

ORIGINAL RESEARCH

Gym-based aerobic exercise with bicycle ergometer contributes to the regression of human papilloma virus infection: a single-center, single-blind and randomized controlled trial

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Abstract

The regression of human papilloma virus is multifactorial and could be possibly targeted by using life-mode form of intervention. The purpose of this trial is to clarify the effect of aerobic exercise on the regression in outpatients firstly diagnosed with human papilloma virus (HPV) infection. Patients were randomly allocated into exercise group (Arm A) and control group (Arm B). Patients in Arm A received the 45-minute aerobic exercise thrice per week using the bicycle ergometer at gym. Patients in Arm B received their usual care at home. The physical exercise spanned a period of 6 weeks. The characteristics at baseline were documented and HPV specimens were tested at initiation, three months and six months after project launching. The primary endpoint was current HPV infection incidents as well as stratified by age at 3-months and 6-months. The secondary endpoint was newly diagnosed HPV incidents. In total, 125 patients in arm A and 136 patients in arm B were enrolled. The mean follow-up period was 7.5 ± 1.5 months (range 6.0–9.2). Post physical training, no significant difference of HPV 16/18 in groups was observed at 3-months or 6-months. As to current rate, physical exercise was superior to the patients with non-16/18 high-risk human papilloma virus (HR-HPV) infections at 6-months ($p = 0.01$). Stratified by age, the strength was still strong regarding age span of 18–25 years, 30–35 years, 35–45 years ($p = 0.02$, $p = 0.04$, $p = 0.01$, respectively). Additionally, the newly diagnosed HPV infection (HPV16/18 excluded) significantly decreased from 29 (21.3%) in Arm B to 14 (11.2%) in Arm A ($p = 0.02$). The current trial indicates that the 6-week aerobic exercise positively affects the regression of HPV infection, especially regarding the non-16/18 HPV infection.

Keywords

Aerobic exercise; Human papilloma virus; Cervical cancer; Regression

1. Introduction

The all-cause morbidity in Curaçao (a Dutch-Caribbean island) attributed to human papillomavirus (HPV)-related cervical cancer (CC) is ranked first among most malignancies, with an age-standardized incident rate of 12–15 per 100,000 over the 2008 to 2014 period [1]. HPV is frequently transmitted during sexual intercourse among reproductive-age women and was found to have an etiological role in CC. Based on DNA sequence, HPV is generally categorized as high-risk HPV (HR-HPV) (genotypes: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82) and low-risk HPV (LR-HPV) (genotypes: 6, 11, 40, 42, 43, 44, 54, 61, 70, 72 and 81). The worldwide prevalence of HR-HPV infection without cytological abnormalities is 10%–13%, with the highest prevalence in Africa (24%), Europe (21%) and Latin America (16%) [2]. The most common population-based HPV prevalence genotypes are HPV 16 (3.2%), HPV18 (1.4%) and HPV 52 (0.9%) [3]. HR-HPV is an

indispensable precursor of CC, whereas LR-HPV is integral for genital mucosal warts. In particular, HR-HPV 16/18 or non-16/18 HR-HPV are considered oncogenic and causative [4–6]. Although occasionally eliminated by immune processes, HR-HPV is now progressively difficult to eliminate due to the influence of smoking, alcohol consumption or irregular sexual activity.

Currently, limited strategies are available for the dislodgement of the remaining HR-HPV infection after colposcopy inspection in combination with cytological examinations. Photodynamic therapy with 5-aminolevulinic acid was reported to relieve the HR-HPV burden. However, the clearance rate was not satisfactory because of the dynamic virus loads and differing HPV genotypes [7, 8]. Thus, recombinant human interferon alpha-2b was suggested as an alternative to eliminate HR-HPV. However, despite the HPV-negative conversion ratio at 3 months after treatment, follow-up studies found that it was

accompanied by hematotoxicity and reduced tolerance, which limited its clinical use [9].

