RESEARCH ARTICLE

Clearance of Cervical Human Papillomavirus Infection by Topical Application of Curcumin and Curcumin Containing Polyherbal Cream: A Phase II Randomized Controlled Study

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Abstract

Curcumin and curcumin containing polyherbal preparations have demonstrated anti-microbial and anti-viral properties in pre-clinical studies. Till date no therapeutic intervention has been proved to be effective and safe in clearing established cervical human papillomavirus (HPV) infection. The present study evaluated the efficacy of *Basant* polyherbal vaginal cream (containing extracts of curcumin, reetha, amla and aloe vera) and of curcumin vaginal capsules to eliminate HPV infection from cervix. Women were screened by Pap smear and HPV DNA test by PCR. HPV positive women without high grade cervical neoplasias (N=287) were randomized to four intervention arms to be treated with vaginal *Basant* cream, vaginal placebo cream, curcumin vaginal capsules and placebo vaginal capsules respectively. All subjects were instructed to use one application of the assigned formulation daily for 30 consecutive days except during menstruation and recalled within seven days of the last application for repeat HPV test, cytology and colposcopy. HPV clearance rate in *Basant* arm (87.7%) was significantly higher than the combined placebo arms (73.3%). Curcumin caused higher rate of clearance (81.3%) than placebo though the difference was not statistically significant. Vaginal irritation and itching, mostly mild to moderate, was significantly higher after *Basant* application. No serious adverse events were noted.

Keywords: Cervical cancer - human papillomavirus (HPV) - clearance - curcumin - polyherbal cream

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Introduction

Human papillomavirus (HPV) infection is the necessary cause of cervical cancer (IARC monograph, 1995). Women persistently infected with high risk HPV types, especially HPV 16 and HPV 18, show a high rate of progression to high grade cervical precancers (Kjaer et al., 2002). Studies have also observed a positive correlation between HPV viral load and risk of developing cervical Neoplasia (van Duin et al., 2002). Till date there is no effective treatment directed towards clearance of HPV. The infected women, if they are 30 years or older, are to be kept under frequent surveillance till they clear the infection or develop cervical neoplasias.

Curcumin [diferuloyl methane {(E,E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione}] is the yellow pigment derived from the rhizomes of turmeric (*Curcuma longa linn*), used as a spice and as a herbal medicine. In preclinical studies curcumin has been observed to be cytotoxic to cervical cancer cells in a concentration-dependent and time-dependent manner (Divya and Pillai, 2006). The cytotoxicity was selectively more in HPV 16 and HPV 18 infected cells compared to non-HPV infected cells. Curcumin treatment inhibits the transcription of HPV 16 E6/E7 as early as 6 hours post-treatment and restores the expression of tumor suppressor proteins p53, retinoblastoma protein, and PTPN13 (Maher et al., 2011).

Talwar et al. (2000) initially developed a polyherbal formulation (*Praneem*™) containing purified extract of neem (*Azadiracta indica*) and saponins extracted from reetha (*Sapindus mukerossi*), which strongly inhibited the growth of *Neisseria gonorrhoea*, multi-drug resistant Escherichia coli and various species of Candida in in-vitro and mice models (Talwar et al., 2000). In progestin sensitized mice, vaginally administered *Praneem*™ exhibited viricidal action against HIV-1 and prevented the transmission of Herpes simplex-2 infection (Vermani and Garg, 2002). Due to the initial success of the formulation, Talwar Research Foundation developed *Basant*™ as the next generation polyherbal cream with bio-adhesive and acid buffering properties containing following ingredients:

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i) Purified curcumin; *ii*) Purified extract of Amla (*Emblica officinalis*); *iii*) Purified saponins from Reetha; *iv*) Aloe Vera; *v*) Rose water.

