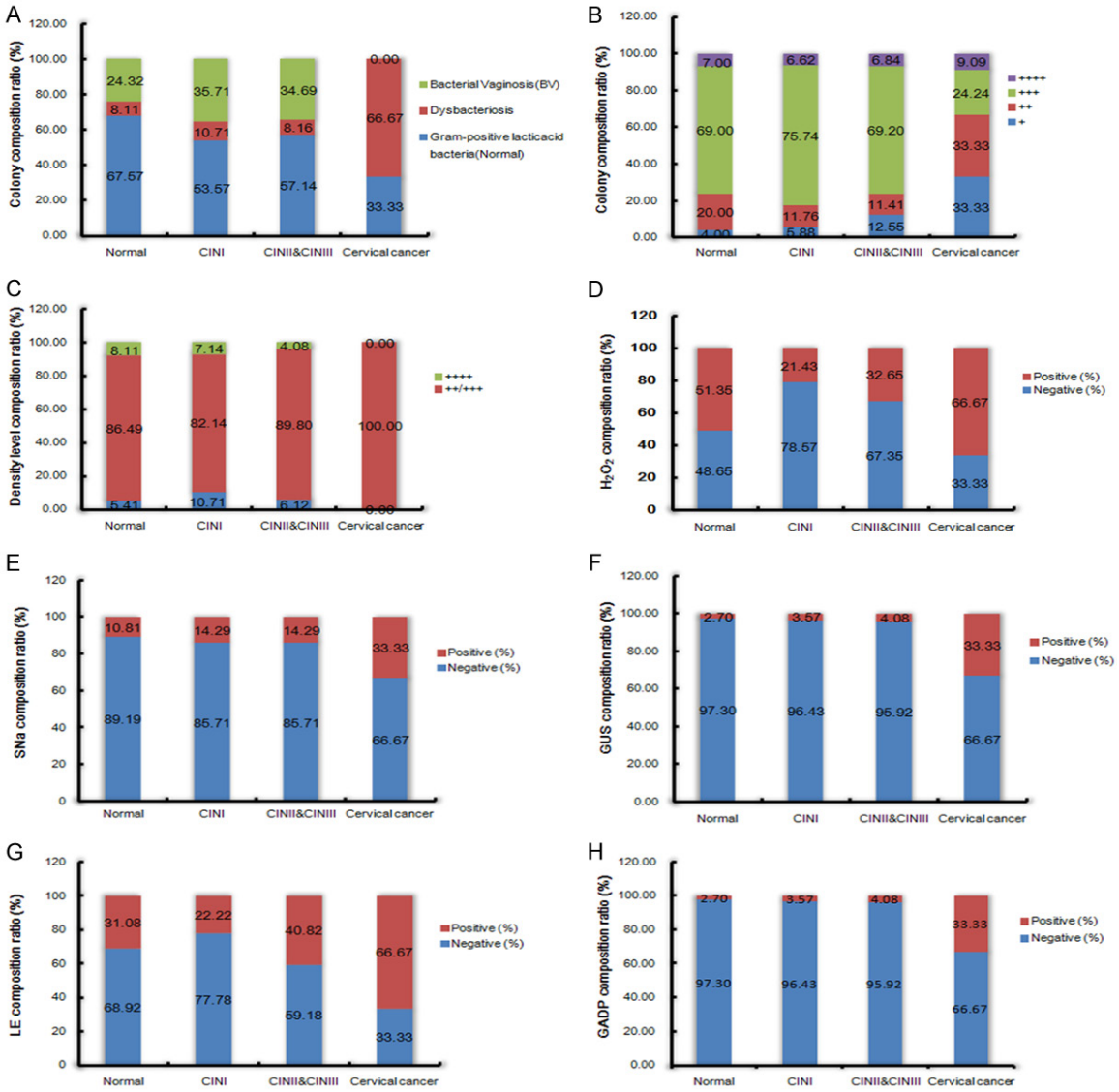

Figure 2. Characteristics of microecology in different subgroups without HPV infection before treatments. A. Dominant bacteria analysis of Normal, CINI, CINII & CINIII, and Cervical cancer. Green color represents bacterial vaginosis (BV); red color represents dysbacteriosis; blue color means Gram-positive lacticacid bacteria (Normal). B. Bacterial diversity analysis of Normal, CINI, CINII & CINIII, and Cervical cancer. C. Colony density analysis of Normal, CINI, CINII & CINIII, and Cervical cancer. D. H_2O_2 analysis of Normal, CINI, CINII & CINIII, and Cervical cancer. E. SNa analysis of Normal, CINI, CINII & CINIII, and Cervical cancer. F. GUS analysis of Normal, CINI, CINII & CINIII, and Cervical cancer. G. LE analysis of Normal, CINI, CINII & CINIII, and Cervical cancer. H. GADP analysis of Normal, CINI, CINII & CINIII, and Cervical cancer.

other indicators of micro-ecology in vagina, including H_2O_2, SNa, LE, GUS and GADP (**Figure 2D-H**). Chi-square test indicates that there are no differences of SNa, LE, GUS and GADP between different subgroups ($P>0.05$). H_2O_2 values in different subgroups met statistical differences ($P<0.05$). The highest values of H_2O_2 positive could be retrieved in CINII & CINIII subgroup. The second highest values of H_2O_2 positive could be found in cervical cancer subgroup. In the group with HR-HPV infection (HPV16 and/or HPV18), 205 female were further divided into four subgroups (Normal: 9 female; CINI: 56 female; CINII & CINIII: 112 female; Cervical cancer: 28 female). Dominant bacteria composition in different subgroups was also analysis by chi-square test. Dominant bacteria differences between subgroups met