Aerobic exercise (AE), defined as rhythmic, oxygen metabolism-balanced and long-playing exercises, roughly includes walking/jogging, Taichi/square dancing, yoga/gym training, badminton/table tennis and running/skating/climbing. It is worth noting that AE has been increasingly documented to be beneficial for cardiovascular circulation, endocrine balance and inflammation or immune activity [10–12]. Behrouz Baghaiee *et al.* [13] reported that 12 weeks of AE was associated with considerable effects on systolic/diastolic hypertension through oxidative stress reduction. Shang-ling Chiang *et al.* [14] confirmed the presence of exercise-induced response in glucose control during morning, afternoon and evening periods. Based on these observations and considering a similar mechanism with HPV infection, we aim to determine the significance of AE in the scheduled management of HR-HPV. Thus, we design this single-center, single-blind clinical trial on HPV regression derived from physical training.

2. Methods

2.1 Subject, ethic and sample size calculation

This is a prospective single-center, single-blind, randomized controlled trial, with assessments performed at initial randomization and 3- and 6- months after exercise training. The exercise training protocol was registered at the Chinese Clinical Trial Registry. The University of Nanjing Chinese Medicine is responsible for the integrity of this study. All participants were adequately notified of the study protocol and provided signed informed consent prior to randomization. A sample size of 250 patients total group (1:1 ratio) was needed in this study with a β of 0.20 and a two-sided α of 0.05, calculated using the Power Analysis and Sample Size (PASS) software (version 15.0, NCSS company, Kaysville, UT, USA).

2.2 Inclusion and exclusion criteria

Patients consented to participate in this study via public advertisement or recommendation by a specialized gynecologist at our outpatient department. The study inclusion criteria were: newly diagnosed HR-HPV infection; pap smear of negative intraepithelial lesion or malignancy (NILM) or simple inflammation; HPV 16/18 infection with colposcopy biopsy showing inflammation; an age span of 18–45 years; had a stable sexual partner; normal sexual activity of 3–5 times per week, and condom use. In addition, outpatients were also included if they had a sedentary lifestyle or spent no leisure time exercising. Patients were excluded if they had/were: recurrent or preexisting HR-HPV infection and undergoing medical therapy; morbid cytology; cervical lesion needing further conization; age of first sexual intercourse \leq 18 years; times of abortion \geq 3; vaginitis including fungous vaginitis, bacterial vaginitis and trichomonas vaginitis; autoimmune disease or immunosuppression status; walking disability; volleyball, football, basketball or other moderate-high intensity ball games; fitness trainer, swimming coach, physical teacher or other moderate-high intensity occupations; performing heavy

manual labor; active exercises \geq 3 times per week; coronary artery disease; severe hypertension (above 160/100 mmHg); stroke; active infection; breastfeeding; mental illness, and pregnancy.

2.3 Randomization and blind methods

Patients fulfilling the inclusion criteria attended three sessions, including an initial HPV examination at randomization and 3- and 6- months after group allocation. The patients' characteristics were obtained mainly through self-assessment questionnaires containing queries on age, sexual life activity, sexual partner number(s), contraception method, age of first sex life, number of abortions, presence of vaginitis and the type if present, occupation, heavy manual labor or not, and accustomed to holding ball games or not. HPV specimens were acquired by the same and trained gynecologist at initial randomization and 3- and 6- months after grouping. The patients were randomly allocated into an exercise (Arm A) or control group (Arm B) using a computer-generated random number table. The conductor involved in the assignment did not participate in patient recruitment, treatment or data collection. The groups were randomized using concealed envelopes numbered with Arabic numbers and were consecutively opened by the treating researcher. The patients were unaware of group allocation and were not disclosed (but could be aware via other sources) of the presence of an intervention-compared study. Similarly, it was hard to fully blind the treating investigators. In this regard, information exchange was forbidden between treatment and assessment researchers. Moreover, it is worth emphasizing that the total attention paid by the treating researchers was balanced between groups.

