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RESEARCH ARTICLE

Integrated Non-invasive Management of Cervical Low-Grade Squamous Intraepithelial Lesions Observed in Papanicolaou Smears with Antimicrobials Followed by Oral *Curcuma Longa* Extract

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Abstract

Background: Prevention of cervical cancer by treatment of precancerous conditions is critical in saving lives and is cost-effective. Turmeric bio-actives have shown potential anticancer activity in vitro, in vivo and in early clinical studies. The activity and safety of non-invasive integrated treatment with antimicrobials and oral turmeric extract was explored clinically in women with Low – grade Squamous Intraepithelial Lesion (LSIL) detected in Papanicolaou (Pap) smears. **Methods:** Women who attended a cancer screening program and who were detected with LSIL were enrolled for the study. Treatment offered was antimicrobials for associated genital infections followed by oral turmeric extract (*Curcuma longa* Linn, Haldone®, 600 mg, BD) for 10 weeks. The end points for activity were the degree of abnormality in Pap smears and colposcopy, micrometry in Pap smears and serum IL-6 assays, which were carried out initially, at 4-6, and at 10-11 weeks. Safety was assessed clinically, and with blood and urine tests. **Results:** Out of 21 enrolled cases, 1 discontinued within 2 weeks, and 3 after 4-6 weeks, due to vaginal irritation. Out of 20 cases treated for 4-10 weeks, LSIL did not progress in any case and 18 showed regression in Pap smear scores ($p<0.003$; Wilcoxan Rank test); 6 showed Cervical Intraepithelial Neoplasia (CIN 1) by colposcopy initially but none post-treatment. No other significant abnormalities were observed clinically, or biochemically. Manual micrometry of Pap smears (N=17) showed a significant reduction in Nuclear diameters & Nucleus/Cytoplasmic ratio ($p<0.03$; paired t test). A non-significant reduction in serum IL-6 levels occurred in 5/15 cases. Post-therapy Pap smears showed persistent benefit in 16 women after 6-36 months. **Conclusions:** Non-invasive integrative therapy with antimicrobials followed by standardised oral turmeric extract for 10 weeks appears to be promising for LSIL management by reversing early carcinogenesis through suppression of inflammation and inhibition of NF-kB pathway.

Keywords: Cervical Cancer Prevention- Turmeric extract- Integrated Treatment of LSIL

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Introduction

Cervical cancer is a preventable disease. Despite this the morbidity and mortality rate in India is still very high [1]. The best strategy for reducing morbidity and mortality is undoubtedly prevention of cervical cancer [2]. The most commonly used screening methods for cervical cancer and precancerous conditions include the Papanicolaou (Pap) smear, High Risk Human Papilloma Virus (HRHPV) detection tests, Colposcopy and Visual Inspection with Acetic Acid (VIA). The Pap smear is cheaper than HRHPV test and is more easily available than colposcopy in most clinics and hospitals in India. Further, it is more useful than VIA for detecting Low-Grade Squamous Intraepithelial

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Lesions (LSIL) or High-Grade Squamous Intraepithelial Lesions (HSIL) which are precancerous conditions [3-4].

Prevention can be achieved by primary means like healthy life style or HPV vaccination, but a large proportion of the population is already HRHPV positive e.g. 16% of healthy controls were HPV 16/18 positive in an Indian city [5]. Vaccination for HPV 16/18 genotypes can prevent 70 % of cervical cancers, not all [6]. Secondary prevention therefore can be life saving for many women when LSIL or HSIL are detected in Pap smears, because their treatment is almost 100% successful. We have been advocating and practising high quality Pap smear screening for decades. It is possible to identify LSIL and HSIL cases in conventional Pap smears, and to confirm the diagnosis with colposcopy/cervical biopsy for confirmatory diagnosis and management [7].

When we reported persistence of LSIL in Pap smears repeated after treatment with antimicrobials in the previous study [8], we further studied the potential of oral Turmeric Oil Extract (TOE) in reversal of the abnormal changes of LSIL. Women who came for long term follow up examination up to 3 years showed persistent benefit in repeat Pap smears [9-10].