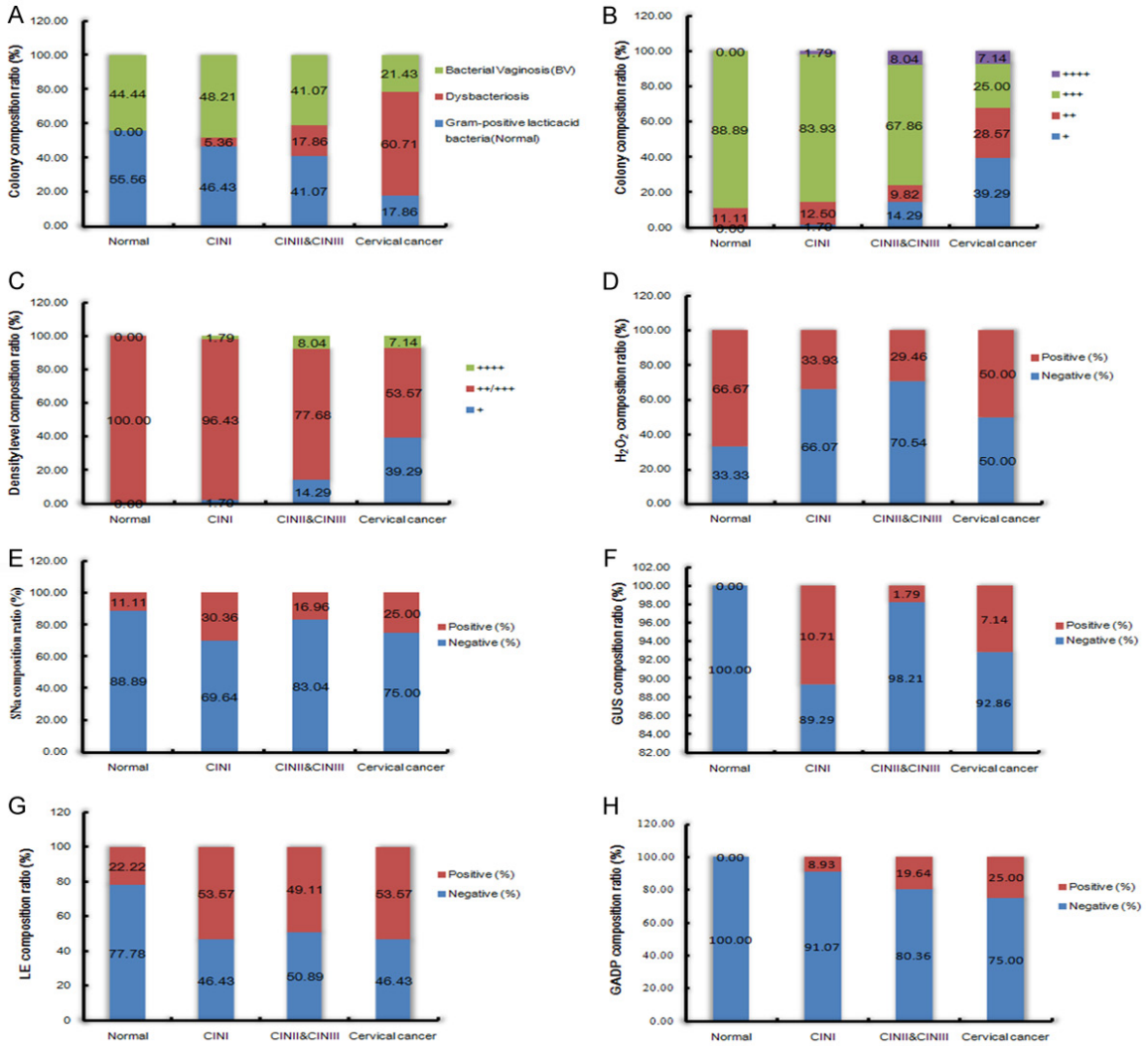

Figure 3. Characteristics of microecology in different subgroups with HPV16 and/or HPV18 infection before treatments. A. Dominant bacteria analysis of Normal, CINI, CINII & CINIII, and Cervical cancer. Green color represents bacterial vaginosis (BV); red color represents dysbacteriosis; blue color means Gram-positive lacticacid bacteria (Normal). B. Bacterial diversity analysis of Normal, CINI, CINII & CINIII, and Cervical cancer. C. Colony density analysis of Normal, CINI, CINII & CINIII, and Cervical cancer. D. H_2O_2 analysis of Normal, CINI, CINII & CINIII, and Cervical cancer. E. SNa analysis of Normal, CINI, CINII & CINIII, and Cervical cancer. F. GUS analysis of Normal, CINI, CINII & CINIII, and Cervical cancer. G. LE analysis of Normal, CINI, CINII & CINIII, and Cervical cancer. H. GADP analysis of Normal, CINI, CINII & CINIII, and Cervical cancer.

statistically significant (X^2=39.99, P<0.0001) (**Figure 3A**). The ratio of bacterial vaginosis (BV) and Gram-positive lacticacid bacteria (Normal) were decreased along with the promoted lesion degree. However, the ratio of dysbacteriosis was increased. Bacterial diversity differences between subgroups met statistically significant (X^2=40.02, P<0.0001) (**Figure 3B**). Level I, level II and level IV ratio were increased along with the promoted lesion degree. However, Level I ratio was decreased. Colony density differences between subgroups met statistically significant (X^2=28.43, P<0.0001) (**Figure 3C**). Level I ratio and level IV ratio were increased along with the promoted lesion degree. Furthermore, we also invest the other indicators of microecology in vagina, including H_2O_2, SNa, LE, GUS and GADP (**Figure 3D-H**). Chi-square test indicated that there were no differences of SNa, LE, GUS and GADP between different subgroups (P> 0.05). H_2O_2 values in different subgroups met statistical differences (P<0.05). The highest values of H_2O_2 positive could be retrieved in Normal subgroup, which suggested that H_2O_2