2.4 Protocols of the exercise group

We performed the supervised AE for 45 minutes, 3 times per week over a total of 6 weeks and without interruption at a gym using a bicycle ergometer (Mini Bicycle M190, Baron Company, Beijing, China). The same treating therapist supervised the exercise training at the same gym. After recruitment, the patients' HRs were recorded via portable heart rate monitor W8 (Dido Company, Shenzhen, China) corresponding to exercise intensity during the first 5 minutes warm-up duration (slightly out of breath but competent for talking) before beginning AE and subsequently 30 minutes of endurance training (out of breath but can still talk in a short sentence) and lastly 10 minutes of cool-down training (slightly out of breath but competent for talking). In the beginning, the patients were instructed to warm themselves by performing Taichi on a yoga mat, followed by bicycle AE with a 60%–75% estimated maximum heart rate (HR_{max} = 220-age). By stretching their arms at their sides, the patients were made to perform lower limb movements through the initial endurance time and progressed to higher intensity exercise based on their performance, up to the prescribed target, although occasionally exceeding until the end time. Then, they had a cool-down period lasting for 10 minutes, during which the patients were guided to fully relax. During and after AE, the patients were prevented from continuing if they had the following: pain, dizziness, dyspnea, several coughs, facial pallor, hypotension, and hard

chest distress. Additionally, patients in the exercise group were monitored via telephone or WeChat on usual physical activity when at home, considering that because they performed AE in the trial, this might make them have reduced activity intensity during their leisure time.

2.5 Control group

The patients were instructed to perform their usual routine and were not given specific interventions. However, their daily footsteps were monitored with a personal smartphone or smartwatch. The investigators provided patients with a footstep recording device (a pedometer, FB-731, Tokyo, Japan). The steps were recorded as an indicator of daily activity rather than a determination of adherence to an exercise program. The investigators corresponded with the patients every four weeks via WeChat or emails for queries on pregnancy status, contraception use, alcohol or cigarette abuse, physical status, presence of acute infection, newly emerging disease and daily activities. Additionally, HPV specimens were acquired by a trained gynecologist at the onset of patient selection, 3- and 6-months after grouping.

2.6 HPV detection

All HR-HPV genotypes were detected using the three-channel Roche Cobas 6800 RealTime PCR assay and Roche HPV Test kit (Roche Diagnostics Ltd., Rotkreuz, Switzerland). After 10 laps of cervical brush, specimens were immediately incubated into formalin-mixed lysate for later extraction. The cervical cell specimens were then vigorously shaken for 30 seconds with the Micro Oscillator (Shjingmi Ltd., Shanghai, China), followed by heating at 100 °C for 10 minutes. Then, the samples were centrifuged at 3000 rpm for 8 minutes, of which 200 μ L of the middle layer supernatant was transferred into prelabelled tubes mixed with deoxy-ribonucleoside triphosphate (dNTP), DNA polymerase, primer and buffer. Amplification and determination of positive HR-HPV were performed according to the manufacturer's instructions, with β -actin as the internal or negative control.

2.7 Data collecting, follow-up and trial endpoints

Data gathered by the blinded investigator included questionnaire queries on demographic and clinical characteristics at the first outpatient visit and 3- and 6- months after grouping for HPV test in Arm A. Arm B's information were obtained via WeChat or email. When the project finished, all patients were followed up via telephone or WeChat for 6–12 months since randomization. The primary endpoint was current HPV incidence ratio (or negative conversion ratio) at 3- and 6- months, along with stratified analysis by age span. The secondary endpoint was newly diagnosed HPV incidence ratio at 3- and 6- months.

