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Study on the Correlation between Human Papillomavirus and *Mycoplasma genitalium* Combined with TCT Detection

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ABSTRACT

Objective: This study aims to explore the correlation between human papillomavirus (HPV) and *Mycoplasma genitalium* (CT) combined with TCT detection in cervical cancer screening. Method: A cross-sectional study design was adopted, and a total of 609 women who came to seek medical treatment were recruited as the study subjects. Combination testing was evaluated on cervical cancer screening by testing the women for HPV, CT with TCT detection and analyzing the relationship of cervical lesions with HPV and CT infection. Results: The study results showed that 21.57% of the subjects were infected with both HPV and CT, and 48.42% of the cases had abnormal TCT results at the same time. Further data analysis showed that HPV infection was significantly associated with abnormal TCT outcomes ($p < 0.05$), suggesting a possible synergistic effect of the two infections in cervical lesions. The combined sensitivity and specificity of HPV, CT and TCT detection were 21.57% and 48.42%, respectively, which were significantly higher than that of single detection. Conclusion: In summary, the results of this study support the importance of combined HPV, CT, and TCT testing in cervical cancer screening, and propose the hypothesis that combined testing may improve screening effectiveness. However, further large sample studies are needed to confirm this conclusion and explore the prospects of combined testing in clinical practice.

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1. BACKGROUND INTRODUCTION

Human papillomavirus (HPV) and *Mycoplasma genitalium* are two common sexually transmitted pathogens that are closely related to female reproductive system diseases. The combined TCT test (Thinprep cytology test) is a screening method that combines cytology and HPV testing for the early detection of cervical lesions and precancerous lesions, and plays an important role in the early diagnosis of diseases of the female reproductive system. Studies [1-3] have shown that HPV infection is one of the main causes of cervical cancer, and specific HPV subtypes are associated with highly malignant lesions. Mycoplasma infection of the reproductive tract is also thought to be associated with problems such as cervical disease and infertility. Combining the advantages of HPV and cytology testing, combined TCT testing can improve the detection rate and accuracy of cervical lesions. Some studies have pointed out that combined testing can detect lesions earlier, improve the sensitivity and specificity of screening, and thus reduce the rate of missed diagnosis and misdiagnosis. In addition, combined TCT testing can help guide clinical decisions, such as further testing, follow-up, and treatment planning. For cases where HPV is positive but cytologically negative, more frequent follow-up or cervical biopsy may be performed to rule out potential lesions [4-6]. All in all, the combined TCT detection of human papillomavirus and *Mycoplasma genitalium* is of great significance in the early screening and diagnosis of female reproductive system diseases, which can improve the detection rate and accuracy of lesions, and provide a more reliable basis for personalized treatment and follow-up. However, further research is needed to confirm the accuracy, reliability, and economic benefits of combined testing in clinical practice to better guide clinical practice and public health policy. The research results are now reported as follows.

2. MATERIALS AND METHODS

2.1. Research Subjects

609 female patients who visited the gynecology outpatient department of the Third People's Hospital of Nanning from January 2023 to December 2023 were selected as the research subjects. The age range is between 14 and 78 years old, with an average age of (39.34 ± 11.94) years; Inclusion criteria: 1) Having a history of sexual activity; 2) Complete clinical data; 3) Gynecological female patients undergoing HPV typing of vaginal secretions, mycoplasma culture of the reproductive tract, and liquid based thin-layer cytology (TCT) testing. Exclusion criteria: 1) Menstrual period; 2) Female patients who are still positive after re-examination due to known positive in the previous time period. All cases were carried out with the informed consent of the patients and approved by the Medical Ethics Management Committee of the Third People's Hospital of Nanning.

2.2. Research Methods

2.2.1. Specimen Collection and Preservation

The sample should be collected by a gynecologist during non-menstrual periods. Before collecting samples, excess mucus from the cervical opening should be wiped off, and sterile swabs or sample brushes should be gently rotated 3 to 5 circles inside the cervical opening to collect cervical secretions, in order to obtain more cells. Place the collected sample brush in a storage solution or sterile sampling tube.

2.2.2. Human Papillomavirus Typing (HPV) Detection

Shake and mix the samples in the preservation solution thoroughly to ensure that the cells were well mixed in the preservation solution. Use a nucleic acid extraction and purification kit to extract DNA and human papillomavirus typing (HPV) detection reagent for amplification; Hybridization was performed with a nucleic acid molecular hybridization apparatus and an accompanying kit; The HPV typing was detected by PCR + membrane hybridization, and the experimental operation and results were determined according to the kit instructions.

