ORIGINAL RESEARCH ARTICLE

Influence of vitamin D supplementation and the vaginal microenvironment on human papillomavirus infection

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Abstract

This was a retrospective study, mainly explored the mediating role of vaginal microenvironment and the influence of vitamin D addition on human papillomavirus (HPV) infection. Five hundred and twelve participants were chosen in this study, followed by dividing into HPV positive (212 cases) and negative groups (300 cases) based on HPV 23 typing results. The high-risk human papillomavirus (HR-HPV) positive group showed higher abnormal rates of lactobacillus, catalase, cleanliness, sialidosidase, and proline aminopeptidase than the HPV negative group. No significant differences were found in pH value, leukocyte esterase, and Acetylglucosaminidase abnormality between 2 groups. The HR-HPV positive group presented a higher percentage of patients with cleanliness III and IV. Relative to low-grade squamous intraepithelial lesion (LSIL) group, HSIL group presented a higher HPV positive infection rate. Mould infection, Gardnerella infection, and catalase were identified as independent risk elements for HR-HPV infection. Vitamin D supplementation was found to potentially reduce HR-HPV infection persistence post-Loop Electrosurgical Excision Procedure (LEEP), improve nutritional health, reduce insulin, insulin resistance (HOMA-IR) and triglyceride levels, as well as reduce high-sensitivity C-reactive protein (hs-CRP) along with malondialdehyde (MDA) levels. Our results indicate that HR-HPV infection is intimately associated with the condition of the vaginal microenvironment, and vitamin D addition potentially reduces the persistence of HR-HPV infection post-LEEP, improves nutritional and metabolic health, reduces inflammation, and be well-tolerated. *(Afr J Reprod Health 2024; 28 [10]: 88-98).*

Keywords: Human Papillomavirus; Nutritional Status; Vaginal Discharge; Vaginal Microenvironment; Vitamin D

Résumé

Il s'agissait d'une étude rétrospective, explorant principalement le rôle médiateur du microenvironnement vaginal et l'influence de l'ajout de vitamine D sur l'infection par le virus du papillome humain (VPH). Cinq cent douze participants ont été choisis dans cette étude, puis divisés en groupes positifs au VPH (212 cas) et groupes négatifs (300 cas) sur la base des résultats du typage HPV 23. Le groupe positif au virus du papillome humain (HR-HPV) à haut risque présentait des taux anormaux de lactobacilles, de catalase, de propreté, de sialidosidase et de proline aminopeptidase plus élevés que le groupe négatif au HPV. Aucune différence significative n'a été trouvée dans la valeur du pH, l'estérase leucocytaire et l'anomalie de l'acétylglucosaminidase entre les 2 groupes. Le groupe HR-HPV positif présentait un pourcentage plus élevé de patients avec une propreté III et IV. Par rapport au groupe de lésions épidermoïdes intraépithéliales de bas grade (LSIL), le groupe HSIL présentait un taux d'infection positive au VPH plus élevé. L'infection par les moisissures, l'infection à Gardnerella et la catalase ont été identifiées comme des éléments de risque indépendants d'infection par le HR-HPV. Il a été démontré que la supplémentation en vitamine D réduisait potentiellement la persistance de l'infection par le VPH-HR après la procédure d'excision électrochirurgicale en boucle (LEEP), améliorait la santé nutritionnelle, réduisait l'insuline, la résistance à l'insuline (HOMA-IR) et les niveaux de triglycérides, ainsi que réduisait la sensibilité élevée aux C- protéine réactive (hs-CRP) ainsi que les niveaux de malondialdéhyde (MDA). Nos résultats indiquent que l'infection par le HR-HPV est intimement associée à l'état du microenvironnement vaginal et que l'ajout de vitamine D réduit potentiellement la persistance de l'infection par le HR-HPV après le RAD, améliore la santé nutritionnelle et métabolique, réduit l'inflammation et est bien toléré.. *(Afr J Reprod Health 2024; 28 [10]: 88-98).*

Mots-clés: Papillomavirus humain; État nutritionnel ; Pertes vaginales ; Microenvironnement vaginal ; Vitamine D