The various active ingredients in the formulation have anti-microbial, anti-inflammatory, anti-HPV and anti-HIV action (Gupta and Gupta, 2011). *Basant*™ inhibits the growth of *Neisseria gonorrhoea*, including those resistant to penicillin, tetracycline, nalidixic acid and ciprofloxacin (Talwar et al., 2008). It has pronounced inhibitory action against *Candida glabrata*, *Candida albicans* and *Candida tropicalis* isolated from women with vulvovaginal candidiasis. Aloe and Amla have been observed to inhibit the transduction of HPV-16 pseudovirus in HeLa cells at concentrations far below those used in the formulation (Talwar et al., 2008).

Based on the preclinical and the early clinical evidences of the anti-HPV effects of curcumin and *Basant*™ cream, the present four arm, placebo controlled, double-blind, randomized phase II trial was designed to evaluate the efficacy of *Basant*™ polyherbal cream and curcumin soft gelatine capsules to clear HPV infections from the uterine cervical epithelium when applied locally in women without any high grade cervical precursor lesions or cervical cancers.

Materials and Methods

Selection of study subjects

Women between 30-60 years of age were screened at the rural community based clinics by conventional Pap smear. A cervical scrape sample was also collected from these women for HPV DNA detection. Trained health workers collected cervical smears for cytology using wooden Ayre's spatulas. The same spatulas were then used to obtain repeat cervical scrapings that were dipped in 2mL of cold phosphate-buffered saline for HPV DNA testing. The cervical smears and the samples for HPV detection were carried to the laboratory at Chittaranjan National Cancer Institute (CNCI) for further processing and reporting. Women positive on either of these tests had colposcopy by trained gynaecologists. Punch biopsies were obtained from any colposcopically suspicious lesions for histopathological evaluation. The women tested positive for any HPV on L1 consensus PCR were invited to participate in the present trial provided they fulfilled the following inclusion and exclusion criteria:

Inclusion criteria: *i*) The cervical sample of the woman should be positive for HPV on L1 consensus PCR; *ii*) There should be no evidence of cervical high grade squamous intraepithelial lesions (HSIL), glandular abnormalities or invasive cancer either on cytology, colposcopy or biopsy; *iii*) The woman and her partner should agree to use barrier method of contraception during the entire duration of the study; *iv*) The woman should give written informed consent to participate in the study voluntarily.

Exclusion criteria: *i*) Pregnant and lactating women; *ii*) Subjects with menstrual flow lasting more than seven days; *iii*) Subjects treated earlier for cervical precancer or cancer; *iv*) Clinical evidence of any serious systemic disease and acute illness.

Plan of randomization: A total of 280 subjects were planned to be randomized in 1:1:1:1 ratio to four treatment/placebo arms as follows: *i*) Arm 1 (*Basant* Arm): to receive *Basant*™, a polyherbal vaginal cream; *ii*) Arm 2 (Placebo Cream Arm): to receive placebo vaginal cream; *iii*) Arm 3 (Curcumin Arm): to receive curcumin soft gelatine vaginal capsules; *iv*) Arm 4 (Placebo Capsule Arm): to receive placebo soft gelatine vaginal capsules.

The sponsor generated a randomization list using PROC PLAN in SAS, "version 9.1.3" for treatment allocation and provided it to the study site. A randomization number was used to uniquely identify the study medication to be administered to a subject. When a subject met the eligibility criteria, the person in charge of drug accountability at the study site sequentially assigned the next available randomization number from the list to determine the study medications to be used for the subject. The placebo preparations were indistinguishable from the corresponding active ingredients. Both the investigators and the patients were blinded to the treatment allocation.

The pharmaceutical company identified to produce the placebo creams for Arm 2 could not provide adequate number of the preparations. With approval from sponsor the recruitment in that arm was stopped prematurely after recruiting 54 subjects.