Recent reports of synergistic anticancer effect of turmerone and curcumin, and immunomodulatory and anticancer activity of curcuma polysaccharides and our recent laboratory study [11-13], prompted us to explore the use of a new holistic extract of Turmeric or *Curcuma longa* Linn (Haldone®) for non-invasive intervention to achieve regression or arrest of LSIL lesions in Pap smears. The extracts have been shown to be safe as far as their ability to induce mutagenicity and/or cytogenetic toxicity is concerned [14-15]. Since chronic inflammation, including HPV infection, is known to be associated with cervical carcinogenesis we also included antimicrobials in the treatment schedule [5-8].

The intervention was planned between initial Pap smear and routine 6 monthly follow up with a repeat Pap smear for LSIL cases which is the standard of care [16-17]. Apart from Pap smears and colposcopy, we also measured serum Interleukin- 6 (IL-6) levels since we had observed a decrease in serum IL-6 levels in users of TOE in the previous study [9]. The study was approved by an Independent Ethics Committee and was registered with the National CTRI registry.

Materials and Methods

Type of study

Open labelled exploratory, single arm study on effect of integrated treatment (antimicrobial kit followed by Haldone® for 10 weeks) for Low-Grade Cervical Squamous Intraepithelial Neoplasia (LSIL) detected in Pap smears, and confirmed as less than Cervical Intraepithelial Neoplasia (CIN) 2 by colposcopy.

Setting

Cervical cancer screening and prevention program of the outpatient department of a General Hospital

Inclusion criteria

i) Age between 30 and 65 years

ii) LSIL reported in Pap smear and confirmed by colposcopy as VIA positive or CIN 1, or less

iii) Effective non-hormonal contraception

iv) Abstinence or condom use, during treatment with antimicrobials to prevent reinfections

v) Women on antidiabetics, antihypertensives, or mineral/vitamin therapy, or under treatment for mild chronic disease, were included if dosage was steady for past 3 months. They were advised to keep an interval of 1.5 hour between their other medication and the intake of Haldone® to avoid probable drug interaction during drug absorption.

Exclusion criteria

i) HSIL, or CIN 2, or cancer in Pap smear or on colposcopy

ii) Unexplained or heavy uterine bleeding, epistaxis, haematuria

iii) Systemic diseases like heart disease, jaundice etc.

iv) Gynaecologic pathology- fibroids, polyps, prolapse, ovarian tumours

v) Positive *Treponema pallidum* hemagglutination assay

vi) Positive *Human Immunodeficiency Virus* (HIV) test

vii) Pregnancy or lactation

viii) Allergy to turmeric, or to antimicrobials

ix) Moderate or severe Pelvic Inflammatory Disease (PID)

Criteria for withdrawal from the study

Women could withdraw from the study anytime if they wished but criteria for investigators to discontinue subjects were as follows:

i) Noncompliance for more > 1 week continuously, or 15 days in total

ii) Serious Adverse Event (SAE)

iii) Incidental illness of moderate severity requiring treatment for > 4 days e.g. Common cold

iv) Investigator's choice- reason to be provided

Criteria for termination of clinical trial were as following

i) Progression in cervical pathology as observed in Pap smears or colposcopy

ii) Noncompliance by majority of subjects

iii) Discontinuation by majority of subjects within 4 weeks

iv) Serious Adverse Event (SAE) related to treatment

Measures of Outcome

Primary end points-

i) Pap smear: Papanicolaou stain was used to stain cervical smears carefully collected with disposable spatula under vision, spread and fixed on labelled frosted slide with a standard commercial fixative. The smears were examined with a Zeiss binocular microscope under low (X10) & high magnification (X45) and under oil immersion lens (X100), and with a camera attachment. Smears were

classified and progression, arrest or regression of LSIL was recorded using updated Bethesda Classification [18].

ii) Pap smear Scores: Pap smears were given severity scores according to diagnosis: Negative or inflammatory = 0; Atypia = 1; Atypical Squamous Cells of Unknown Significance (ASCUS) = 2; Borderline LSIL = 3 (<20 abnormal cells/ smear); LSIL = 4; HSIL/ Cancer = 5. This helped us in assessing the degree to which the Pap smear improved or deteriorated during treatment.