Introduction

There are several microbial groups in the vagina of healthy women, which coexist and restrict each other, forming a vaginal micro ecological environment. Although a variety of microorganisms coexist in the vaginal microenvironment, there exists a balanced ecological relationship between the vagina and microorganisms, as well as among different microorganisms themselves[1-3]. High-risk

human papillomavirus (HR-HPV) infection belongs to a precancerous condition of the cervix and is one of the principal factors contributing to cervical cancer progression. Nevertheless, it is essential to mention that HR-HPV is not the only factor involved in cervical carcinogenesis. A previous study[4] demonstrated that various diseases causing vaginitis play crucial roles in the gradual transformation of normal cervical tissues into malignant ones. Their findings underscored the significance of the vaginal microenvironment in this pathological process. Moreover, another study highlighted that an imbalance in the vaginal microenvironment is strongly linked to the progression of cervical cancer[5].

One of the promising interventions in the management of HPV infections is the loop electrosurgical excision procedure (LEEP)[6]. LEEP belongs to a medical procedure used to examine and treat the abnormal cell growth on cervix's surface. The procedure involves the use of electrically heated insulated coils to remove the abnormal tissue[7].

Apart from medical interventions, there is growing interest in micronutrients' role, especially vitamin D, in the management of HPV infections[8]. Vitamin D deficiency affects 40% of adults in the United States. Sufficient vitamin D levels are related to better health outcomes, lower incidence of some cancers, and lower risk of respiratory infections[9]. In the context of HPV, lower serum 25 dehydrovitamin (OH)D concentrations decrease innate immune function and heighten susceptibility to infection[10].
The objective of this research is to delve deeper into the role of vitamin D addition in HPV infection management. The intricate interplay between vitamin D, HPV infection, and the vaginal microenvironment will be explored. By shedding light on these complex interactions, this investigation hopes to contribute to the existing body of knowledge and potentially pave the way for novel preventive and therapeutic strategies against HPV infections.

Methods

Five hundred and twelve patients who visited the Beijing Hospital of Traditional Chinese Medicine, Capital Medical University (Beijing, China) from June 2019 to June 2022 were selected, followed by dividing into two groups according to the results of HPV 23 typing. The HPV negative group included 300 cases, aged 22-65 years, with a median age of 37 years; The HR-HPV positive group contained 212 cases, ranging in age 20-65 years, with a median age of 38 years.

No significant difference was exhibited in age between 2 groups ($P > 0.05$). Cervical exfoliated cells and vaginal secretions of all patients were collected for HPV 23 typing and gynecological five joint tests. All patients underwent pathological section examinations, and those presenting with both high-grade squamous intraepithelial lesion (HSIL) on pathology and a PCR result underwent loop electrosurgical excision procedure (LEEP, n = 194). Subsequently, these 194 patients were randomly divided into Group A and Group B, each consisting of 97 cases, utilizing the random number table method.
Cervical exfoliated cells were collected for HPV detection. Secretions from the posterior vaginal fornix were collected with sterile cotton swab, shaken evenly, and 500 μ L sample was aspirated with a screw mouth in a centrifugal tube. Cervical tissues were obtained through colposcopically directed biopsy or cervical conization for histopathological analysis.

Vaginal microecology: A sample was collected from the lateral wall of the vagina utilizing sterile cotton swabs, and was detected for aerobic vaginitis (AV, Donders diagnostic scale score ≥ 3 points is AV positive), bacterial vaginosis (BV, Nugent ≥ 7 points is positive) using a vaginal microecological test kit (Shandong Shidasi biological industry Co., LTD., Shandong, China) through enzyme chemical reaction method.
The occurrence of vaginal candidiasis (VVC, was determined by budding spores or pseudohyphae). The pH value of vaginal secretions in both study groups was measured. Lactobacillus detection was categorized as either deficient [≤ 30/ high-power field (HPF)] or normal (> 30/HPF).