Diagnostic work up and treatment procedures

The eligible subjects were assigned to the treatment/placebo arms within 14 days of being screened. Prior to initiation of treatment all subjects had pregnancy test in urine, general physical examination and routine blood tests. The route of administration and dosage of the medication/placebo in each arm are given in Table 1. While dispensing, a trained female study coordinator explained the vaginal use of the medications using pictorial demonstration cards. All the women were instructed to retain the used tubes/capsule packs and return them during their next visit. They were also advised to contact the principal investigator or the study coordinator if they had any problem while taking the medicines. All the women were recalled for the final visit within 14 days of completion of the medications or earlier if they experienced any problem. At the final visit the following procedures were performed: *i*) Collection of Pap smear for cytology; *ii*) Obtaining cervical scrapes in PBS vial for detection of HPV; *iii*) Colposcopy; *iv*) Cervical biopsy was obtained if there was any abnormality on colposcopy and also if there was a pre-treatment abnormal histology histology; *v*) Hemogram, liver function test and renal function test.

All these procedures were performed even if the patient did not complete the course of medications and attended for the final visit. The patients were asked about any adverse reactions that occurred after initiating the medications. The adverse events were graded into mild, moderate and severe by the study investigators and their relatedness to the study medications (not related, possible, probable and definite) was ascribed as per standard criteria. The compliance to the dosage schedule was checked by measuring the contents of the returned tubes or counting the number of capsules returned.

Even if some of the women did not complete the full course of medications/placebo and were not willing to undergo the scheduled procedures of the final visit, the study coordinator enquired about the adverse events and collected the unused medications/placebo, if necessary by making home visits.

Detection of HPV

The steps of HPV DNA detection and typing have been described in details in our earlier publication (Dutta et al., 2012). Briefly, the cervical samples were tested for the presence of HPV by PCR in GeneAmp PCR System 9700 (Applied BioSystems, Foster City, CA) using primers from the L1 consensus region of the HPV genome and AmpliTaq Gold™ DNA polymerase enzyme. MY 09/11 primers of the L1 region were used to detect 450 bp amplicons of HPV. For confirmation of HPV presence in the samples, nested PCR was done on the PCR products using GP5+/6+ primers having complementary sequence within the amplicons of MY 09/11. The samples that showed PCR products of 155 bp size in the agarose gel electrophoresis were confirmed as HPV positive. The HPV-positive samples were then tested for the presence of HPV types 16 and 18 by PCR using HPV 16 type-specific primers from the E6 region and HPV 18-specific primer from the long control region. The HPV types were confirmed by Southern hybridization using 32P-labeled HPV type-specific probes.

Statistical analysis

Sample size was calculated to demonstrate superiority of each of the treatment groups (*Basant* cream and Curcumin soft gelatin capsule) relative to corresponding placebo groups in enhancing the clearance rate of HPV infection. The spontaneous clearance rate of HPV infection has been observed to be about 23% at 2 months and 30% at 3 months (Sterling et al., 2001). Assuming a spontaneous clearance rate of 20% in the placebo group and about 45% in each of the treatment groups after 30 applications, 60 subjects were required in each arm to have at least 78% power to demonstrate superiority, using a two-sided significance level of 5%. To make up for the possible losses due to non-compliance the sample size was enhanced to 70 in each arm.

For efficacy analysis the primary end point was elimination of HPV from cervical cells of subjects based on molecular detection of HPV after 30 applications of treatment. Subjects who tested positive for HPV at baseline and negative for HPV at the final visit were considered as subjects with elimination of HPV.

The efficacy endpoints were analyzed for the Modified-Intention-to-Treat (MITT) and the Per-Protocol (PP) population of which the MITT population was the primary population. All randomized subjects who had the primary endpoint (the results of HPV analysis) available at Visit 1 (screening) and at Final Visit (follow up) were included in the MITT population. All randomized subjects who completed the course of medicines as prescribed and have the follow up visit have been included in the PP population. All subjects who were dispensed with the study drug and had at least one application of the drug were included in the safety analysis. The efficacy and the safety outcomes were analyzed separately for Arm 1 Vs Arm 2 and Arm 3 Vs Arm 4 using Chi-square test