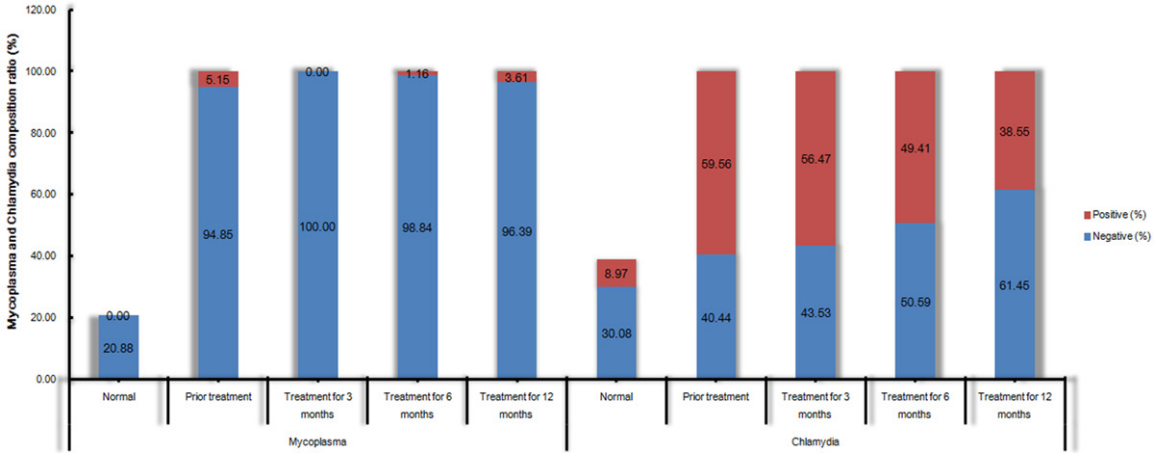

Figure 4. Characteristics of mycoplasma and chlamydia composition before and after treatments.

Table 2. HPV (HPV16 and HPV18) infection and chlamydia distribution in different time phases

Groups				Chlamydia before treatment		Total
				Negative	Positive	
Normal	HPV	Negative	Count	60	14	74
			% of Total	60.0%	14.0%	74.0%
		Positive	Count	20	6	26
			% of Total	20.0%	6.0%	26.0%
	Total		Count	80	20	100
			% of Total	80.0%	20.0%	100.0%
Prior treatment	HPV	Positive	Count	26	30	56
			% of Total	46.4%	53.6%	100.0%
	Total		Count	26	30	56
			% of Total	46.4%	53.6%	100.0%
Treatment for 6 months	HPV	Negative	Count	8	17	25
			% of Total	22.9%	48.6%	71.4%
		Positive	Count	4	6	10
			% of Total	11.4%	17.1%	28.6%
	Total		Count	12	23	35
			% of Total	34.3%	65.7%	100.0%
Treatment for 6 months	HPV	Negative	Count	10	16	26
			% of Total	32.3%	51.6%	83.9%
		Positive	Count	3	2	5
			% of Total	9.7%	6.5%	16.1%
	Total		Count	13	18	31
			% of Total	41.9%	58.1%	100.0%
Treatment for 12 months	HPV	Negative	Count	24	8	32
			% of Total	68.6%	22.9%	91.4%
		Positive	Count	2	1	3
			% of Total	5.7%	2.9%	8.6%
	Total		Count	26	9	35
			% of Total	74.3%	25.7%	100.0%

would potentially play a protective role for cancer.

Characteristics of mycoplasma and chlamydia in CINI subgroup before and after treatments

In this study, we focused on the mycoplasma and chlamydia composition in CINI subgroup before and after treatments. The mycoplasma and chlamydia composition in Control subgroup (100 healthy female) without treatments, CINI subgroup before treatments, CINI subgroup after treatments 3 months, CINI subgroup after treatments 6 months, and CINI subgroup after treatments 12 months were observed. **Figure 4** showed the detailed mycoplasma and chlamydia composition information. The result indicated that there were differences of mycoplasma and chlamydia composition between different groups ($P<0.05$). The highest my-

Table 3. HPV (HPV16 and HPV18) infection and mycoplasma distribution in different time phases