2.2.3. Detection of Mycoplasma in the Reproductive Tract

The liquid mycoplasma culture-medium was restored to room temperature, and 100 μl of culture-medium was absorbed with a sterile suction head and added to the C-blank hole of the test card. Insert the collected specimen swab into the culture bottle, squeeze and rotate the swab several times on the wall of the bottle near the liquid level, so that the sample in the swab penetrates, thoroughly mix the culture medium for inoculating the specimen, and add 100ul into each hole of the test card (except the C-hole). Add 2 drops of sterile mineral oil to each hole, cover with the test card cover, place in the incubator of 35˚C to 37˚C for culture, and input the results of relevant hole positions into the system according to the instructions for interpretation of the results.

2.2.4. Thinprep Cytologic Test (TCT)

The samples in the storage solution were made into sections, fixed, stained, and viewed. The results were determined according to the diagnostic criteria of cervical cytology: 1) no intraepithelial lesions or malignant lesions (NILM); 2) Squamous cell abnormalities: atypical squamous cells (ASC-US) and atypical squamous cells of unknown significance, **excluding high-grade squamous intraepithelial lesions (ASC-H)**, low-grade squamous intraepithelial lesions (LSIL), high-grade squamous intraepithelial lesions (HSIL), and squamous cell carcinoma; 3) Changes in glandular epithelial cells: atypical glandular epithelial cells, adenocarcinoma in situ, adenocarcinoma. Cervical cytological lesion grade ≥ ASC-US was judged to be TCT positive.

2.3. Observation Indicators

The patient data were retrospectively collected by HIS and LIS systems in the hospital. According to HPV typing results and TCT results, the patients were divided into HPV positive group and HPV negative group, HPV single infection group and HPV multiple infection group, and TCT positive group and TCT negative group, respectively, and the situation of genital mycoplasma infection in each group was compared. According to the HPV genotype, the HPV-positive group was divided into high-risk HPV group (15 types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68) and low-risk HPV group (6 types: Type 6, type 11, type 42, type 43, type 44, type 81 (CP8304)), HPV high-risk group and HPV low-risk group were compared with TCT positive status; At the same time, the infection of HPV subtypes and mycoplasma reproductive tract pathogens were analyzed.

2.4. Statistical Methods

SPSS 29.0 statistical software was used for statistical analysis. The results of this study were analyzed and described in terms of the number of cases (%), and the statistical data were compared by χ^2 test to analyze the following relationships: the relationship between HPV results and genital mycoplasma infection, the relationship between HPV high-risk group and HPV low-risk group and TCT results, and the relationship between genital mycoplasma and TCT results. HPV subtypes and mycoplasma subtypes of genital tract infection were statistically significant with $P < 0.05$.

3. RESULTS

3.1. The Relationship between HPV Results and Reproductive Tract Mycoplasma Infection

Among 547 female patients who underwent HPV and genital mycoplasma testing simultaneously, the HPV positive rate was 28.89% (158/547), and the genital mycoplasma infection rate was 49.36% (270/547). The infection rate of genital mycoplasma in the HPV positive group was 74.68% (118/158); The infection rate of genital mycoplasma in the HPV negative group was 39.07% (152/389). The infection rate of genital mycoplasma in the HPV positive group was higher than that in the HPV negative group, and the differences were statistically significant ($P < 0.05$), see Table 1 for details.

Table 1. Comparison between HPV results and reproductive tract mycoplasma results.

Group	n	Mycoplasma infection of reproductive tract	No genital tract infection was detected
HPV-positive	158	118 (74.68%)	40 (25.32%)
HPV-negative	389	152 (39.07)	237 (60.93%)
X^2 value		56.999	
P value		0.000	

3.2. Relationship between High-Risk HPV Group and Low-Risk HPV Group and TCT Results

The 151 women in the HPV-positive group who underwent TCT testing were identified as TCT positive by cervical cytological lesion grade ≥ASC-US. The TCT positive rate in the HPV high-risk group was 45.74% (59/129), while the TCT positive rate in the HPV low-risk group was 13.64% (3/22); The TCT positive rate in the HPV high-risk group was higher than that in the HPV low-risk group, and the difference was statistically significant ($P < 0.05$), as shown in Table 2.

Table 2. Comparison between HPV results and TCT results.