2.8 Data analysis

Continuous variables are displayed as mean \pm standard deviation or median (inter-quartile range), whereas categorical variables as n (%). Unpaired Student's *t*-test was used to assess

continuous variables confirmed by the Shapiro-Wilk test and Levene test. For non-normally-distributed or heterogeneous values, the Mann-Whitney U test was used. The Pearson's χ^2 -test or Fisher's exact test was used to compare categorical variables. The endpoints were evaluated mainly based on per protocol principle, meaning all available variables of patients progressed in this protocol were integrally pooled. A 2-sided $\alpha < 0.05$ was considered to determine significant difference between comparisons. Statistical analyses were performed using the SPSS software version 16.0 (SPSS Inc., Chicago, IL, USA).

3. Results

From 01 January 2020 through 01 June 2020, 1230 outpatients were screened for fulfilling the inclusion criteria, of whom 702 patients were excluded depending on the exclusion criteria (main cited reasons were morbid cytology ($n = 280$); age of first sex life ≤ 18 years or abortion times ≥ 3 ($n = 123$); heavy manual labor ($n = 79$)). Of the remaining 528 patients, 224 patients declined to participate for the inconvenience of being treated or assessed, time constraints, no payment, disability of sticking, psychological and other not shown reasons. Finally, 304 patients were randomized: 152 assigned to exercise group and 152 assigned to control group. 7 patients in exercise group and 4 patients in control group retracted their written consents after project launching. 9 and 7 patients were lost whereas 11 and 5 cases were discontinued during physical training in exercise group and control group, respectively. The mean follow-up period was 7.5 ± 1.5 months (range 6.0–9.2). In the end, total of 125 in arm A and 136 in arm B were completely performed and brought into ultimate analysis, with data collection ending in January 2021 (Fig. 1).

Detailed characteristics at baseline were balanced in Table 1. The mean age was 31.9 ± 4.8 in Arm A and 32.8 ± 5.3 in Arm B. Little amount of patients (10%–20%) were abuse of alcohol or cigarette in both Arms. Most patients were married, not menopausal, of 1-time parity and 1-time abortion and experienced the natural labor delivery. As presented in Table 2, 187 HPV infection incidents (all subtypes summarized as total incidents) in Arm A and 197 incidents in Arm B were observed. The infection rate of HPV16 and HPV18 was 15.2% (19/125) and 12.8% (16/125) in Arm A and 12.5% (17/136) and 12.5% (17/136) in Arm B ($p = 0.52$, $p = 0.94$, respectively). The non-16/18 HR-HPV infection was 121.6% (152/125) and 119.8% (163/136) between groups ($p = 0.77$).

As presented in Table 3, between-group comparisons showed non-significant difference in current HPV infection incidents at 3-months. However, the discrepancy in total HPV infection at 6-months was observed in Arm A than in Arm B (134 (107.2%) vs. 161 (118.4%), respectively; $p = 0.01$). Similarly, non-16/18 HR-HPV markedly decreased in Arm A than in Arm B (105 (84.0%) vs. 137(100.7%), respectively; $p < 0.01$). Stratified by age span (Table 4), we observed notable decrease in the age span of 18–25 years, 30–35 years, 35–45 years ($p = 0.02$, $p = 0.04$, $p = 0.01$, respectively). But the HPV infection rate was not differentiated in groups regarding HPV16/18 regardless of age stratification. The newly diagnosed HPV infection incident was shown in