The presence of Candida was confirmed if spores and hyphae were visible under the microscope, and Trichomonas was considered positive if Trichomonas vaginalis was observed microscopically. Additionally, the overall cleanliness of vaginal secretions in both groups was comprehensively evaluated. Vaginal cleanliness is an indicator of vaginal inflammation. Vaginal cleanliness is divided into four levels based on the type and number of bacteria and cells in vaginal secretions, including: Cleanliness I: there were

abundant vaginal bacteria, epithelial cells, no miscellaneous bacteria white blood cells, the observation field was clean, indicating the nature of secretions was normal; Cleanliness II: there were some vaginal bacilli and epithelial cells in the secretions, and a small number of miscellaneous bacteria and white blood cells, indicating normal secretions; Cleanliness III: there were a few vaginal bacilli, epithelial cells, white blood cells, miscellaneous bacteria rich, indicating inflammation; Cleanliness IVleno vaginal bacillus, there were a few epithelial cells, white blood cells, miscellaneous bacteria rich, indicating heavy inflammation.

HPV positive rate: The exfoliated cells underwent centrifugation at room temperature at 12,000 R/min for 1 minute. Subsequently, HPV-DNA extraction was conducted using the DNA Extraction Kit[11]. HPV genotypes were determined using polymerase chain reaction (PCR)-based hybridization microarray technology. This genotyping assay allows for the qualitative detection of 18 high-risk types and 3 low-risk types of HPV.

Pathological examination results: In the absence of high-risk HPV-DNA testing results, two pathology experts jointly reviewed the slides and subsequently reported the pathological sections according to the Bethesda System (TBS) classification recommended by the International Cancer Institute (NCI) in 2001[12]. Pathological findings were categorized into several groups: normal or inflammatory, cervical intraepithelial neoplasia (CIN) I-III, and cervical cancer. A cervix deemed normal or showing signs of inflammation was characterized as NC, while CIN grade I was designated to be low-grade squamous intraepithelial lesions (LSIL). CIN grades II and III were classified to be high-grade squamous intraepithelial lesions (HSIL).

Routine detection of vaginal secretions: two copies of vaginal secretions were taken from the posterior fornix of the patient's vagina with a sterile swab, one for saline wet film microscopy and the other for BV detection. (1) Saline wet slide microscopy: a vaginal secretion swab was taken, 0.9% sodium chloride injection was added, and was mixed well. The sample was aspirated on the glass slide for direct microscopy, using the national clinical inspection operation specification (version 3)[13]. The test items included leukocytes, epithelial cells, trichomonas, Candida, Lactobacillus, miscellaneous bacteria, and clue cells. (2) The detection was carried out according to the kit instructions.

Treatment

Participants (n = 97 per group) were randomly assigned to accept either 50,000 IU vitamin D3 supplements (Group B) or matching placebo (Group A) every 2 weeks for 6 months, with both formulations provided by Zhejiang Garden Biochemical High-Tech Co., Ltd (China). Placebo capsules were indistinguishable from the active supplements in appearance, including color, shape, size, and packaging. Dosage selection aligned with prior research[14,15], and randomization employed computer-generated numbers, with allocation were hidden from both researchers and patients until final analyses. Trained staff managed the randomization sequence, patient enrollment, and intervention allocation. All participants were guided to maintain their regular diet and physical activity throughout the study period.

Outcome measures

HR-HPV Persistence Post-LEEP: The main metric was the continuation of HR-HPV presence after the LEEP. Tests for HPV DNA were conducted initially and then at intervals of 6, 12, and 18 months post-LEEP.

Nutritional Indicators: (1) Vitamin D: The concentration of Vitamin D was determined using a specific blood test kit (D751004, Sangon, China), with blood drawn from the study participants. (2) Albumin: The level of serum albumin, an indicator of nutritional health, was measured using a blood sample from the participants and the Bromocresol Green (BCG) Albumin Assay Kit (MAK124, Sigma-Aldrich, USA). (3) Prealbumin: The concentration of prealbumin, a more sensitive nutritional marker, was also determined. This was done using a blood sample and the Human PreAlbumin Kit (ab108895, Abcam, UK).

Metabolic Wellness: (1) Insulin Resistance (HOMA-IR): Blood samples were gathered from fasting participants to measure glucose and insulin levels. (2) Triglycerides: The concentration of triglycerides was determined using a blood test. A blood sample was taken after a 12-hour fast and the triglyceride level was measured using the Serum Triglyceride Determination Kit.