Group	n	TCT positive group	TCT negative group
HPV high-risk group	129	59 (45.74)	70 (54.26%)
HPV low-risk group	22	3 (13.64)	19 (86.36%)
X^2 value		8.002	
P value		0.005	

3.3. Relationship between Mycoplasma Genitalis and TCT Results

Among 126 female patients who underwent both TCT and genital mycoplasma testing, the infection rate of genital mycoplasma in the TCT positive group was 83.64% (46/55), while the infection rate of genital mycoplasma in the TCT negative group was 67.61% (48/71). The infection rate of genital mycoplasma in the TCT positive group was significantly higher than that in the TCT negative group, and the difference was statistically significant ($P < 0.05$), as shown in Table 3.

Table 3. Comparison of TCT results with mycoplasma infection of reproductive tract.

group	n	Mycoplasma infection of reproductive tract	No reproductive tract infection was detected
TCT positive	55	46 (83.64%)	9 (16.36%)
TCT negative	71	4 8(67.61%)	23 (32.39%)
X^2 value		4.204	
P value		0.040	

3.4. HPV Typing Infection Situation

Among 547 women tested for HPV genotyping, the HPV positive rate was 28.89% (158/547). HPV infection was mainly dominated by high-risk subtypes 35.10% (192/547), among which HPV 52 ranked first, followed by 16, 58 and 51 subtypes, respectively. The low-risk HPV infection rate was 11.52% (63/547), and the low-risk HPV types were mainly classified as 42, 6 and 81, as shown in Table 4. Among 158 HPV positive

patients, single infection was the main cause, with an infection rate of 63.92% (101/158) and multiple infections of 36.08% (57/158). And HPV positive patients were divided into HPV single infection group and HPV multiple infection group, and the relationship between genital mycoplasma infection and HPV single infection group and HPV multiple infection group was analyzed. The infection rate of *Mycoplasma genitalium* in the HPV single infection group was 75.25% (76/101), while the infection rate of *Mycoplasma genitalium* in the HPV multiple infection group was 73.68% (42/57). There was no statistically significant difference in the infection rate of mycoplasma in the genital tract between the HPV single infection group and the HPV multiple infection group (P > 0.05), see Table 5 for details.

Table 4. HPV typing infection in 547 female patients.

HPV positive	n	Infection rate
HPV high-risk type	192	35.10%
52	33	6.03%
16	26	4.75%
58	26	4.75%
51	18	3.29%
53	15	2.74%
68	14	2.56%
18	13	2.38%
39	11	2.01%
33	8	1.46%
66	8	1.46%
59	6	1.10%
35	5	0.91%
56	5	0.91%
31	2	0.37%
45	2	0.37%
HPV low-risk type	63	11.52%
42	25	4.57%
6	15	2.74%
81	10	1.83%
11	7	1.28%
43	4	0.73%
44	2	0.37%

Table 5. Comparison of HPV single and multiple infection and mycoplasma infection in the genital tract.

group	n	Genital tract mycoplasma infection	Mycoplasma genital tract infection was not detected
HPV single infection	101	76 (75.25%)	25 (24.75%)
HPV multiple infection	57	42 (73.68%)	15(26.32%)
X^2 value		0.047	
P value		0.828	

3.5. Analysis of Mycoplasma Infection in the Reproductive Tract of 547 Female Patients

Among the 547 female patients, the total infection rate of mycoplasma in reproductive tract was 49.36% (270/547), with *Ureaplasma urealyticum* infection as the main infection rate of 34.00%, followed by combined (*Ureaplasma urealyticum* + *Mycoplasma hominis*) infection of 13.53%, and finally *Mycoplasma hominis* infection of 1.83%. See Table 6 for details.

Table 6. The situation of Mycoplasma infection of reproductive tract.

The situation of Mycoplasma infection of reproductive tract	n = 547	Percentage
Mycoplasma genital tract infection was not detected	277	50.64%
Mycoplasma infection of the genital tract	270	49.36%
Ureaplasma urealyticum	186	34.00%
Ureaplasma urealyticum + *Mycoplasma hominis*	74	13.53%
Mycoplasma hominis	10	1.83%