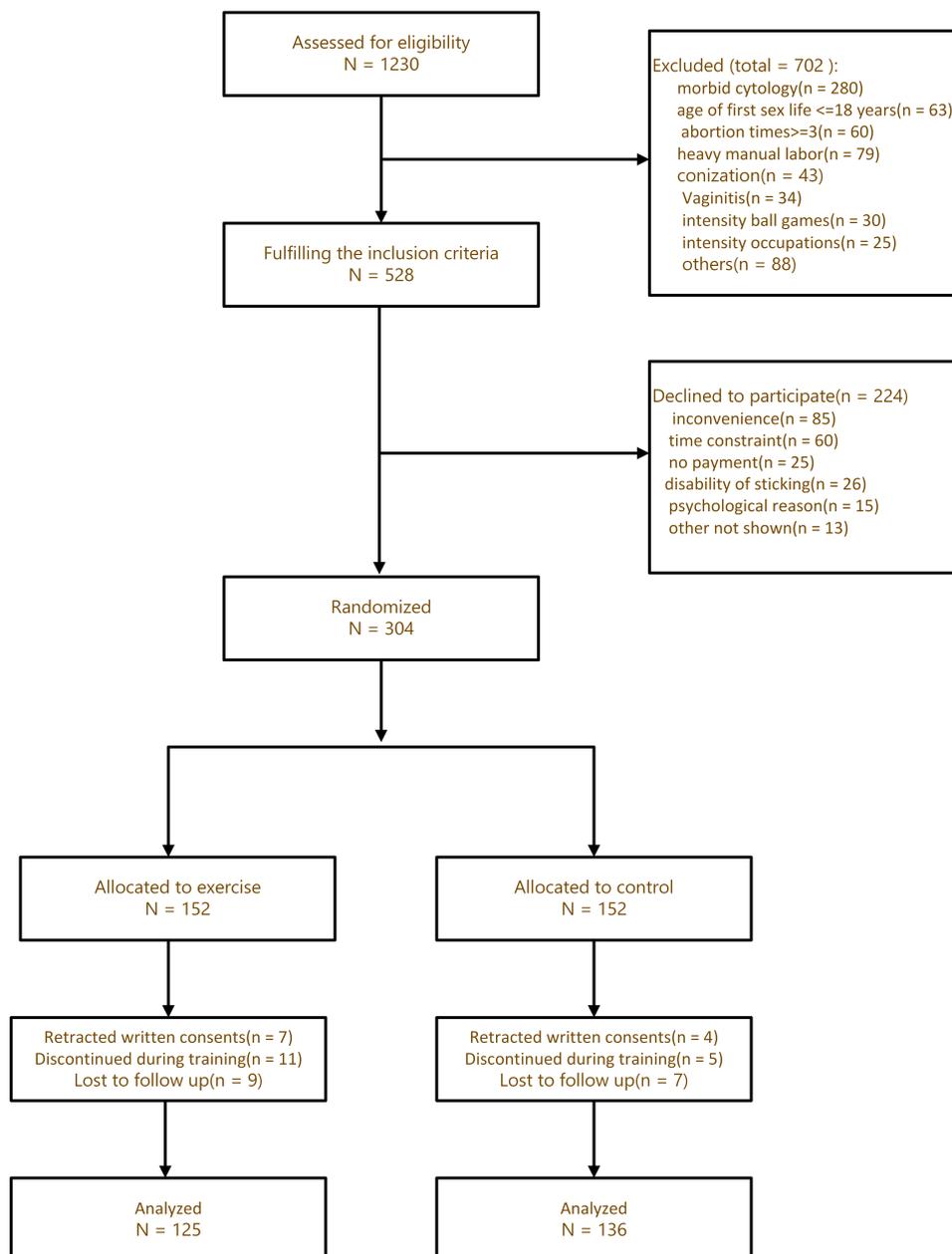


FIGURE 1. Flow diagram of patients through the various periods.

Table 5. We observed 25 (20%) total incidents in Arm A and 43 (31.6%) incidents in Arm B ($p = 0.03$), and 14 (11.2%) non-16/18 HPV infection incidents in Arm A and 29 (21.3%) incidents in Arm B ($p = 0.02$).

4. Discussion

Here we report our findings on a two-arm prospective single-center, single-blind and randomized controlled trial (RCT targeting, for which Arm A was based on gym AE over a period of 6 weeks. To the best of our knowledge, this is the first RCT to investigate exercise-related HPV regression exclusively in patients diagnosed with HPV infection without morbid cytology. The trial follows the Consolidated Standards of Reporting Trials guidelines for RCT (modified in 2010). Compared with other trials on exercise training, research bias of this study is reliably maintained via the type of research and the supervision

at the gym by a therapist or home-used monitor schedule.