Inflammatory and Immune Markers: (1) High-sensitivity C-reactive protein (hs-CRP): hs-CRP, an inflammation marker, was measured using a blood sample from the participants and the Hs-CRP+CRP fast test kit. (2) Malondialdehyde (MDA): MDA, an oxidative stress marker, was measured using a blood sample from the participants and a thiobarbituric acid reactive substances (TBARS) assay.
Reported side effects: Participants were asked to record anyadverse occurrences, such as bleeding, site of excision infection, and pelvic discomfort.

Statistical analysis

Data analysis was implemented using SPSS version 27.0 statistical software. Measurement data were presented as mean ± standard deviation (x ± s) and analyzed using t-tests for intergroup comparisons. Counting data were expressed as [n (%)] and analyzed using the χ^2 test for intergroup comparisons. A significance level of $P < 0.05$ meant the difference was statistical significance.

Ethical considerations

Our study was approved by the Ethics Committee of The Beijing Hospital of Traditional Chinese Medicine, Capital Medical University.

Results

Vaginal microecology between 2 groups

As Table 1 revealed, relative to HPV negative group, HR-HPV positive group presented higher abnormal ratios of Lactobacillus, catalase, cleanliness, neuraminidase and proline aminopeptidase ($P < 0.05$). Simultaneously, no difference was exhibited in pH value, leukocyte esterase and Acetylglucosaminidase abnormality between 2 groups ($P > 0.05$).

Cleanliness of vaginal secretions

As Figure 1 revealed, relative to HPV negative group, HR-HPV positive group presented higher proportion of patients with cleanliness III and IV and lower proportion of patients with cleanliness I and II ($P < 0.05$).

Pathogen detection between 2 groups

As Figure 2 revealed, relative to HPV negative group, HR-HPV positive group presented higher detection ratios of moulds and Gardnerella ($P < 0.05$). Simultaneously, no difference was found in the detection rate of Trichomonas between 2 groups ($P > 0.05$).

HPV positive rate in patients with different grades of cervical lesions

As Figure 3 revealed, there were 217 patients in the NC group, 76 in the LSIL group, 209 in the HSIL group, as well as 10 in the CC group. The positive rates of HPV infection in 4 groups were 75.6% (164/217), 84.2% (64/76), 92.8% (194/209) and 90.0% (9/10). Significant differences were exhibited in HPV positive rate among 4 groups ($P < 0.001$). Besides, relative to NC and LSIL groups, HSIL group presented higher HPV positive rate ($P < 0.01$).

Correlation between HR-HPV infection and vaginal microecology

Table 2 displayed, multivariate logistic regression analysis manifested that mold infection, Gardnerella infection and catalase were independent risk elements for HR-HPV infection ($P < 0.05$).

Persistence rate of HR-HPV infection after LEEP

At the 6-month mark, the persistence rate in Group A (40.2%) presented higher as comparing with Group B (32.0%). This trend continued at the 12-month follow-up, with a persistence rate of 20.6% in Group A, and a persistence rate of 11.3% in Group B. At 18 months, the persistence rate for Groups A and B dropped to 11.3% and 3.1%, respectively. Statistical analysis manifested significant differences in persistence rates between 2 groups at all time points, with Group A consistently exhibiting higher HR-HPV infection persistence rates as comparing with Group B ($P < 0.05$, Table 3).

Nutritional status between 2 groups

Prior to intervention, no significant differences were seen in vitamin D, albumin, and prealbumin levels between 2 groups ($P > 0.05$).

Table 1: Comparison of vaginal microecology between two groups

Index	HR -HPV Positive group **(n=212)**	HPV Negative group (n = 300)	**r**	P
pH				
≥4. 6	**14 (6.6)**	**17 (5.7)**	0.1	0.8
<4.6	**198 (93.4)**	**283 (94.3)**		
Lactobacillus				
abnormal	**149 (70.3)**	**157 (52.3)**	5.7	0.0
normal	**63 (29.7)**	**143 (47.7)**		
Catalase				
abnormal	**152 (71.7)**	**146 (48.7)**	9.4	0.0
normal	**21 (28.3)**	**154 (51.3)**		
Cleanliness				
abnormal	**129 (60.9)**	**128 (42.7)**	5.6	0.0
normal	**83 (39.1)**	**172 (57.3)**		
Leukocyte esterase				
abnormal	**160 (75.5)**	**207 (69.0)**	1.0	0.3
normal	**52 (24.5)**	**93 (31.0)**		
Neuraminidase				
abnormal	**54 (25.5)**	**29 (9.7)**	8.0	0.0
normal	**158 (74.5)**	**271 (90.3)**		
Proline aminopeptidase				
abnormal	**37 (17.5)**	**17 (5.7)**	6.2	0.0
normal	**175 (82.5)**	**283 (94.3)**		
Acetylglucosamine Fu enzyme				
abnormal	**34 (16.0)**	**26 (8.7)**	2.3	0.1
normal	**178 (84.0)**	**274 (91.3)**		