Table 1. Mode of Administration and Dosage of Study Medications/Placebo

Treatment Arm	Study Treatment	Total No. of Applications	Dosing Regimen
Arm 1	*Basant*™, a polyherbal vaginal cream (5 ml. per application)	30 intra-vaginal applications	One application per day at bed time, excluding the days of menstruation
Arm 2	Placebo vaginal cream (5 ml. per application)		
Arm 3	Curcumin soft gelatin vaginal capsules (500 mg curcumin per capsule)		
Arm 4	Placebo vaginal capsules		

Table 2. Summary of Subject Demographic Characteristics and HPV 16 and HPV 18 Positivity at Baseline

Variable, Categories	Arm 1 (N=72)	Arm 2 (N=54)	Arm 3 (N=79)	Arm 4 (N=82)
Age (years), Mean (95% CI)	36.5 (35.1-37.9)	38.7 (36.6-40.9)	37.5 (35.8-39.2)	38.3 (35.7-38.9)
Parity, Mean (95% CI)	2.3 (2.0-2.6)	2.5 (2.2-2.8)	2.5 (2.2-2.9)	2.4 (2.0-2.7)
Post-menopausal, n (%)	6 (8.3)	13 (24.1)	11 (13.9)	11 (13.4)
HPV 16 positive, n (%)	12 (16.6)	4 (7.4)	6 (7.6)	11 (13.4)
HPV 18 positive, n (%)	6 (8.3)	6 (11.1)	6 (7.6)	5 (6.1)
HPV 16 & 18 positive, n (%)	1 (1.4)	0 (0)	4 (5.1)	1 (1.2)

Table 3. Rate of Clearance of HPV 16 and HPV 18 in the MITT Population*

	Arm 1 (*Basant* cream)	Arm 2 (Placebo cream)	Arm 3 (Curcumin Capsule)	Arm 4 (Placebo capsule)
HPV 16	11/11 (100.0%)	3/3 (100.0%)	4/6 (66.7%)	6/10 (60.0%)*
HPV 18	5/5 (100.0%)	6/6 (100.0%)	5/5 (100.0%)	5/5 (100.0%)#
HPV 16 & 18	1/1 (100.0%)	0/0	3/3 (100.0%)	1/1 (100.0%)
Overall for HPV 16 or/and 18	17/17 (100.0%)	10/12 (83.3%)	12/14 (85.7%)	12/16 (75.0%)

*One case cleared type 16 but was found to be infected with Non 16/18 type at follow up; #Once case cleared type 18 but was found to be infected with Non 16/18 type at follow up

or Fisher's exact test as appropriate. Since in the Arm 2 less than the target number of subjects was recruited, the subjects belonging to placebo arms (Arm 2 and Arm 4) were grouped together to estimate HPV clearance in control subjects since subjects belonging to both these arms did not receive any ingredient that could influence HPV elimination. This combined control group was separately compared with the two study arms. Statistical significance was defined if the p value <0.05.

Ethical considerations

The study protocol was approved by the Research Ethics Committee of Chittaranjan National Cancer Institute and was reviewed by the Technical Advisory Group of Department of Biotechnology, Government of India. The study was listed in the Indian Council for Medical Research Clinical Trials Registry (registration number CTRI/2008/091/000095). All the potential subjects for the study were explained the study procedure including the implications of being in the placebo arm by one of the authorized investigators. All subjects either signed an informed consent form themselves or provided thumb impression (if illiterate) in the presence of their legally accepted representatives. The study was monitored by an external agency (Manipal Acunova Clinical Research Organization) employed by Department of Biotechnology to ensure that the appropriate ethical and Good Clinical Practice guidelines were adhered to.