The multifaceted effects of exercise capacity have been verified by preceding RCTs. However, exercise focusing on HPV infection has not been previously described, although several trials had recruited patients with pelvic girdle pain or adolescents receiving vaccinations, showing exercise as more beneficial to specific pain [15, 16]. In this trial, we noted preferred endpoints regarding current and newly diagnosed HPV infection in the exercise group. Despite no significant difference between the two groups at 3-months, the current non-16/18 HPV infection was 105 (84.0%) in Arm A, compared with 137 (100.7%) in Arm B at 6-month ($p < 0.01$), resulting in an apparent decrease in confirmed total concurrent infection (134 (107.2%) vs. 161 (118.4%); $p = 0.01$). We also observed that the newly diagnosed non-16/18 HPV infection rate was 14 (11.2%) in Arm A and 29 (21.3%) in Arm B ($p = 0.02$), consistent with the total rate estimate ($p = 0.03$). These

TABLE 1. Comparison of demographic and clinical data at baseline.

	Arm A (n = 125)	Arm B (n = 136)	<i>P</i>
Age(years)	31.9 ± 4.8	32.8 ± 5.3	0.15
BMI (kg/m ²)	22.8 ± 4.4	23.0 ± 5.3	0.73
Cigarette smoking			
Yes	13 (10.4%)	15 (11.0%)	0.86
No	112 (89.6%)	121 (89.0%)	
Alcohol consumption			
Yes	22 (17.6%)	21 (15.4%)	0.63
No	103 (82.4%)	115 (84.6%)	
Education (years)	13.6 ± 3.7	14.3 ± 5.5	0.22
Marital status			
Single	28 (22.4%)	27 (19.9%)	0.43
Married	78 (62.4%)	80 (58.8%)	
Divorced	19 (15.2%)	29 (21.3%)	
Annual income (USD)	16,500 ± 3150	16,350 ± 3875	0.73
Partner has other sexual partner(s)			
Yes	11 (8.8%)	14 (10.3%)	0.89
No	80 (64.0%)	84 (61.8%)	
Don't know	34 (27.2%)	38 (27.9%)	
Menopausal			
Yes	10 (8.0%)	15 (11.0%)	0.40
No	115 (92.0%)	121 (89.0%)	
Parity			
0	34 (27.2%)	37 (27.2%)	0.94
1	69 (55.2%)	73 (53.7%)	
2	22 (17.6%)	26 (19.1%)	
Abortion			
0	57 (45.6%)	55 (40.4%)	0.69
1	36 (28.8%)	42 (30.8%)	
2	32 (25.6%)	39 (28.8%)	
Delivery way (if parous)			
Cesarean	31 (24.8%)	38 (28.0%)	0.53
Natural labor	60 (75.2%)	61 (72.0%)	

Abbreviation: BMI, body mass index.

TABLE 2. HR-HPV infection incidents of 261 patients prior to initiation.

	Arm A (n = 125)	Arm B (n = 136)	<i>p</i> -value
Total	187 (149.6%)	197 (144.8%)	0.64
HPV16	19 (15.2%)	17 (12.5%)	0.52
HPV18	16 (12.8%)	17 (12.5%)	0.94
Non-16/18 HR-HPV	152 (121.6%)	163 (119.8%)	0.77

Abbreviation: HR-HPV, high-risk human papilloma virus.

findings showed a gradual decrease in HPV infection when exposed to AE for 6 months, displaying a high value of AE

for HPV-infected females. In regard to the independent effects in either the regressing or newly diagnosed HPV infection, it is

TABLE 3. HR-HPV infection according to follow-up regardless of newly diagnosed incident.