iii) VIA & Colposcopy: Acetic acid (5%) precipitates the proteins in the cervical epithelial cells in a hyperplastic condition like LSIL, HSIL or cancer so that the abnormal areas are seen as white or thickened areas. VIA was followed by colposcopy (magnified view of the vagina and cervix during gynaecologic examination) using Digital Video Colposcope (Model COLpro 222DX-OZ View). Only those patients with a colposcopy report of Cervical Intraepithelial Neoplasia (CIN) 1 or lesser severity were included. When colposcopy was not possible VIA was documented by cervicography using a high pixel camera which produced excellent pictures [19]. The action of acetic acid is reversible.

Secondary end points-

i) Micrometry- Nuclear diameter and Nucleus/ Cytoplasmic (N/C) diameter ratio in Pap smears were measured in microns by manual micrometry with the Zeiss microscope under X1000 magnification under oil immersion lens [9].

ii) Serum IL-6 levels: These were measured in nanogram quantity by Enzyme Linked Immuno- Sorbent Assay (ELISA) using a standardised marketed kit (Diasource) in duplicate along with standards. Samples were collected between 10:30 am and 12:00 noon, sera were separated and preserved at -80°C.

Criteria for assessment of tolerability and safety:

A special Case Record Form, approved by the Ethics Committee, was designed to record the details of participant's history, clinical and biochemical examinations- initially, at 4-6 weeks, 10-11 weeks, and any unscheduled visit.

i) Clinical tolerability- Symptoms, weight, pulse rate, blood pressure, and gynaecological complaints like discharge, itching, dysuria, vaginal bleeding, were recorded at 0, 4-6, and 10 weeks.

ii) Gynecological check up– Speculum examination, amount and type of vaginal discharge, any vulvo-vaginal lesions, size of the cervical erosions, uterine size, mobility and tenderness, were recorded at 0, 4-6 and 10 weeks.

iii) Blood tests- Organ function tests: Complete Blood Count, Random Blood Sugar, Serum Alanine Transaminase, Serum Thyroid Stimulating Hormone (TSH), Serum Creatinine, Total cholesterol, TPHA, HIV, Bleeding time (BT), and Clotting time (CT) were assessed at 0, 4-6 and 10 weeks using standard methodology in our laboratory with International Standards Organization (ISO) certification. TPHA, HIV, and TSH tests were not repeated.

iv) Routine urinalysis - Protein, sugar, and microscopic examination for red blood cells, pus cells, and casts were evaluated at 0, 4-6 and 10 weeks.

Treatment schedule for Integrative Treatment

A. Antimicrobial treatment-Initial syndromic antimicrobial treatment was given to the couple as per National Guidelines [20] for associated Reproductive Tract Infections: single dose of Forcanazole (150 mg), Azithromycin (1000 mg) and Secnidazole (2000 mg) as FAS-3® kit tablets (Hetero Health Care). Women with mild PID were give treatment for 2 weeks and Cephadroxyl if needed. Those with moderate or severe PID were excluded from study.

B. Study drug: Holistic extract of *Curcuma longa* Linn (Haldone®)- Capsules of 600 mg of Turmeric extract, Haldone® were given in labelled bottles and preserved at room temperature. These were started on the morning after the antimicrobial kit was used. Women were issued 2 weeks supply at each visit and compliance was noted at each visit.

C. Standardization of Extract: Haldone® was standardised by quantitative HPTLC for 3 bioactives: curcumin as Solid Lipid Curcumin Particles, SLCP [21], turmerone and polysaccharides (about 200 mg each) per 600 mg of extract per soft gelatin capsule, by GMP certified and registered Pharmanza Herbals Private Ltd.

Dose and Duration of use

One capsule (600 mg) twice daily after breakfast and dinner for 10 weeks.

Counselling

All participants were counselled for discontinuation of tobacco, prevention of reinfections of RTIs and a follow up with Pap smears for long term benefits.

Results

A total of 624 women were screened between 1st March 2016 and 31st March 2019. Out of these LSIL was detected in 47 cases by Pap smears. All were counselled, and advised antimicrobial therapy, colposcopy and a 6 monthly follow up. Of these 21 women agreed to participate in the study after inclusion/ exclusion criteria and after informed consent.