Results

A total of 287 HPV positive women were randomized to the study. The subject demographic characteristics and the prevalence of HPV 16 and HPV 18 infections in different study arms are provided in Table 2. The study and the control arms were comparable for age and parity. There were significantly more post-menopausal women in the placebo cream arm compared to the *Basant* cream arm (p=0.02), whereas there was no significant difference between the curcumin capsule and placebo capsule arms in this regard. The proportions of women positive for HPV 16 and HPV 18 (either single or in combination) were 26.3% for *Basant* cream arm, 18.5% for placebo cream arm, 20.3% for the curcumin capsule arm and 20.7% for the placebo capsule arm. There was no significant difference between the arms in this aspect.

The distribution of the study subjects in different arms and reasons for some of their exclusion from the per-protocol and MITT populations are shown in Figure 1. Out of the total 287 women randomized, 255 (88.8%) completed all the study related procedures and were included in the per-protocol analysis. There were 14 women who did not use complete dosage of the formulations, yet they completed the last follow up visit. The MITT analysis included these 14 women along with the 255 women included in the per-protocol analysis (N=269). All the 287 randomized women were included in the safety analysis.

In the MITT analysis HPV was eliminated in 57/65 (87.7%) subjects in the *Basant* cream arm compared to 36/48 (75.0%) subjects in the placebo cream arm and the difference was not statistically significant (p=0.08). In the Curcumin capsule arm, 61/75 (81.3%) subjects showed elimination of HPV when compared to 58/80 (72.5%) subjects in the placebo capsule arm. The difference was not statistically significant either (p=0.19). When the HPV clearance rate of *Basant*™ cream was compared to two placebo arms grouped together (cream and capsule; N=128), the difference was statistically significant (87.7% vs 73.4%; p=0.03). The HPV elimination rate of the curcumin arm was higher than the combined placebo arm but the difference still failed to reach the level of clinical significance.

The median duration of clearance of HPV infection was similar in all the arms (Arm 1: 38 days; Arm 2: 39.5 days; Arm 3: 39.0 days; Arm 4: 38.5 days) of the MITT