Groups				Mycoplasma before treatment		Total
				Negative	Positive	
Normal	HPV	Negative	Count	74	0	74
			% of Total	74.0%	0%	74.0%
		Positive	Count	26	0	26
			% of Total	26.0%	0%	26.0%
	Total		Count	100	0	100
			% of Total	100.0%	0%	100.0%
Prior treatment	HPV	Positive	Count	54	2	56
			% of Total	96.4%	3.6%	100.0%
	Total		Count	54	2	56
			% of Total	96.4%	3.6%	100.0%
Treatment for 6 months	HPV	Negative	Count	25	0	25
			% of Total	71.4%	0%	71.4%
		Positive	Count	10	0	10
			% of Total	28.6%	0%	28.6%
	Total		Count	35	0	35
			% of Total	100.0%	0%	100.0%
Treatment for 6 months	HPV	Negative	Count	27	0	27
			% of Total	84.4%	0%	84.4%
		Positive	Count	5	0	5
			% of Total	15.6%	0%	15.6%
	Total		Count	32	0	32
			% of Total	100.0%	0%	100.0%
Treatment for 12 months	HPV	Negative	Count	31	1	32
			% of Total	88.6%	2.9%	91.4%
		Positive	Count	3	0	3
			% of Total	8.6%	0%	8.6%
	Total		Count	34	1	35
			% of Total	97.1%	2.9%	100.0%

coplasma (positive) and chlamydia (positive) ratio could be simultaneously found in CINI subgroup before treatments. After treatments, mycoplasma (negative) and chlamydia (negative) ratio were gradually increased. Moreover, we also examined the connections between HPV (HPV16 and HPV18) infection and chlamydia/mycoplasma distribution. In the chlamydia distribution and HR-HPV analysis, there are no differences between different subgroups with chi square analyses (F=0.0313, P=0.8596) (**Table 2**). Along with the treatment time extension, the proportion of HPV (positive) tended to be similar with that in Control subgroup after 12 months treatment. Meanwhile, the proportion of HPV (negative) tended to be similar with that in Control subgroup after 3 months treatment. The results suggested that chlamydia infection may affect HPV changes. In the analysis of mycoplasma distribution and HR-HPV, there were no differences between different subgroups with chi square analyses (F=0.0937, P=0.7595) (**Table 3**). Along with the time extension, the proportion of HPV (positive) tended to be similar with that in Control subgroup after 3 months treatment. Meanwhile, the proportion of HPV (negative) tended to be similar with that in Control subgroup after 6 months treatment.

Discussion

Micro-ecology is an emerging discipline in recent 40 years. Micro-ecological balance and imbalance theory are the core issues. Vaginal micro-ecology has become to be an independent branch of micro-ecology [14]. Vaginal microflora is a dynamic equilibrium micro-ecosystem, which is related to the mutual promotion, coordination and mutualism between dominant bacteria and other bacterial species. The broken dynamic balance would lead to vaginal disease. Therefore, understanding the potential relationships between vaginal microflora, HPV infection, CIN even cervical cancer are becoming very important. Dominant bacteria in female vagina are *Lactobacillus*, accounting for 95% of the whole vaginal microflora. *Lactobacillus* could be widely found in the mucosal surface of the reproductive tract. This feature make this bacterium could adhesion to the mucosa and matrix forming a protective biofilm, which could effectively inhabit the pathogen adhesion and infection. Meanwhile, *Lactobacillus* could decompose glycogen in vaginal mucosal epithelial and pro-

duct lactic acid, that is mainly contributor to maintain the pH value (3.8-4.4). Acidic environment ensure most bacteria in vagina belonging to acidophilus and acid-fast bacteria. On contrary, the growth of other pathogenic bacteria, including *Candida albicans*, *Escherichia coli* and *Gardnerella*, would be inhabited [15]. Therefore, academic perspectives think *Lactobacillus* is indispensable to keep vaginal healthy [16]. In recently, the formation and development of cervical cancer is considerate to keep close relationships with dysbacteriosis in vagina. *Lactobacillus* reduction and gardnerella vaginalis multiply would generate a large number of toxic metabolites, which together with other carcinogenic factors (human papillomavirus, human cytomegalovirus infection, etc.) would accelerate the cervical cancer occurrence [17]. Korshunov et al. had studied the distribution of vaginal flora in patients with CIN and HPV infection [18]. The results indicated that the CIN occurrence was closely related to the *Lactobacilli* reduction. Meanwhile, other researchers think cervical cancer is associated to the *Lactobacilli* reduction and dysbacteriosis in vagina. Vaginal *Lactobacilli* could active the immune system, which would inhabit the cancer formation and development by cell apoptosis pathway [19-21].