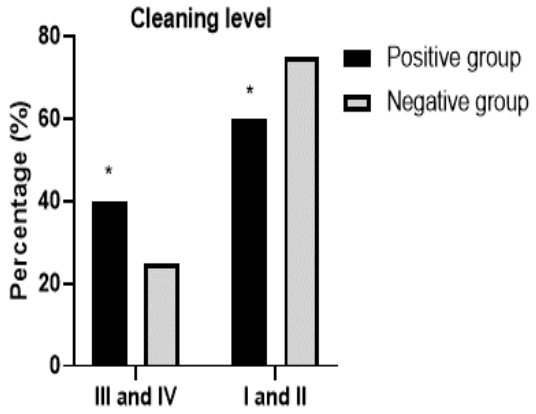


Figure 1: Detection of cleanliness of vaginal secretions. *P < 0.05.

However, after intervention, vitamin D levels were elevated in Group B, while albumin and prealbumin levels were elevated in both groups (P < 0.05). Besides, after the intervention, vitamin D levels as well as albumin and prealbumin levels in Group B presented higher as comparing with Group A (P < 0.05, Figure 4).

Metabolic-health between 2 groups

Prior to intervention, no significant differences were seen in insulin, HOMA-IR, and triglycerides levels between 2 groups (P > 0.05). However, after intervention, both groups displayed a statistically significant decrease in these parameters (P < 0.05). Specifically, relative to Group A, Group B had significantly lower levels of insulin, HOMA-IR as well as triglycerides after the intervention (P < 0.05, Figure 5).

Inflammation and immune response between 2 groups

Prior to intervention, no significant differences were seen in hs-CRP along with MDA levels between 2 groups (P > 0.05).

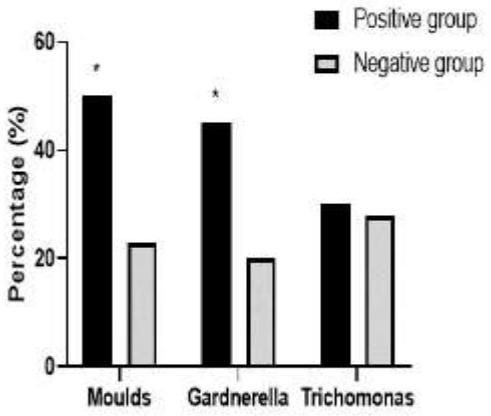


Figure 2: Comparison of pathogen detection between two groups. *P < 0.05.

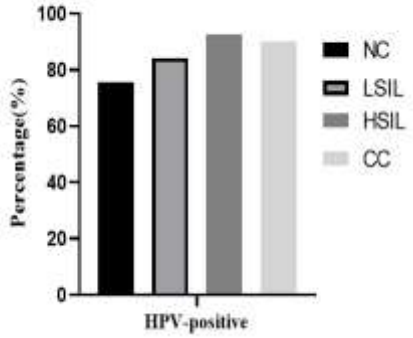


Figure 3: Comparison of HPV positive rate in patients with different grades of cervical lesions

Table 2: Multivariate logistic regression analysis of HR-HPV infection

Factors	β	SE	Wald	OR	95% CI	P
mould	1.0	0.5	4.2	2.7	1.0~7.0	0.0
Gardnerella	1.2	0.4	7.9	3.4	1.4~7.8	0.0
catalase	0.9	0.3	7.1	2.4	1.3~4.6	0.0

Note: SE is standard error; OR is the odds ratio; CI is the confidence interval.

However, hs-CRP along with MDA levels in both groups were declined after intervention (P < 0.05), and relative to Group A, Group B presented lower hs-CRP along with MDA levels (P < 0.05, Figure 6).