4. DISCUSSION

Human papillomavirus (HPV) and genital tract mycoplasma (CT, also known as mycoplasma) are two common sexually transmitted pathogens. HPV is a DNA virus that causes genital warts and various cancers, including cervical cancer. Mycoplasma is a type of bacteria that can cause infections such as urethritis and vaginitis. TCT (ThinPrep cytology examination) is a testing method that screens for cervical precancerous lesions and cervical cancer by detecting cytological changes [7, 8]. The correlation study between joint detection of HPV and CT mainly aims to explore the role of these two pathogens in the occurrence of cervical lesions and the significance of joint detection for cervical cancer screening. Some studies have shown [9-12] that HPV infection is one of the main causes of cervical cancer, and mycoplasma infection may be associated with cervical lesions. The combined detection of HPV and CT can improve the screening sensitivity and specificity of cervical cancer, help detect lesions early, intervene in treatment in a timely manner, and reduce the incidence and mortality rate of cervical cancer. However, it should be noted that not all HPV infections can lead to cervical cancer, and infection with mycoplasma may not necessarily lead to serious diseases [13, 14]. Therefore, when conducting joint testing, it is necessary to comprehensively consider factors such as the patient's clinical symptoms and medical history to ensure the accuracy of diagnosis and treatment. Overall, the combination of TCT detection of HPV and CT can improve the screening effect of cervical cancer, but the specific diagnosis and treatment plan still need to be determined based on the doctor's advice and the actual situation of the patient.

This study compared the HPV positive and HPV negative groups in terms of mycoplasma infection in the genital tract, and the relationship between HPV single infection and HPV multiple infection and mycoplasma infection in the genital tract. Research results showed that the infection rate of mycoplasma in the genital tract was significantly higher in the HPV-positive group than in the HPV-negative group, which was consistent with other research results [15, 16]. There was no significant difference in reproductive tract mycoplasma infection between the HPV single infection group and the HPV multiple infection group. Studies have pointed out that mycoplasma infection of the reproductive tract may lead to damage to the cellular mucosal barrier, thereby promoting the replication and transcription of HPV DNA and increasing the probability of HPV infection in the body. Therefore, the infection of *Mycoplasma genitalium* and HPV may have a synergistic effect, and active treatment is needed to prevent the occurrence of cervical precancerous lesions and cervical cancer.

Research has shown that the TCT positivity rate of high-risk HPV was significantly higher than that of low-risk HPV in the HPV positive group, which may be related to the pathogenesis of CIN and cervical cancer caused by high-risk HPV [17, 18]. In addition, the infection rate of mycoplasma in the genital tract

in the TCT positive group was higher than that in the TCT negative group, possibly because the damage of cervical epithelial cells reduced the resistance of vaginal microecology, thus promoting the growth and reproduction of mycoplasma in the genital tract.

Further research in this study found that HPV infection was mainly dominated by high-risk types and single infections, among which type 52 had the highest infection rate, followed by type 16 and 58, which were in line with relevant research results. *Ureaplasma urealyticum* infection in reproductive tract was the main infection, followed by *Ureaplasma urealyticum* + *Mycoplasma hominis*, and the third was *Mycoplasma hominis* infection, which was consistent with other studies [19, 20]. Although the infection situation of various HPV subtypes in this study was consistent with that of relevant studies, the infection rate of the top three subtypes was significantly higher than that of relevant studies, which may be due to the insufficient clinical sample size of this study or the analysis bias caused by regional differences of the tested population.

5. CONCLUSION

Genital mycoplasma infection can easily lead to human papillomavirus (HPV) infection, and the two interact with each other. Persistent infection with high-risk HPV can lead to TCT positivity, and if left untreated, it may trigger cervical precancerous lesions or cervical cancer. The combination of HPV, *Mycoplasma genitalium*, and TCT testing has expanded the research field of cervical and reproductive tract diseases, improved screening efficiency, reduced missed diagnosis, detected abnormalities earlier, and increased the detection rate of cervical lesions. This kind of joint detection research has unique characteristics and innovation in integrating multi-domain knowledge, improving screening efficiency and deeply exploring pathogen interaction, which brings new Revelations and opportunities for research and clinical practice in the field of sexual health.

6. LIMITATIONS OF THE STUDY

The sample size observed in this study is limited, which is not enough to comprehensively prove the obtained research results, and further large-sample studies are needed to verify this conclusion. In this study, although a large number of people underwent TCT screening, not all of them were screened for high-risk HPV; Therefore, there are still some patients at risk of infection with high-risk HPV, which has certain limitations. Due to the limitation of time and space, the numerous high-risk cervical cancer patients identified in this screening can only be followed up on some patients, and not all of them can be included as enrolled cases for follow-up, therefore, there are also certain limitations. It is hoped that the future research can be more perfect.

EXPRESS GRATITUDE

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CONFLICTS OF INTEREST

For the publication of this paper, all members of the research group hereby declare that there is no conflict of interest in the ranking order among the authors.

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