	3-months			6-months		
	Arm A (n = 125) (Inc. = 187)	Arm B (n = 136) (Inc. = 197)	<i>p</i> -value	Arm A (n = 125) (Inc. = 187)	Arm B (n = 136) (Inc. = 197)	<i>p</i> -value
Total	173 (138.4%)	184 (135.3%)	0.72	134 (107.2%)	161 (118.4%)	0.01*
HPV16	18 (14.4%)	16 (11.8%)	0.52	15 (12.0%)	14 (10.2%)	0.66
HPV18	16 (12.8%)	15 (11.0%)	0.65	14 (11.2%)	13 (9.6%)	0.66
Non-16/18 HR-HPV	139 (111.2%)	153 (112.5%)	0.77	105 (84.0%)	137 (100.7%)	<0.01*

Data presented as n (%), Inc.: incident, **p* < 0.05. Abbreviation: HR-HPV, high-risk human papilloma virus.

TABLE 4. HR-HPV infection distribution according to age subgroup at 6-months.

	Patients at initiation			Patients at 6-months		
	Arm A N = 125 Inc. = 187	Arm B N = 136 Inc. = 197	<i>p</i> -value	Arm A N = 125 Inc. = 134	Arm B N = 136 Inc. = 161	<i>p</i> -value
18–25 (y)	58/38 (152.6%)	52/34 (167.6%)	0.98	44/38 (115.8%)	51/34 (150.0%)	0.02*
25–30 (y)	30/27 (111.1%)	35/30 (116.7%)	0.60	28/27 (103.4%)	35/30 (116.7%)	0.15
30–35 (y)	34/25 (136.0%)	38/27 (140.7%)	0.81	27/25 (108.0%)	37/27 (137.0%)	0.04*
35–45 (y)	65/35 (185.7%)	72/45 (160.0%)	0.30	35/35 (100.0%)	38/45 (84.4%)	0.01*

Data presented as n (%), Inc.: incident, **p* < 0.05. Abbreviation: HR-HPV, high-risk human papilloma virus.

TABLE 5. Newly diagnosed HR-HPV infection incident according to follow-up.

	3-months			6-months		
	Arm A (n = 125)	Arm B (n = 136)	<i>p</i> -value	Arm A (n = 125)	Arm B (n = 136)	<i>p</i> -value
Total	21 (16.8%)	27 (19.8%)	0.52	25 (20.0%)	43 (31.6%)	0.03*
HPV16	3 (2.4%)	5 (3.7%)	0.55	4 (3.2%)	6 (4.4%)	0.61
HPV18	5 (4.0%)	7 (5.1%)	0.65	7 (5.6%)	8 (5.9%)	0.92
Non-16/18 HR-HPV	13 (10.4%)	15 (11.0%)	0.86	14 (11.2%)	29 (21.3%)	0.02*

Data presented as n (%), **p* < 0.05. Abbreviation: HR-HPV, high-risk human papilloma virus.

assumed that performing AE could be propagable and without adverse events, providing new insights to the understanding of physical training on HPV infection.

Improvement in the disappearance of HPV infection with AE might lead to accessible outcomes involving decreased HPV-referred precancer lesions, simultaneously promoting the degradation of cervical intraepithelial neoplasias. Indeed, the effects of different levels of exercise intensity remain unknown, mainly due to the absence of described design and interim analysis. Therefore, future trials on discriminative intensity are necessary to determine their influence. Second, in this trial, we performed 6-week training followed by a 3- and 6-month follow-up for HPV examination at the hospital and a 12-month follow-up via WeChat/telephone. Significant differences between the two groups were confirmed by the 6-month rather than the 3-month follow-up. We hypothesized that the delayed significance between the groups was

due to exercise-induced rehabilitation of vaginal environment disturbance. However, HPV was not eradicated at or after 12 months in our trial. Future trials using a longer follow-up period would be needed to demonstrate an interim or persistent regression after initiation. Third, although the patients had different occupations, they were instructed to maintain their usual activity in both groups. The different levels of compliance to the exercise protocol or adherence to instructions at home may be associated with certain levels of grouping bias, despite measuring the patients' footsteps via a pedometer to decrease inherent bias. The footsteps were monitored and corresponded between therapist and patients, and if the number of footsteps extremely exceeded the routine footsteps, they were corrected after feedback.