Adverse reactions in 2 groups

As Table 4 displayed, in Group A, 12 patients (12.4%) experienced bleeding, 10 patients (10.3%) had infections, and 9 patients (9.3%) reported pelvic pain. This resulted in a total incidence rate of 31 patients (32.0%).

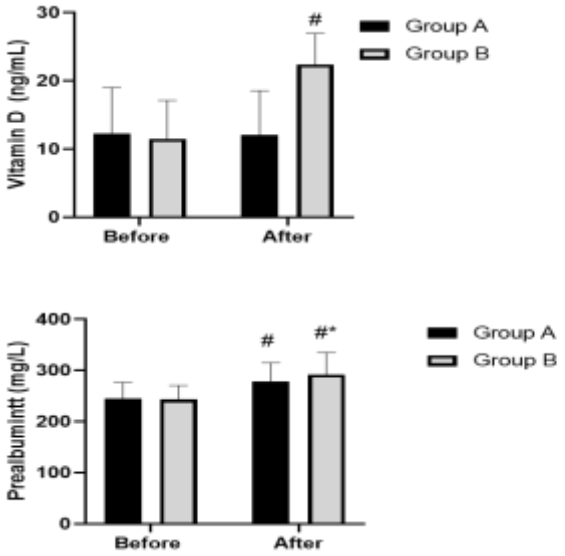
Table 3: persistence rate of HR-HPV infection after LEEP

Groups	N	6 months	12months	18 months
Group A	97	39 (40.2%)	20(20.6%)	11(11.3%)
Group B	97	31 (32.0%)	11(11.3%)	3 (3.1%)

Conversely, Group B exhibited lower adverse reaction rates, with 5 patients (5.2%) reporting bleeding, 4 patients (4.1%) experiencing infections, and another 4 patients (4.1%) reporting pelvic pain. The total incidence rate of adverse reactions in Group B was 13 patients (13.4%). This suggested that patients relative to Group A, Group B experienced a lower incidence of adverse reactions (P < 0.05).

Discussion

HPV, characterized by its small spherical shape and DNA composition without an envelope, has the capacity to trigger abnormal proliferation of the squamous epithelium within the cervical mucosa. This aberrant cellular growth can progress to the formation of cervical lesions and, in advanced stages, may culminate in cervical cancer. In the vaginal environment of normal women, there are microorganisms beneficial to the human body, mainly Lactobacillus acidogenic, which maintain a dynamic balance between the human body and the vaginal environment. Both exogenous and endogenous factors can disrupt the delicate balance of the vaginal microecology. While most susceptible individuals can experience transient HPV infections that resolve spontaneously, imbalances in the vaginal microecology may predispose individuals to persistent HR-HPV infection. The results revealed that relative to HPV negative group, HR-HPV positive group presented higher prevalence of thunderbolt and Gardnerella infections. No statistical difference was confirmed between the prevalence of Trichomonas in the two groups, which was consistent with the existing research conclusions[16-18].



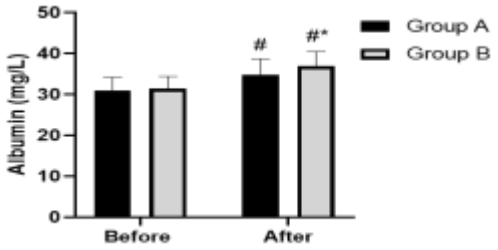
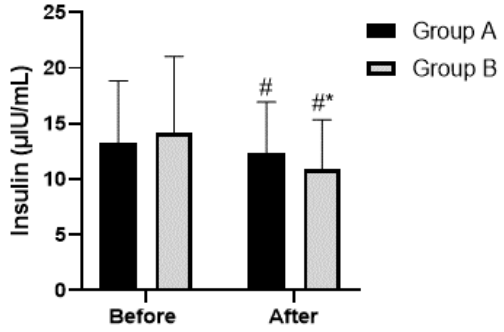
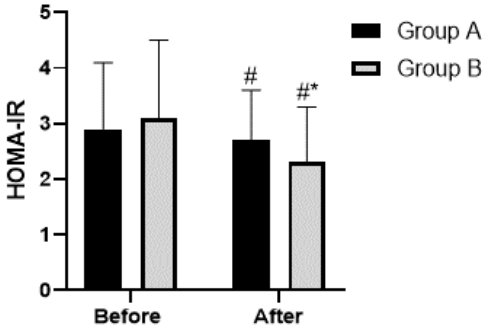


