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A Preliminary clinical observation on the effect of the gel containing black raspberry extract on patients with persistent human papilloma virus infection

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Abstract

To investigate the negative rate and effectiveness of black raspberry extract in clearing persistent infection with human papilloma virus (HPV), three hundred cases are included in the multicenter, randomized, double-blind, placebo-controlled trial. The distribution ratio of the tested group versus the control group is 2: 1 and the subject patients were given a preparation in the vagina before bed every other day, each dose was 3 g containing black raspberry extract (tested group) or placebo (control group). SPSS 20.0 was used for statistical analysis. Differences between groups were analyzed by the Rank Sum method or Chi-square method. The results showed that 191 subjects in the tested group completed the trial, of which 139 subjects (72.8%) were identified to have an effective clearance of HPV; 95 subjects in the control group completed the trial, of which only 15 subjects (15.8%) were identified to have effective clearance of HPV. The difference between the two groups was statistically significant ($\chi^2 = 82.9$, $P < 0.001$). The HPV viral load of the tested group was decreased from 224.7 RLU/CO to 88.6 RLU/CO whereas HPV viral load of the control group was ascended from 218.4 RLU/CO to 266.1 RLU/CO. Thus, the present clinical observation suggested that black raspberry extract may be a potential candidate in targeting persistent infection with HPV.

Keywords: Human papillomavirus virus; Cervical cancer; Black raspberry extract.

1. Introduction

Human papillomavirus (HPV) infects epithelial cells of human skin and mucosa tissues, especially in the genitalia of men and women. HPV infection can lead to condyloma acuminatum, cervical erosion, chronic cervicitis, and even tumors (Wardak, 2016). As one of the most common malignant tumors in women, the incidence rate of cervical cancer is only inferior to that of breast cancer and colorectal cancer (Chan, 2012). In China, an estimated 135,000 new cases and 80,000 deaths from cervical cancer per year. Epidemiological and molecular biological studies have confirmed that persistent infection of high-risk human papillomavirus (HPV) is a necessary condition for the occurrence of cervical cancer (Yang, 2013). According to statistics, 99.7% of cervical cancer is caused by persistent HPV infection (Walboomers et al., 1999). Therefore, control of HPV infection is the key to the prevention and treatment of cervical cancer.

At present, there are few drugs effectively controlling HPV infection in the world except for the preventive vaccine (Nicol et al., 2015). It has been clinically confirmed that the HPV vaccine can almost 100% prevent the infection of some HPV strains. However, the current vaccines do not cover all types of virus strains to thor-

oughly prevent the infection of HPV. As for treatment, laser microwave and drug are mainly used. The negative rate remains low in patients albeit with a long course of drug treatment. Invasive Laser microwave therapy is constrained due to local wound and infection. Therefore, it is urgent to develop products and biological agents effectively against HPV infection.

A number of basic researches and animal experiments have confirmed that black raspberry and its active ingredients have a chemopreventive effect on the tumor. Hecht et al. (2006) identified that the active compounds of the black raspberry extract by high-performance liquid chromatography are mainly cyanidin-3-O-glucoside, cyanidin 3-O-(2^{G}-xylosylrutinoside) and cyanidin 3-O-rutinoside. These chemical components from raspberry are promising inhibitors to target BPDE-induced NF-κB activation. Wang et al. (2009) confirmed that black raspberry anthocyanins inhibit the occurrence of chemically induced esophageal cancer in rats. In recent years, it has been reported that black raspberry extract effectively target a panel of cancer cells such as human oral cancer cells, colorectal cancer cells, and cervical cancer cells (Rodrigo et al., 2006; Cho et al., 2015; Zhang et al., 2011). Notably, mounting evidence suggested that black raspberry extract potentially target a number of viruses such as influenza A virus and norovirus (Baatartsogt et al., 2016; Lee et al., 2016b). Therefore, formulated preparation of black raspberry extract might be suitable for HPV.

In this study, we tried to investigate the effect of the formulated black raspberry extract gel on remove of HPV virus to patients with persistent human papilloma virus infection.

2. Materials and methods

2.1. Formulated black raspberry extract gel

The gel (SanuGene Vaginal Gel) applied in the test group is mainly composed of black raspberry extract, lactoglobulin, gel matrix and other components. Accordingly, the gel applied in the control group is just without black raspberry extract. The gels were provided free of charge by Medicare Biotechnology (Tianjin) Co., Ltd. The study Is completely independent and the company has no declared requests or interest conflicts.

2.2. Subjects

The 300 outpatients with HPV infection were recruited in the clinical observation confirmed by HPV E6/E7 mRNA or HPV DNA test and were divided into test group (n = 200) and control group (n = 100). These patients were detected in Tianjin Cancer Hospital, Tianjin Heping District Obstetrics and Gynecology Hospital, Tianjin Central maternity hospital, the fourth hospital of Hebei Medical University, and Harbin cancer hospital. The mean age of the participants was similar in both cohorts, 37.73 ± 3.67 and 36.36 ± 4.89 years in the control and rtest cohorts, respectively. The trial is approved by the medical ethic committees of Tianjin Medical University Cancer Institute and Hospital (Ethic Approval Protocol Number: E2018260).