Previous studies provided added evidence to the association between smoking history [17], numbers of sexual partners [18], and contraception methods [19] with HPV infection,

and accordingly with reinfection or regression. To rule out these biases, we controlled and subsequently excluded cases with specific identifications before or during the trial. It has been documented that the proportion of genotypes detected by primary cervical screening independently increased with age [20–22]. The regression of HPV infection is likely referred to age-specific self-restoration in the transformation zone. Despite balancing baseline on age using Student's *t*-test, we could not draw a reliable conclusion from the non-age—stratified analysis. Therefore, we divided the pooled patients into separate age groups: 18–25, 25–30, 30–35 and 35–45, where the endpoints were found to be relatively comparative. We observed a marked decrease in HPV infection in the 18–25, 30–35 and 35–45 age groups ($p = 0.02$, $p = 0.04$, $p = 0.01$, respectively), supporting the potential significance of AE.

The underlying mechanism by which AE may ameliorate HPV remains to be clarified. Previous literature delineated intrinsic factors in the benefits to cardiovascular and endocrine cycles. Regitse Højgaard Christensen *et al.* [23] revealed that AE could induce cardiac fat loss and decrease the occurrence of cardiac hypertrophy through the interleukin-6 receptor-dependent signal; Xudong Chen *et al.* [24] reported that AE ameliorated fibrosis and apoptosis via inflammasome activation of P2X7 purinergic receptors. We hypothesized that the gradual disappearance of HPV infection was attributed to immune surveillance or anti-inflammatory cytokine reactivated by physical training, allowing for further detection of circulating biological cytokines. Similar to our findings, Na Li *et al.* [25] reported that AE could alleviate internal inflammation by decreasing tumor necrosis factor- α (TNF- α) or Interleukin-6 (IL-6) in mouse polycystic ovarian syndrome. Additionally, a recent study indicated that B7H4 acted as an immune checkpoint and participated in enhancing T cell proliferation and interferon- γ (IFN- γ) production against HPV infection [26]. Nevertheless, although the link between B7H4 and AE might be unclear, a possible link is biologically plausible.

The trial is strengthened by its randomized controlled design, comprehensive baseline balance, and age-stratified analysis. The aim was to investigate the effects among females with AE. The sample size was based on the primary endpoint. Subgroup analysis stratified by age span may have resulted in bias derived from patients completing the prescribed program. On the other hand, AE in our trial was limited to performing AE using a bicycle ergometer, which may not be generalized to people not performing AE. Moreover, we did not determine whether the regression of HPV was persistent or interim. However, the conclusion is relatively reliable because of a similar low dropout rate between the groups, regardless of intention-to-treat or per-protocol test. Although we focused on the effects of AE on cervical HPV infection, this study lacked mechanistic, molecular or immune function experiments. Future investigations should focus on the specific mechanisms of AE on gynecological infectious diseases, *i.e.*, by analyzing the levels of TNF- α , IL-6 or B7H4 in the peripheral blood.

5. Conclusions

In conclusion, this randomized controlled trial shows that gym-based AE via bicycle ergometer is associated with HPV regression, suggesting AE as a potentially effective intervention for female patients with newly diagnosed or current HPV infection.

AUTHOR CONTRIBUTIONS

AWX—Manuscript writing, data collection, data analysis; TM—Data collection; KHW—Protocol development; MMY—Revision of manuscript, support of funding; SLZ—Data analysis.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The research was authorized by the Institutional Review Board of Nanjing Second Hospital (2021-LY-kt082). Exercise training protocol was registered at Chinese Clinical Trial Registry.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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