Figure 4: Comparison of nutritional status in patients with different treatments. #P < 0.05, compared with before intervention; *P < 0.05, compared with Group A





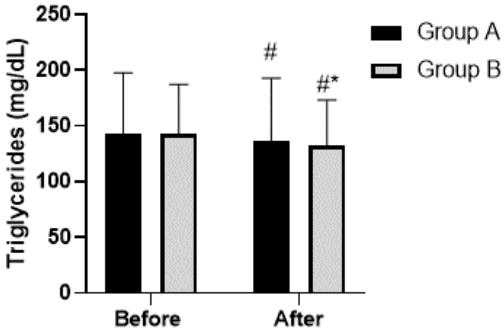


Figure 5: Comparison of metabolic-health in patients with different treatments. #P < 0.05, compared with before intervention; *P < 0.05, compared with Group A

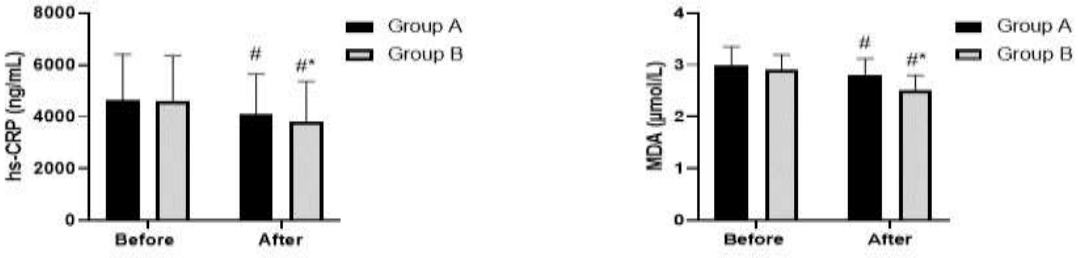


Figure 6: Comparison of metabolic-health in patients with different treatments. $^{\#}P < 0.05$, compared with before intervention; $^{*}P < 0.05$, compared with Group A.

The comparative results of vaginal microecology showed that relative to HPV negative group, HR-HPV positive group presented higher abnormal ratios of Lactobacillus, catalase, cleanliness, neuraminidase and proline aminopeptidase. Further multivariate logistic regression analysis manifested that thunderbolt infection, Gardnerella infection, and catalase positive were independent risk elements for HR-HPV infection. All these findings imply that HPV infection can lead to the disorder of vaginal microenvironment. In addition, HPV infection may also reduce the levels of antioxidants, and damage cellular DNA. In the process of cellular DNA replication, HPV DNA can be transferred to host cell DNA, aggravating the damage of mucosa and further aggravating the abnormality of vaginal microenvironment[19,20].

The normal vaginal microbiome serves a crucial role in in the acidic environment necessary to maintain vaginal health, ensuring the vagina's natural self-purification by producing lactic acid[21,22]. The typical pH resistance range for the female vagina is 3.8 to 4.5. However, a decrease in Lactobacillus abundance disrupts this balance, promoting an increase in vaginal pH, further exacerbating the vaginal microenvironmental imbalance and increasing susceptibility to reproductive system infections. These infections perpetuate a cycle of disruption, affecting vaginal cleanliness and enzyme levels, such as sialidase, and heightening the risk of HPV acquisition. Notably, infections like Gardnerella can disrupt the protective mucosal barrier, induce microdamage, and alter epithelial cells, thereby amplifying susceptibility to HPV[23]. Additionally, the presence of catalase diminishes the antimicrobial effects of hydrogen peroxide, enabling the proliferation of pathogenic bacteria in the vagina, further increasing susceptibility to HPV infection.