2.3. Inclusion criteria

The detection of HPV E6/E7 mRNA or HPV DNA positively lasted for more than 6 months.

Cervical cytology is negative or inflammatory.

The patients with ASC-US or LISL showed cervical inflammatory histopathology.

Female adults with sexual activity.

The patients should have proper nutritional status, body mass index ≥ 18 kg/m^2, body weight > 40 kg, serum albumin ≥ 3 g/dL.

Patients should be easy to accept the study and participate in follow-up. Patients who participated in the trial must be followed up in the research center where they participated. This means that patients participating in the trial must have reasonable geographical restrictions. The investigator should ensure that the patients randomly enrolled in the study can complete all the studies, HPV virus evaluation, adverse event evaluation and follow-up.

Patients participating in other clinical trials simultaneously during the study are not included.

Informed consent must be signed by the patient.

3. Drug administration and virus assessments

Cervical cells of all subjects were taken for HPV DNA test, HPV E6/E7 mRNA detection, TCT test, secretion test, epidemiological examination, and quality of life assessment form 1 day before the study. Every subject conducted 3 g gel administration *Quaque Other Die* (qod) for 3 months, except for the menstrual period. The administration periodically conducted for 20 days and ceased for 10 days within 3 months. After that, all the subjects received determinations for TCT, HPV DNA, HPV E6/E7 mRNA and secretion.

4. Evaluation criteria of study endpoint

4.1. HPV clearance negative rate and effective rate evaluation standard

Turn negative: HPV E6/E7 mRNA and HPV DNA test turned negative (3 points).

Remarkable effect: the viral load of HPV DNA detection was reduced by two or more orders of magnitude (2 points).

Improvement: the viral load of HPV DNA detection decreased by an order of magnitude (1 point).

Ineffective: HPV E6/E7 mRNA and HPV DNA detection did not turn negative, and HPV viral load did not decrease or aggravate (0 point).

4.2. Secondary evaluation criteria

No inflammation was detected by TCT (2 points).

TCT was used to detect inflammation by one level or more (1 point).

TCT test showed that the inflammatory phenomenon aggravated or decreased by less than one level (0 point).

Secretion: normal (1 point), abnormal (−1 point).

Recovery: 3 points.

Remarkable effect: the score is 2 points.

Invalid: score ≤1 point.

4.3. Statistics

The subjects in each group were described by means and frequency counting methods. SPSS (version 20.0) software was used for all statistical analysis. The difference between groups was analyzed by the rank-sum test or chi-square test. It was considered that a P

Table 1. Comparison of effective clearance of HPV between two groups

	Test group	Control group	X^2	*P*
Number of participants	200	100		
Number of clinical trials completed	191	95		
Number of HPV positive patients before the test (%)	191 (100%)	95 (100%)		
Effective number of HPV clearance after test (%)	139 (72.8%)	15 (15.8%)	82.9	<0.001

X^2, Chi-square value; *P, P* value.

Table 2. The average viral load of HPV was compared before and after the two sets of tests

	Test group (RLU/CO)	Control group (RLU/CO)	*Z*	*P*
Before the test	224.7	218.4	0.762	0.446
After the test	88.6	266.1	7.551	<0.001

Z, Rank sum; *P, P* value.

value less than 0.05 was statistically significant.

5. Results

5.1. Black raspberry extract can effectively prevent persistent HPV infection

In this study, no serious side effects, including burning, tingling or other serious symptoms, were observed. Black raspberry extract is generally considered to be more comfortable, soft and moist in the body. The results of HPV clearance efficiency in the test group and control group are shown in Table 1.

As shown in Table 1, 191 patients in the test group completed the test, of which 139 patients had HPV effectively eliminated after using the black raspberry extract preparation. In the placebo control group, 5 patients were dropped off and 95 patients completed the test with 15 patients effectively cleared HPV virus. The chi-square test showed that the test group and the control group had a significant difference ($P < 0.001$). Therefore, black raspberry extract can effectively eliminate persistent HPV infection.

5.2. The viral load of HPV decreased significantly after using black raspberry extract

As shown in Table 2, the HPV DNA viral load of each subject was detected by HPV DNA quantitative method. Before the experiment, the average viral load of 191 subjects in the test group was 224.7 RLU/Co, while the average viral load of each person in the control group was 218.4 RLU/Co. After the test, the average viral load of each person in the test group decreased to 88.6 RLU/CO. The control group had an increase in the average viral load of HPV to 266.1 RLU/CO. These data prove that the black raspberry extract can effectively reduce the viral load in patients and even eliminate them directly.