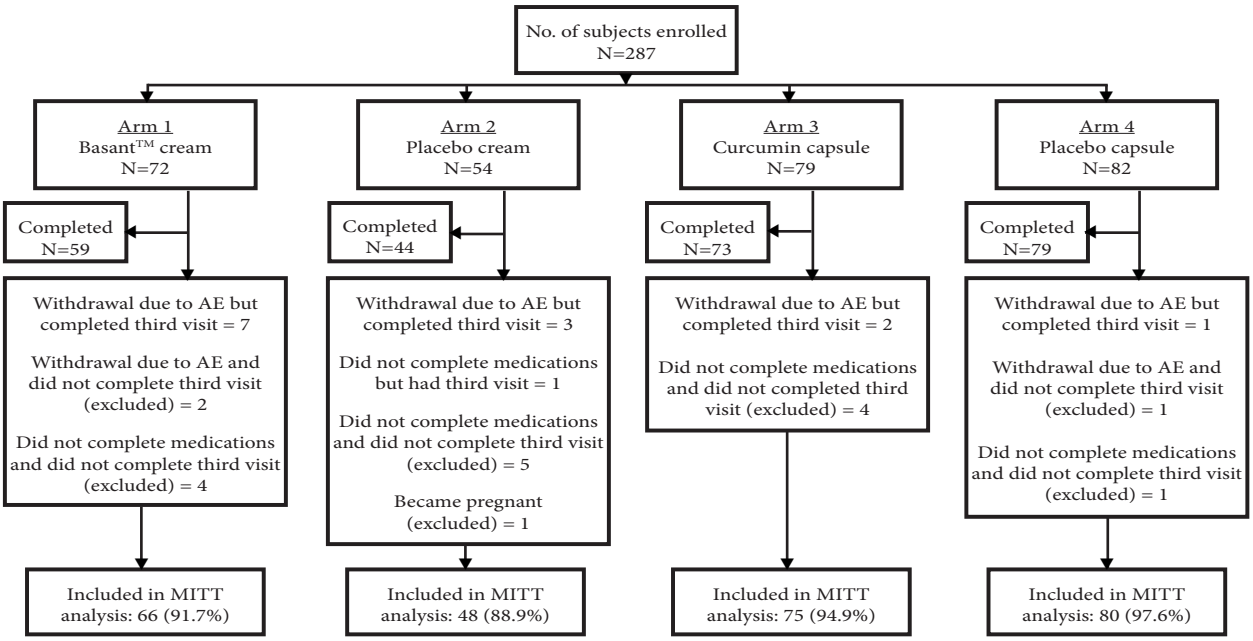

Figure 1. Number of Subjects Completing Treatment and Reasons for Exclusion of Subjects from MITT Analysis

Table 4. Frequency of Treatment Emergent Adverse Events during the Study

	Arm 1 (N=72)	Arm 2 (N=54)	Arm 3 (N=79)	Arm 4 (N=82)
Total no. of AEs in each study arm	33	5	12	9
The relatedness of AEs with study medications as established by principal investigator				
Not related	4	0	4	1
Possibly related	15	5	6	3
Probably related	11	0	2	5
Definitely related	3	0	0	0
No. of subjects experiencing at least one AE	20 (27.8%)	4 (7.4%)	7 (8.9%)	7 (8.5%)
No. of subjects experiencing at least one severe AE	4 (5.6%)	0	2 (2.5%)	0
No. of subjects who withdrew due to AEs	6 (8.3%)	2 (3.7%)	2 (2.5%)	2 (2.4%)
Serious adverse events	0	0	0	0

Table 5. System Organ Wise Listing of All Adverse Events

System organ class, Preferred term		Arm 1 (N=72)	Arm 2 (N=54)	Arm 3 (N=79)	Arm 4 (N=82)
Gastrointestinal Disorders	Abdominal Pain	3	0	0	0
	Abdominal Distension	0	0	0	1
	Diarrhoea	0	0	0	1
Hepatobiliary Disorders	Cholelithiasis	1	0	0	0
Reproductive System Disorders	Genital Rash	2	0	0	0
	Menorrhagia	1	0	0	0
	Uterine Cervical Pain	1	0	0	0
	Vaginal Discharge/infection	0	1	1	0
	Vulvovaginal Burning Sensation	12	1	3	1
	Vulvovaginal Discomfort	1	0	0	0
	Vulvovaginal Pruritus	10	3	6	4
Nervous system and psychiatric disorders	Dizziness	0	0	0	1
	Insomnia	0	0	1	0
Ear And Labyrinth Disorders	Vertigo	0	0	0	1
General Disorders And Administration Site Conditions					
	Pyrexia	0	0	1	0
Skin And Subcutaneous Tissue Disorders	Pruritus Generalized	1	0	0	0
	Rash Generalized	1	0	0	0

population.

Even in the per-protocol analysis population no significant difference in HPV clearance rates was observed between the *Basant* cream versus the placebo cream arms (p=0.18) and the curcumin capsule versus the placebo capsule (p=0.19) arms.

The rates of clearance of HPV 16 and HPV 18 in the MITT populations of the four arms are shown in Table 3. All the women who received *Basant* cream cleared the HPV 16/HPV 18 infections, whereas, the clearance rate for the women receiving curcumin capsules was 85.7%. The clearance rate for HPV was 78.6% among the HPV 16/HPV 18 positive women (N=28) who received placebo either in the form of cream or capsule. There was no statistically significant difference in the clearance rates between the *Basant* cream arm and the placebo (p=0.07) or the curcumin capsule arm and the placebo (p=0.69).

All women with biopsy proved HSIL or worse lesion or women suspected to have HSIL or worse lesion on cytology and/or colposcopy were excluded from the study. There were only few biopsy proved LSIL lesions (N=15) in the MITT study population at baseline. In the *Basant* cream arm, four out of the seven cases (57.1%) with LSIL histopathology had regression of lesion following treatment. In the placebo cream arm there were only two cases with LSIL histopathology at baseline and both the patients (100%) had normal findings at the end of study. In the Curcumin capsule arm two out of six (33.3%) subjects with LSIL histology had post-treatment regression of lesion. None of the subjects in the placebo capsule arm had LSIL histopathology at baseline. The p values were not determined as data was insufficient for the analysis.