Sociodemographic data

The average age of participants was 38.2 years ± 5.9 (range, 30-49 years), and the average weight was 62.02 kg ± 10.02 (range, 46-76 kg). Mean parity was 2 (range, 1-3). All belonged to the low or low - middle class section of society.

Clinical evaluation

The weight, or blood pressure of the participants was not altered significantly during 10 weeks of treatment (p Not Significant; paired t test). Only 2 cases needed treatment for mild PID. In 3 instances participants, or their husbands, complained of mild gastrointestinal symptoms (nausea, occasional loose motions for 1-2 days) during antimicrobial therapy. One case discontinued

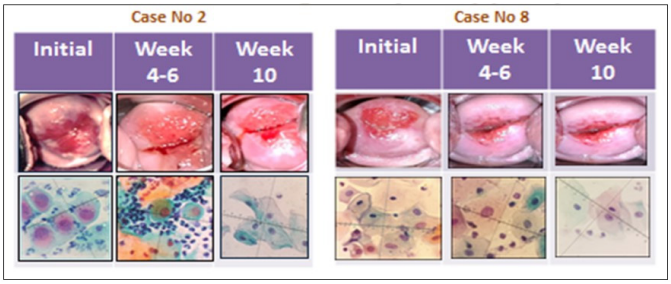

Figure 1. Colposcopy and Pap smear Changes in 2 Cases which Show Improvement and Regression in Pap smear and Colposcopy Changes

within 2 weeks due to local genital irritation, itching and dryness and was excluded from analysis for evaluation of efficacy. Another 3 cases discontinued after a follow up examination at 4-6 weeks due to similar complaints. One case was discontinued by investigators for suspected endometrial carcinoma on repeat smear at 5 weeks. This case was reported to the Ethics Committee as an unrelated Adverse Event. Another case left city after 4 weeks (nonmedical reason). A total of 20 cases continued treatment for a period of 4 to 10 weeks. There were no side effects in women who continued treatment for 10 weeks. Two cases had Diabetes and hypertension and their blood sugars or blood pressure did not increase during 10 weeks of therapy. One case had mild psoriasis which was not aggravated by treatment.

Primary end points

Pap smears

Out of 20 cases, none showed progression to HSIL or cancer in Pap smears during treatment at 4-6 weeks or after 10 weeks. In 18 cases Pap smears regressed and were reported as Atypical Squamous Cells of Unknown Significance (ASCUS), Atypia, Inflammation or Negative smear, whilst 2 cases had persistent borderline LSIL after 10 weeks of use. Sixteen cases came for follow up Pap smears from 6 to 36 months. None of these had showed progression to LSIL, HSIL or Cancer. One case, who was excluded from analysis because of early discontinuation within 2 weeks had LSIL reported in Pap smear when she came for follow up after 6 months and she was advised surgical management.

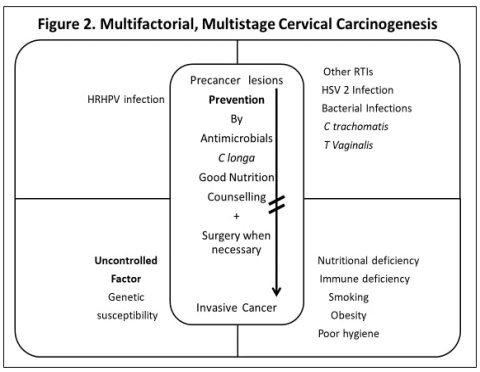

Figure 2. A Schematic Presentation of Cervical Carcinogenesis and Preventive Measures Possible in Secondary Prevention of Cervical Cancer

Pap smear scores

The mean Pap smear scores were significantly reduced from 4±0 to 1.5±0.28 (SEM) after 4-10 weeks of treatment ($P < 0.0001$, paired t test; $P < 0.003$, Wilcoxan Rank test; highly significant).