In this study, 432 female with HPV infection and 100 healthy female were selected as research object. Cervical cancer subgroup owned the highest mean age (50.70±11.06), which was significantly different with other subgroups. Previous study had revealed that cervical cancer in elderly patients showed an increasing trend in Europe. Meanwhile, older women account to over 40% of the deaths with cervical cancer per year [22], which is adapted to our results. Therefore, more attentions should be paid to the screening, prevention and treatment of cervical cancer in elderly women. Dominant bacteria analysis indicated that there existed differences between subgroups. Dysbacteriosis ratio was gradually increased along with the promoted lesion degree, which was adaptable to the previous study [23]. In this study, bacterial diversity and colony density differences between subgroups met statistically significant. Furthermore, we also invested other indicators, including H_2O_2, SNa, LE, GUS and GADP. There were differences of H_2O_2, SNa and GADP between different subgroups. The highest values of H_2O_2 positive and SNa positive could be retrieved in CINII & CINIII subgroup. Meanwhile, the highest values of GADP could be detected in cervical cancer subgroup. These results suggested that vaginal microecological imbalance was the most serious issue in cervical cancer group. We consider that HPV infection and other cervical diseases are related to the infection of vaginal pathogenic bacteria [24]. Therefore, we studied the microecology characteristics in people with or without HR-HPV infection before treatments. In members without HR-HPV infection, dominant bacteria, bacterial diversity, SNa, LE, GUS and GADP between subgroups could not meet statistically significant. Colony density and H_2O_2 in different subgroups meet statistical differences. In the group with HR-HPV infection, dominant bacteria, bacterial diversity, colony density, H_2O_2 could meet statistically significant. SNa, LE, GUS and GADP could not meet statistically significant. The results indicated that HPV infection should be an important factor in the dynamic balance of vaginal microbe system [25]. In addition, we focused on whether mycoplasma and chlamydia composition in CINI subgroup would be influenced before and after treatments. There are differences of mycoplasma and chlamydia composition between different groups. The highest mycoplasma (positive) and chlamydia (positive) ratio could be found in CINI subgroup before treatments. After treatments, mycoplasma (negative) and chlamydia (negative) ratio are gradually increased. In the chlamydia distribution and HR-HPV analysis, the proportion of HPV (positive) tended to be similar with that in Control subgroup after 12 months treatment. The proportion of HPV (negative) tended to be similar with that in Control subgroup after 3 months treatment. The results suggested that chlamydia infection may change HPV infection to negative. In the mycoplasma distribution and HR-HPV analysis, HPV (positive) tended to be similar with that in Control subgroup after 3 months treatment. The proportion of HPV (negative) tended to be similar with that in Control subgroup after 6 months treatment. In summary, mycoplasma and chlamydia infection might be the factor to persistent high risk HPV infection.

Through detecting the vaginal micro-ecology in 432 research subjects and 100 controls, we found that vaginal micro-ecological imbalance

was gradually increased along with the promoted lesion degree. The most serious vaginal microecological imbalance was the cervical cancer group. Meanwhile, we also study the micro-ecology characteristics in patients with or without HR-HPV infection before treatments. The results indicated that HPV infection should be an important factor affecting the dynamic balance of vaginal microbe system. In addition, we invested the relationships between HPV and mycoplasma/chlamydia composition. Mycoplasma and chlamydia infection might be the factor to persistent high risk HPV infection. Based on the results above, we consider vaginal micro-ecological detection could be a potential indicator to predict and evaluate cervical lesion. The results of this study would provide important information for further research.

Acknowledgements

We acknowledge the work of patients involved in this study.

Disclosure of conflict of interest

None.

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