The frequency of the treatment emergent adverse events (AEs) along with their relationships with the study medications are given is Table 4. There were total 33 adverse events (AEs) in 20 (27.8%) subjects in the *Basant* cream arm and total five AEs in 4 (7.4%) subjects in the placebo cream arm (p=0.005). There were total 12 AEs in seven (8.9%) subjects In the Curcumin capsule arm compared to total nine AEs in seven (8.5%) subjects in the placebo capsule arm (p=1.00). There were no SAEs reported in the study. Although there were significantly higher numbers of AEs observed with *Basant*™ cream compared to placebo cream, most of the AEs were assessed as mild to moderate in severity. AEs for only four subjects in *Basant* cream were assessed as severe, all of which were application site reactions. With Curcumin capsules there were slightly more AEs as compared to placebo capsules, however only two subjects had severe AEs in the form of application site reactions.

The frequency of various AEs classified according to various organ systems is shown in Table 5. The most frequently observed AE in the *Basant* cream arm was vulvo-vaginal burning sensation seen in 11 (15.3%) subjects compared to one (1.8%) subject in the placebo cream arm; followed by vulvo-vaginal pruritus seen in nine (12.5%) subjects compared to three (5.6%) subjects in the placebo cream arm. The most frequently observed AE in the Curcumin capsule arm was vulvo-vaginal pruritus seen in five (6.3%) subjects compared to four (4.9%) subjects in the placebo capsule arm. The second most common AE, vulvo-vaginal burning sensation was

seen in three (3.8%) subjects in the Curcumin capsule arm and one (1.2%) subject in the placebo capsule arm.

The AEs that led to withdrawal were vulvo-vaginal burning sensation, vulvo-vaginal pruritus and abdominal pain in the *Basant* cream arm and vulvo-vaginal pruritus and vaginal discharge in the placebo cream arm. The AEs that led to withdrawal in the Curcumin capsule arm were vulvo-vaginal pruritus and sleeplessness, whereas, in placebo capsule arm these were dizziness, vertigo, diarrhoea and abdominal pain. Only vulvo-vaginal burning sensation and vulvo-vaginal pruritus were assessed as severe and while others were assessed as mild to moderate. All the subjects who discontinued treatment due to AEs were followed up till recovery and the outcomes were reported as "recovered" at the end of the study.

Discussion

This was the first placebo controlled clinical trial on the efficacy of curcumin and a curcumin containing polyherbal formulation to clear HPV infection of the uterine cervix. Key baseline characteristics and prevalence of the foremost oncogenic types of HPV (types 16 and 18) were similar between subjects in the four study arms.

Women treated with *Basant*™ polyherbal cream had the higher clearance rate for any HPV as well as for HPV types 16 and 18. Superior HPV elimination rate observed following *Basant*™ application was of greater clinical significance because compared to all other arms these women had higher prevalence of HPV 16 and HPV 18 infections. These two HPV types are most oncogenic, tend to persist more often and the infected women have high risk of developing cervical neoplasias even several years after initial infection (Khan et al., 2005). Women treated with Curcumin capsules had higher clearance rate compared to the women receiving placebo only, but the difference was not statistically significant. It is possible that the improved performance of *Basant* cream was due a synergistic effect of curcumin with other herbal ingredients, which have independently shown anti-HPV effect in earlier study (Talwar et al., 2008).