Colposcopy Follow up

Colposcopy was possible in 12 cases. Six cases had CIN 1; all regressed to VIA positive or inflammatory pattern after treatment. In another 6 cases colposcopy did not show CIN, but VIA was positive, and there was no deterioration after treatment. In the remaining cases colposcopy was unsatisfactory due to burning sensation with acetic acid, or slight bleeding on examination.

Overall, both Pap smears and colposcopy showed significant improvement after integrative treatment for 4-10 weeks (Figure 1).

Secondary End points

Micrometry (N=17)

The mean nuclear diameter and the nucleo/cytoplasmic ratio (NCR) decreased significantly after treatment ($p < 0.03$, paired t test).

Interleukin-6 levels (N=15)

Serum IL-6 could be measured in 15 cases before and after treatment. In five cases the sample was inadequate for duplicate assays. IL-6 was within normal limits in 10 cases before as well as after Haldone® therapy. In four cases it reduced by 10 to 250 ng/ml after treatment. In one case it increased after 10 weeks even though the Pap smear was negative, and she had no fever or complaints. She spontaneously reported that she had no complaints with the treatment but was stressed because her adolescent son was addicted to the phone and did not study. After excluding this case mean serum IL-6 levels (N=14) showed a non-significant decrease from 48.0±29.0 ng/ml to 17.9±2.6 ng/ml after treatment (p value- Not significant; paired t test).

Safety assessment

All biochemical tests, bleeding and clotting time, and urine tests remained within normal limits during treatment

(data not shown; p value- Not significant; paired t test).

Discussion

Cervical carcinogenesis is a gradually progressive multistage multifactorial process starting with HPV infection and may reach the stage of invasive cancer after 6 to 11 years. Hence there is every possibility of detecting precancerous stages if women are screened at regular intervals. Treatment, surgical or pharmacological, in precancerous stage i.e. secondary prevention, is likely to be more successful and, less traumatic, and more economical for the patients and for the health systems, than treatment of invasive cancer.

The scope for secondary prevention of cervical cancer should be expanded from invasive surgical methods to include non-invasive chemoprophylaxis because the number of women who are detected with precancerous Squamous Intraepithelial Lesions (LSIL and HSIL) is huge, several times larger than cancer cases. Many women in poor sections of the society, and in remote areas do not have access to surgical treatment. Surgical treatment is expensive, and not easily available due to a lack of adequately trained personnel and equipment, to many. It may be unsuccessful in removal of total abnormal tissue in about 10% of cases. It may also be associated with complications like postoperative discharge, bleeding, infections, infertility, cervical stenosis or incompetent cervix, and may be followed by recurrences [22-23]. LSIL lesions of cervix are usually detected in younger women up to 45 years of age and it is desirable to avoid the complications in this age group. Our present and previous studies indicate that antimicrobials followed by standardised oral extracts of turmeric have the potential to reverse early nuclear abnormalities detected as LSIL in Pap smears and may have persistent benefit till 3 years after treatment, may be longer if women are counselled and they come for follow ups. In case a recurrence of LSIL is observed, this non-invasive therapy may possibly be safely and effectively repeated [9-10]. Even then this needs confirmation in long term clinical studies in larger sample size. In any case immediate surgical treatment and possible complications can be avoided in majority of cases.

In the present study the duration of treatment was reduced to 10 weeks, vs 12 weeks in past, because the extract was standardised for three bioactive compounds, instead of one, and the curcumin was encapsulated in lipid particles to increase its bioavailability. It is always desirable to have a minimum duration of prophylactic therapy because it is proposed for a large number of asymptomatic women, it should be safe, and compliance is expected to be superior for shorter duration. Whilst turmeric extracts are usually safe, compliance and safety need to be ensured. Although not exactly known for cervical cells, the life span of keratinocytes is approximately 2-3 weeks [24] and in our experience regression occurred after 4-5 weeks in most cases. Yet, considering the wide range of normal biological individual variations we would suggest treatment with standardised turmeric extract at least for 10 weeks. During this integrative therapy all clinical and investigational variables remained within normal range. Despite this we strongly recommend its use under medical supervision and after initial screening because of possible co-morbid conditions and side effects in individual cases. The use of antimicrobials also requires screening for medical conditions and follow up for side effects due to antimicrobials. In the present study there were no significant side effects to the antimicrobials commonly advised in public health programmes. Local vaginal itching, burning or dryness due to turmeric extracts for cervical precancer have been reported by other investigators after vaginal use for chemoprevention [25-26]. In standard Ayurvedic texts, turmeric is reported to cause dryness and burning in susceptible individuals and in damaged tissues [27]. Some dose adjustment or additional treatment may be required in women who manifest this side effect and this deserves further clinical exploration.

