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## RESEARCH COMMUNICATION

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# Response of Cervical Intra-epithelial Lesions to Vitamin E Supplementation - A Preliminary Report

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### Abstract

Carcinoma of the uterine cervix is preceded by well characterized pre-cancerous lesions which if left untreated may progress to invasive carcinoma. In the present study women in the age group of 35-55 years with cervical intraepithelial lesions (CIN I & II) were treated with vitamin E and advised to come for follow up after every three months for one year. This preliminary report shows vitamin E can restrict and regress CIN I & II lesions with elevation in circulating vitamin E levels. Improvement in immune status as reflected in mitogenic responses of peripheral blood lymphocytes was also noted.

**Key words:** Cervical intraepithelial neoplasia - vitamin E - chemoprevention

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### Introduction

Numerous epidemiological studies have strongly suggested that high intake of food rich in antioxidant vitamins ascorbic acid, carotenoids and tocopherols may reduce cancer risk. However assessment based on epidemiological data concerning antioxidant vitamins revealed that with respect to vitamin E, reduced cancer risk was associated with supplemental vitamin E rather than that obtained from dietary sources (Machlin,1995).

Vitamin E was shown to be associated with reduced risk for cancer at several sites including the prostate, lung, colorectum and oral cavity (Heinonen et al,1998, ABCP Study Gr.,1994, Benner et al,1993) but to date no reports are available with respect to uterine cervix which is the second most common female cancer representing approximately 15% of all neoplasms.

Cancer of the uterine cervix accounts for 26% of female cancers in India and about 90,000 Indian women develop the disease annually (ICMR NCR Ann.Rep.1998). At the tissue level, the onset or progression of neoplasia is initially

confined to the intraepithelial compartment when it has not yet become invasive (cervical intraepithelial neoplasia or CIN). The histopathological classification of CIN into I, II, and III and the increasing age at diagnosis with CIN grade imply that CIN has the potential to progress in severity and lead to invasive cancer (Syrjanen,1992). In the last decade human papilloma viruses (HPVs) have emerged as the principal etiological factor in cervical cancer and cervical intraepithelial neoplasia (Schiffman,1995). But some other co-factors such as antioxidants, micronutrients, nutritional and immunological status may also modulate the progression of HPV infection. In case-control and cohort studies, inverse associations between plasma carotenoids, ascorbate, alpha-tocopherol and risk for CIN and cancer have been observed (Potischman & Brinton 1996, Manetta et al ,1996). Palan et al (1996) reported a trend of lower plasma carotenoids and tocopherol levels in CIN and cervical cancer patients which suggests that deficiency of antioxidant vitamins may have a role in pathogenesis of the disease. It has been reported that persistence of HPV infection is important in the etiology of cervical carcinoma (Koutsky et

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al,1992) The significant inverse association of some vitamins with CIN and cervical cancer may be related to HPV persistence.

CNCI is conducting a prospective case control study, perhaps the first of its kind, to clinically assess the chemopreventive role of antioxidant vitamins in cancer of the uterine cervix. The study is conducted with approval from the institute's ethical committee and with informed consent from the subjects recruited. The present communication furnishes a preliminary report of intervention treatment of CIN I and II cases.

### Subjects and Treatment

Women in the age group of 35-55 years diagnosed following screening were recruited. The screening procedure include clinical examination, PAP test, colposcopy and histopathological confirmation.

Confirmed subjects were randomly assigned to two groups. One group received oral vitamin E supplementation (Evion 400 capsules, Merck, tocopherol acetate 400mg), every day continuously for one year, while the other group was maintained on placebo with close follow up. All were advised to report for check up at 3, 6 and 12 months of recruitment. Lesions showing progression to higher grades were treated by conventional methods.

The clinical response data was statistically evaluated using Log rank test.

### Determination of Plasma Vitamin E Level and Mitogenic Stimulation of PBL

Plasma levels of vitamin E, before and after treatment with supplemental vitamin E, were determined by reverse phase HPLC (Waters, Millipore) and UV detection at 320 nm. Mitogenic response of peripheral blood lymphocytes were assessed *in vitro* using phytohaemagglutinin (PHA M, Gibco).

Statistical analysis of vitamin levels was made by Wilcoxon Composite Rank Sum Test and mitogenic stimulation by Student's t test.

## Results and Discussion

Among the 122 patients, 88 cases were diagnosed with CIN I lesions and the remaining 34 had CIN II lesions. 6 of the CIN I cases were lost due to non compliance. As shown in Table 1, in the first group (CIN I) regression of lesion was highly significant (73.58 %  $P < 0.005$ ) as compared to the placebo where 71.42 % of the lesion remained unaltered. In the second group (CIN II) also significant regression was obtained ( $P < 0.025$ ) although higher non-responsive lesions were noted. It is possible that continuation of supplemental vitamin may result in complete regression in both groups. Since generally the progression of CIN lesions from lower to higher grades is a slow process and occur over a period of 8-10 years if left untreated, longer treatment may be required to ensure complete regression. The feasibility and cost-benefit of this approach for prophylaxis of cancer cervix can be ascertained only after further study which is in progress.