It is already documented that the majority of HPV infections are self-limiting and cell-mediated immunity is responsible for spontaneous clearance. There are several determinants of HPV clearance like age, parity, use of oral contraceptive pills, smoking, HPV type etc (IARC monograph, 1995). The duration of follow up and interval between the tests can also influence the observed rate of clearance of the virus. The subjects enrolled in the placebo arms in our study served as controls to know the rate of spontaneous clearance of HPV in the untreated women. Comparison with these control subjects allowed us to assess the effectiveness of the study drugs to clear HPV infection directly. The HPV clearance rate with *Basant*™ cream was higher than the spontaneous clearance rates documented from other studies as well. In a large population based study from Columbia, HPV positive women were systematically followed up every 6-9 months to look for HPV clearance (Molano et al., 2003). The clearance rate was highest in the first 6 months of follow-up and by one year 77% of the women cleared the infection spontaneously. We observed similar HPV clearance rate (73.4%) in the women who received placebo only but much higher rate (87.7%) for women treated with *Basant*™. Interestingly, the median duration of clearance in our study was just over one month, possibly because our follow up interval was much shorter compared to other studies.

We observed that the number of subjects reported with AEs was significantly greater in the *Basant* cream arm when compared with the placebo cream arm. This was not the case with curcumin capsule arm. Most of the AEs of *Basant* cream and curcumin capsules were assessed as mild to moderate in severity. There were 12 subjects who were withdrawn due to AE out of which 6 subjects belonged to *Basant* cream arm and 2 subjects each to other arms in the study. There was more withdrawal of subjects in the *Basant* cream arm due to vulvovaginal burning sensation and pruritus. It should be noted that the subjects were selected from a screening population who were asymptomatic at the time of recruitment to the study. For some of them even mild to moderate vaginal discomfort was unacceptable and they decided to discontinue the study medications.

Till date all the therapeutic techniques for HPV induced conditions are directed towards treatment of established precursor diseases rather than clearance of HPV infection and prevention of progression to disease. Immunomodulators, such as imidazoquinolones that induce production of inflammatory cytokines have been used to potentiate the innate immune mechanism mediated through macrophages and dendritic cells (Bharti et al., 2009). Imiquimod™, one of the imidazoquinolone compounds has shown efficacy and safety in clinical trials for the treatment of external HPV-infected genital warts (Beutner et al., 1998). In a phase II trial women treated with Imiquimod vaginal suppositories had significantly higher complete or partial regression of CIN 2/CIN 3 compared to the placebo group (Grimm et al., 2012). HPV clearance rates in these women with CIN were significantly higher in the Imiquimod group (60%) compared with the placebo group (14%). However, the local irritation and burning sensation are significant adverse effects of Imiquimod. Even this compound has not been evaluated in women having only HPV infection but no HPV induced disease.

One major limitation of the study was that there was incomplete recruitment in the placebo cream arm. This may influence the comparison of the adverse events with the *Basant* cream arm. As far as the primary end point of viral clearance is concerned, this is unlikely to affect the study. The subjects from the placebo cream arm and the placebo capsule arm did not receive any active ingredient that could influence viral clearance. However, we accept that clubbing the data from two different randomization arms is strictly against the principle of randomized controlled trials and leaves behind scope of biased estimation.

As far as the natural history of HPV infection is concerned, the follow up interval of just over one month may be considered as too short. The subjects were retested immediately after completion of the one month course of study medications to eliminate the theoretical possibility

of re-infection with HPV, either the already existent type or some other type.

Another shortcoming of the study was its inability to come to a conclusion about the efficacy of the formulations to cause regression of histology proved CIN 1, though that was not our primary objective. The time interval between the baseline tests and the follow up investigations was too low to show any meaningful effect of the formulations on the regression of such lesions. The total number of low grade lesions at baseline in the study cohort was also quite inadequate.

It can be concluded that the women treated with *Basant*™ cream had higher but statistically non-significant. HPV elimination compared to those treated with placebo. The study medications (*Basant*™ cream and Curcumin capsule) were found to be safe and tolerable although some application site adverse events, more commonly seen in the *Basant* cream arm, made some of the subjects to discontinue treatment.

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