Since we have observed an arrest or regression of LSIL in all cases (100%) with turmeric extracts in previous and the present study combined (totally 39 cases), we would like to propose this approach of integrative treatment as a potential chemo-preventive therapy at LSIL or early precancer phase. It is better to address an abnormality in an earlier phase than to await progression to high-grade which usually requires surgery or more expensive therapy. Those options have to be available for women who are detected with high-grade abnormality or cancer in their first Pap smear screening reports.

Probable mechanisms of action of integrative therapy: Chronic inflammation with HPV, and other RTIs, is a known culprit in initiation of carcinogenesis in cervical cancer, and in some other cancers. HPV related carcinogenesis, it's association with other RTIs as reported in some major studies [28-33], and other cofactors are depicted schematically in Figure 2. The possible mechanisms of actions of Curcuma extracts have been reviewed extensively in various reports and are recapitulated below so that the basic mechanism of action of integrative therapy is understood.

Briefly when the HRHPV virus infects the cervical epithelial cells, the viral oncogenic proteins, E6 and E7, which are present in the HRHPV subtypes, attack the intranuclear oncopreventive proteins present in epithelial cells. E7 HPV protein inhibits the Retinoblastoma (Rb) Protein which has Oncosuppressor activity, and the E6 protein inactivates P 53 pathway which controls the normal apoptotic pathway. Both these inhibitory activities lead to mutation, dedifferentiation (loss of normal cellular functions) and uncontrolled proliferation which are characteristic of malignant cells. Both Rb and P 53 proteins control important cell cycle check points during the normal apoptotic processes. It is known that a few mutations lead to abnormal cells, and these may manifest as intraepithelial lesions, but do not lead to malignancy of the cervix. Occasionally they may remain static or can be repaired by the body's defence mechanisms of DNA repair. A series of mutations, usually 5 or more, are necessary before the cells are transformed into malignant cells and acquire immortality, dedifferentiation, and

invasive quality. Mutations are promoted by simultaneous injury due to the Oxidative stress, e.g. due to Bacterial vaginitis, or other cofactors depicted in Figure 2. The most vulnerable cells for adverse effects in the cervix are in the basal stem cell layer of the squamo-columnar junction as they are not fully matured or differentiated into squamous or columnar cells. The virus can easily replicate in the least differentiated epithelial cells starting from the basal layer. The E7 protein can also induce normal gene silencing which prevents differentiation and the physiological process of apoptosis. The E6 viral protein inactivates the P 53 protein with inhibition of apoptosis and the cells acquire immortality. The HPV DNA integrates with the host cell DNA and this leads to mutations so that dedifferentiation occurs simultaneously with uncontrolled proliferation. The E6 and E7 proteins also interfere with the normal immune system responses like induction of interferon, activity of Natural Killer (NK) cells, and promote overexpression of proinflammatory cytokines like IL-1α, IL-6, IL-8, TGF-β, and TNF-α. There is also a compensatory increase in IL-10 and IL-4 which are protective cytokines. Dysregulation of IL-12, IL-17, IL-22 has also been observed. Recently gene expression of micro-RNAs (μRNA) have been found to be dysregulated and these are involved in the initiation, promotion, transformation and metastasis in cancer. In addition, the E5 oncogene expression is increased and this interferes with several normal immune protective pathways which would have otherwise eliminated the abnormal cells. The local immune responses are inhibited because of the highly unfavourable microenvironment and upregulation of IL-10 and TGF-β which suppress local immune response and help the abnormal cells escape from the attack from immune system. Sometimes, but not always, the excess of local cytokines may spill over into general circulation and higher serum levels of cytokines may be observed in women with cervical precancerous conditions and in cancer. The Nuclear- Factor- Kappa-β (NFkβ) pathway is activated by HPV infection and also by co-factors like chronic local infections and oxidative stress. The E1 viral protein of is also associated with the activation of NFkβ pathway thereby compromising the normal balance of the immune system. One of the biomarkers for this pathway is IL-6 which has been found to be overexpressed in precancerous and cancerous tissues causing increased levels in local tissues and sometimes in serum, both in precancer and cancer, particularly metastatic cancer. As stated earlier many of these impaired biological processes can occur sequentially or simultaneously [28-32].