5.3. Effect of black raspberry extract on vaginal microecology

As shown in Table 3, we studied the effect of the black raspberry extract on vaginal microecology. Before the experiment, the vaginal microecology of the test group and the control group had different proportions of abnormalities, including bacterial vaginitis, abnormal vaginal pH, increased vaginal cleanliness index, abnormal white blood cell count of vaginal secretions, and esterase activity of leukocyte. After the three-month treatment of black raspberry extract, although there was no significant difference in vaginal pH, white blood cell count, and esterase activity of leukocyte ($P > 0.05$), the percentage of bacterial vaginitis decreased greatly from 20.9% to 7.9%, which was significantly lower than the control group with a value of 18.9% ($P < 0.05$). The significant difference of vaginal cleanliness was also observed between test and control groups, with the percentage of 29.3% and 94.7%, respectively ($P < 0.05$). These results confirm that black raspberry extract has the

Table 3. The distribution and proportion of vaginal micro-ecological indicators

Index	Number of abnormal vaginal samples (%)					
	Before the test			After the test		
	Test group	Control group	*P*	Test group	Control group	*P*
Vaginal pH	126 (65.9%)	61 (64.2%)	0.768	96 (50.3%)	54 (56.8%)	0.316
bacterial vaginitis	40 (20.9%)	24 (25.3%)	0.409	15 (7.9%)	18 (18.9%)	<0.05
Vaginal cleanliness	163 (85.3%)	80 (84.2%)	0.861	56 (29.3%)	90 (94.7%)	<0.05
White blood cell count of vaginal secretion	171 (89.5%)	87 (91.6%)	0.583	164 (85.9%)	89 (93.7%)	0.075
Esterase activity of leukocyte	168 (87.9%)	89 (93.7%)	0.131	148 (77.5%)	83 (87.4%)	0.056

P, P value.

effect of improving vaginal micro ecological environment.

6. Discussion

Human papillomavirus infection is the main cause of cervical squamous intraepithelial neoplasia and cervical cancer, and persistent high-risk HPV infection is a necessary condition for the occurrence of cervical cancer (Yang, 2013). The risk of HPV infection in a woman's life is 80%. Most HPV infections are transient and can be cleared by the immune system. However, 10–15% of patients can not clear HPV virus in time, resulting in persistent infection of HPV virus, cervical cell lesions, and even developing into cervical cancer (Thomas et al., 2002). The speculated population of female with HPV infection might be with a quite large base in China. The exact reasons mainly include: 1) women have a long-term lifetime probability of being infected with HPV as even high as 80%□90%; 2) according to a recent report from China (Lu et al., 2020), 110,650 new cancer cases and 36,714 cancer deaths in China were attributable to HPV infection in 2015; 3) although HPV vaccination into immunization program are well performed in the world, the actual HPV vaccination for Chinese adolescent women had just begun in recent year. Therefore, active prevention and treatment of HPV infection are necessary to prevent and reduce the occurrence of cervical cancer.

Black raspberry contains a variety of bioactive compounds belonging to polyphenols, which are efficient free radical scavengers and related to tumor chemoprevention and virus clearance (Pan et al., 2017). Through the antioxidant system, black raspberry powder and extract can reduce the growth rate of precancerous cells, promote cell apoptosis and cell differentiation, reduce inflammation and angiogenesis, eliminate the damage of oxygen free radical accumulation on DNA, reduce the risk of carcinogenesis, and restore the metabolic function of cells. Interestingly, polyphenolic compounds derived from black raspberry extract, cyanidin-3-glucoside and gallic acid, were identified to have inhibitory effects against influenza A and B type viruses and food-borne murine norovirus-1 and feline calicivirus-F9 (Lee et al., 2016a; Lee et al., 2016b).

Meanwhile, some studies have found that chemically modified lactoglobulin has highly effective conjugate activity to HPV6, HPV16, and HPV18 typing (Lu et al., 2013) and can be used as an auxiliary treatment and prevention of HPV infection. In addition, bovine β-lactoglobulin modified by 3-hydroxyphthalic anhydride has the potential to inhibit human immunodeficiency virus (HIV), HIV, HSV-1, HSV-2 and some chlamydia (Jiang et al., 1996; Neurath et al., 1998). These auxiliary antiviral activities were related to the percentage of lysine and arginine residues on lactoglobulin. The chemically modified lactoglobulin was no cytotoxicity at a concentration of 1 mg/ml and was highly stable at room temperature and 37 °C for at least 12 weeks. As for gel applied in this study, lactoglobulin is acidic under the action of raspberry powder. The acidified lactoglobulin carries a large number of negative charges on its surface, binding to human papillomavirus HPV L1 which has a large number of positively charged regions and forming a complex. Therefore, after adsorption, the virus cannot bind to the transport glycoprotein on the cell surface or enter the cell to form a new infection, leading to the blocking of the infection pathway of human papillomavirus. In line with these reports, our present observation indicated that the black raspberry extract gel containing chemically modified lactoglobulin has potential as an effective and safe antiviral strategy for HPV infection.

In summary, our present preliminary clinical observation seems to promote that the black raspberry extract gel showed some promising in targeting HPV prevention and clearance. Certainly, in-depth study regarding the effective compounds and mechanisms of black raspberry extract and more stringent and comprehensive double-blind randomized controlled trials are obviously worthy of conducting in future.

Conflict of interest

All authors declare that there is no conflict of interest.

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