The literature suggests a direct association between the incidence of HR-HPV infection and severity of cervical lesions[24,25]. Patients with CIN Grade I have significantly lower rates of HPV infection compared to patients with higher CIN grade[26]. In the initial stages of HPV infection, active viral replication triggers immune recognition and clearance. However, in cases where vaginal environment disruption leads to mucosal and cervical epithelial damage, persistent HPV infection can occur, reducing clearance rates and escalating CIN risk[5,27]. Our study displayed that the positive HPV rate in patients undergoing cervical lesions (LSIL, HSI, CC group) presented higher as comparing with NC group.

Statistically significant differences were exhibited in the abnormal rates of Lactobacillus, cleanliness, neuraminidase, along with proline aminopeptidase between 2 groups, while no statistical significances were found in the multivariate logistic regression analysis. The reason may be that there is a certain spurious or indirect correlation between the vaginal microenvironment and HPV infection. PH, Lactobacillus, cleanliness, leukocyte esterase, neuraminidase, Proline aminopeptidase may not have a significant or direct impact on HPV infection. Mold and Gardnerella are independent factors affecting HPV infection.

Lactobacillus, cleanliness, neuraminidase, and proline aminopeptidase may have a certain correlation with mold and Gardnerella infection, resulting in a significant difference between 2 groups in single factor analysis, but the influence of mold and Gardnerella was adjusted in multi factor analysis, Lactobacillus, cleanliness, neuraminidase and proline aminopeptidase were excluded as confounders.

Vitamin D's role in HPV infections has been a subject of interest in recent studies.

Vitamin D has a critical function in activating killer T cells of the immune system, enabling them to detect and eradicate invading pathogens, potentially averting severe infections. A study published in The Journal of Infectious Diseases identified that various Vitamin D biomarkers that were related to short-term persistence of HR-HPV in 14 clinically relevant strains[28]. In this research, Vitamin D addition showed a significant impact on the persistence rate of HR-HPV infection post-LEEP. At all follow-up sites, the persistence rate was found to be lower in the Vitamin D supplement group, suggesting that Vitamin D supplementation may reduce the persistence rate of HR-HPV infection.

Nutritional status has been proved to be a potential cofactor involved in HPV persistence and carcinogenesis[29,30]. Researchers have investigated the associations between HPV and nutritional disorders along with their impacts on the risk of all-cause mortality[31]. In our results, the levels of nutritional status showed an increase in both groups after the intervention, with higher levels observed in the Vitamin D supplement group. This suggests that Vitamin D supplementation may improve the nutritional status of HPV-infected individuals.

Recent studies have highlighted the potential link between metabolic health and HPV infection. One study investigated the associations between HPV and metabolic syndrome (MetS) and their impacts on the risk of all-cause death[32]. In our results, vitamin D addition caused a decline in insulin, HOMA-IR, as well as triglyceride levels. This suggests Vitamin D addition may improve the metabolic health in HPV-infected individuals.

The immune reaction to HPV infection is complicate that involves both the innate and adaptive immune systems. Inflammation is a key aspect of this immune response. A study published in Frontiers in Microbiology suggested that microbes can trigger inflammation in certain parts of the host body, promoting cervical cancer development[33]. In our results, vitamin D supplementation decreased hs-CRP and MDA levels, indicating a potential anti-inflammation role of Vitamin D.

Strengths and limitations

A major strength of our study lied in the design: a well-organized study with large study size and multiple evaluation indicators. The second strength belonged to the intervention applied, namely vitamin D, which is simple, easily accessible, as well as fairly inexpensive. The major limitation of our study was that we did not carry out long-term studies to observe the influence of vitamin D supplementation on persistence of HR-HPV infection, so further long-term studies should be performed in the near future.

Conclusions

HR-HPV infection demonstrates a close association with the vaginal microenvironment, wherein mold and Gardnerella infections play pivotal roles in facilitating HPV infection. The finding was then expanded with vitamin D interference, suggesting that vitamin D supplementation could reduce the persistence of HR-HPV infection post-LEEP, improve nutritional and metabolic health, reduce inflammation, and be well-tolerated. These findings provide a new direction for clinical practice in the management of HPV infections.

Authors contribution

Yanling Sun and Li Li: conceived and designed the study, as well as collected and analysed the data. Wenxin Xu and Cen Ma: prepared the manuscript" change into "Caiyan Xu: conceived and designed the study, as well as collected and analysed the data. Jun Liu: prepared the manuscript".

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