This therefore brings us to the probable mechanisms of action of the integrated therapy. There are three essential components of this therapy i) Antimicrobials, ii) Counselling and follow up and iii) Standardised extract of *Curcuma longa*. Antimicrobials form an essential component of this therapy because chronic inflammation is known to be associated with carcinogenesis, particularly viral infections. There is no cure for viral diseases like HSV and HPV but it is known that all women who are infected with HPV do not develop cancer. Research in this area has brought to the forefront the chronicity of HRHPV and other RTI infections, the severity of pro-inflammatory response vs anti-inflammatory response, and the role of co-factors like nutritional deficiencies and use of tobacco. Genetic factors like P53 or IL-6 gene mutations also play a role but are nonmodifiable. Associated genital infections are common and can be demonstrated by special investigations in about 30% of adult women in our population [8-33-34]. Many of these are sexually transmitted whilst others are not. These infections can cause other possible complications like chronic Pelvic pain, Tubo-ovarian masses, chronic discharge or cervical stenosis, infertility etc. In clinical practice it is practically not feasible to screen all women for all causative organisms (more than 15-20 common organisms) and treat them with different antimicrobials as this becomes expensive (investigations and specific antimicrobials) and facilities are not available in common public health programs. Antibiotic complications, compliance and resistance are other problems with this approach. All cases with LSIL report in the study had inflammation associated with the nuclear abnormality in the Pap smears. We have therefore followed the national guidelines for treatment for RTIs to control the local infections and have taken particular care to include the sexual partner in this therapy [20]. This is accompanied by information on prevention of reinfections, and other co-factors like nutritional deficiencies, and use of tobacco, so that the disease does not progress. There are several studies on significant association of RTIs with cervical neoplasia and cancer and a detailed review is out of the scope of this article. Treatment of genital infections alone does not prevent the progression of carcinogenesis in all cases, particularly in older women as has been reviewed briefly in our previous communication [8].

This brings us to the last, but not the least important, component of integrated therapy i.e. the use of turmeric extracts. The extract used in the present study was standardised for 3 bioactive compounds: curcumin, turmeric oil and polysaccharides. The biological activities of curcumin have been reviewed extensively and its use has been proposed for indications other than cervical cancer. There are numerous molecules and enzymes in the human body with which curcumin can interact and this may produce a pharmacological response. The problem of poor absorption in the gastrointestinal tract and consequent poor bioavailability is circumvented in this study because of the use of Solid Lipid Curcumin Particle (SLCP) instead of the regular or traditionally extracted curcumin [21]. Briefly curcumin inhibits several metabolic pathways and enzymes and growth factors like Protein Kinase C (PKC), Cyclo-oxygenase-2 (Cox-2), Epidermal Growth Factor Receptor (EGFR), Mitogen Activated Protein Kinase (MAPK), Vascular Endothelial Growth Factor (VEGF). One of major pathways which is up-regulated during cervical carcinogenesis is NFkβ and Cyclin D1 and curcumin has been shown to suppress this and the consequent overexpression of IL-6 and other proinflammatory cytokines. It has been shown to induce apoptosis and reduce proliferation of cervical cancer cell lines, C33a (HPV-ve) and SiHa (HPV +ve). There are other

important activities of curcumin like the anti-oxidant and anti-inflammatory activities and curcumin is shown to attenuate several pathways associated with HPV initiated carcinogenesis including expression of µRNA-21. Curcumin can inhibit transcription of HPV-16 and HPV-18 viral types and can potentiate the apoptotic activity of the chemotherapeutic agent paclitaxel in HPV positive cancer [13-35-38]. Topical vaginal application of curcumin formulation in HPV positive women for 1 month was associated with a demonstrable reduction in HPV shedding and the local side effect of itching and irritation was also reported in these cases [25].

The second bioactive compound is turmeric oil of which turmerone is the major component. Turmeric oil was earlier discarded as a by-product of curcumin extraction but studies have shown that it is pharmacologically active, and has anticancer, antioxidant, antimicrobial and DNA protective effects [9-12-14-38-39]. In addition to these it promotes the transport and bioavailability of curcumin as curcumin is lipid soluble and the two compounds given together have synergistic activity in cancer cell lines [12-13]. In our earlier clinical study turmeric oil had significantly reduced the circulatory level of IL-6 indicating inhibition through NFkβ pathway [9], and in the current study a marginal reduction was seen in circulating levels of IL-6. Study of local tissue levels of IL-6 or other cytokines was not possible as a cervical biopsy is usually not indicated after the first diagnosis of LSIL in Pap smears. Moreover, it is known that the cytokine alterations are dependent on individual response and are not seen in 100% of LSIL cases. In vitro studies have also shown that turmerone has anticancer activity, independent of curcumin against cervical cancer [12-13-38-39]. The third component in this extract was the Curcuma polysaccharides which are normally present in a holistic extract. Recently immunomodulatory and antimutagenic activities have been demonstrated with the polysaccharides [11-13-15] and the extract was standardised to contain all 3 components. HPV specific anticancer activity of curcuma extracts has been reported in *in vitro* and clinical studies [13-25-36].

Serum IL-6 level: Serum IL-6, which is a proinflammatory and pro-carcinogenic biomarker was reduced significantly after treatment with TOE in earlier study [9]. In the present study there was no significant reduction. It is known that circulating cytokine levels may not be increased in all cases of LSIL or HSIL [30]. In the present study it increased in 1 case. It was increased at 10 weeks probably due to the stress factor [40] because her Pap smear remained negative and there was positive history of stress in her home. It is possible that IL-6 is a more constant biomarker for invasion and metastasis [28-30], nevertheless it is likely to be attenuated by holistic Turmeric extract and this may contribute to the arrest of or regression of early carcinogenesis.

Limitations of the study: i) The study was conducted in low grade precancer (LSIL) cases which have a high spontaneous regression rate in young women [22], however this treatment succeeded in arresting or reversing 100% of cases who were above 30 years of age in both our studies and this is reassuring. ii) In this study cases did not undergo a biopsy however they underwent colposcopy and had 3 carefully collected Pap smears, within 3 months, which were negative for HSIL or cancer. Sixteen cases also came for at least one follows up Pap smear, and all remained regressed. iii) The conclusion is limited to LSIL cases. Nevertheless, it is noteworthy that the number of women with an LSIL or Atypia diagnosis in Pap smears is several times larger than the number of women with high grade cytological abnormality. If we address the carcinogenesis at this very early stage a large number of women can be helped by this preventive strategy and fewer women are likely to develop the high-grade abnormality. All women with an HPV positive report or abnormal Pap smear are required to undergo follow up screening as per the updated guidelines. iv) Due to inherent difficulties in the clinical setting with patients from poor socio-economic background and limited funds we could not analyse multiple mechanisms of action of the bioactive extract in these cases. Our primary aim was to establish safety and activity of the integrated therapy because whilst there is prolific data in experimental studies to show the multifaceted mechanisms of actions of *Curcuma longa* extracts the translation of experimental studies to clinical program requires consideration of the multifactorial carcinogenesis and progression along with feasibility of implementation.

In conclusion, integrative non-invasive method with antimicrobials and standardised turmeric extracts has a great potential in cervical cancer chemoprevention and can be evaluated in further clinical studies. A dose adjustment for curcumin in the bioavailable LSCP form can possibly minimize local side effects so that most women can complete the course of treatment.

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Note

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Ethical aspects & Conflict of Interest

The study was approved by an Independent ethics committee and was registered with CTRI, ICMR. Dr Lal Hingorani is Executive Director of a Pharmaceutical company and collaborated in development and standardization of the formulation, and supply, but was not involved in any other aspects of the study. None of the other authors had any conflict of interest.

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