In an earlier study (Ganguly et al 1999) we reported that the serum AST and ALT, protein profile and lipid profile, estimated using biochemical assay kits (E Merck), were found to remain within the normal range following continuous treatment with vitamin E for upto one year, suggesting no adverse effects on general health and liver function .

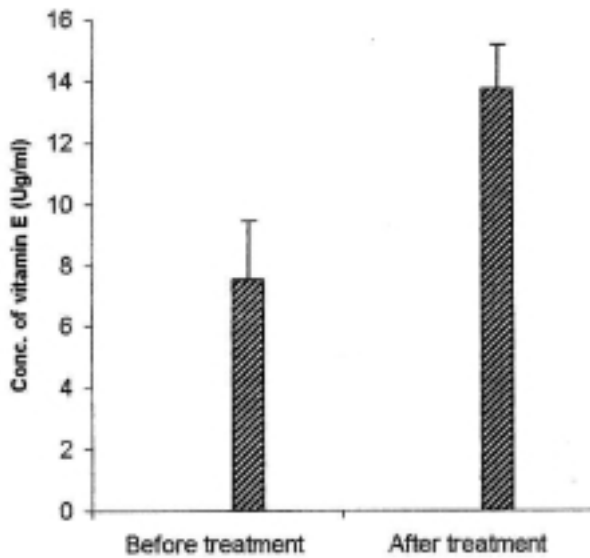
Since the difference in plasma alpha tocopherol (vitamin E) level between CIN I and CIN II were marginal, the values were taken together for representation (Fig 1)

Circulating level of vitamin E (alpha tocopherol) in CIN patients was found to be on the lower side of the normal range (5-14 ug/ml); supplementation significantly elevated the level ( $P < 0.005$ ). Low levels of antioxidant vitamins have been associated with cervical dysplasia and considered to be one of its risk factors along with persistent HPV infection (Giuliano et al,1997). We had also noted low plasma vitamin A and E in both CIN and cancer cervix patients (unpublished data). Marginal improvement in PBL response towards PHA, which is an indicator of cell mediated immunity, was noted following treatment with vitamin E (Fig.2). Several studies have indicated an immune

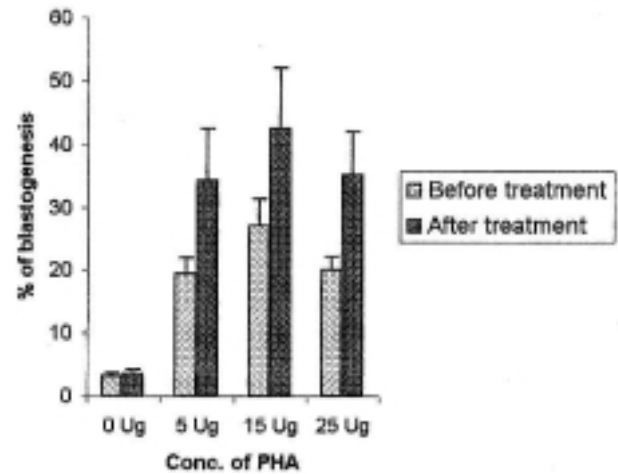
**Table 1. Response of Cervical Intraepithelial Lesions to Vitamin E Supplementation.**

Outcome of treatment	CIN I*		CIN II**	
	Number of cases (%)		Number of cases (%)	
	PL n=35	vit E n=53	PL n=15	vit E n=19
Regression	6(17.14)	39(73.58)	4(26.66)	7(36.84)
Progression	1(2.85)	1(1.8)	3(20.0)	1(5.26)
No Response	25(71.42)	10(18.86)	8(53.33)	11(57.8)

\*  $P < 0.005$     \*\* $P < 0.025$



**Figure 1. Levels of Vitamin E in Plasma of CIN Patients Before and After Treatment with Vitamin E.**



**Figure 2. PBL Response Towards Mitogenic Stimulation Before and After Treatment with Vitamin E.**

stimulating action of this vitamin (Meydani,1995).

The regression of preneoplastic lesion of the cervix observed in the study may be attributed to the varied biological actions of the vitamin. Vitamin E may serve to protect cellular membranes from damage and prevent or reduce HPV infection which is a risk factor for cancer cervix. It would be of interest to correlate the level of vitamin E with the prevalence of HPV in lesions prior to and following vitamin supplementation. Alternatively the vitamin may help to reduce inflammation and infections in the cervix by improving the general body resistance by the immune system. Further studies are in required to clarify the role of vitamin E in chemoprevention of cervix cancer.

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**Personal Profile: Chaiti Ganguly**

Dr. Chaiti Ganguly, a Ph.D. of the Calcutta University, had been engaged in Cancer Research at the Chittaranjan National Cancer Institute, Kolkata, since 1986 when she joined as a Junior Research Fellow. Her field of specialization is Cell and Molecular Biology and her area of research interest include Cancer Chemoprevention.

Currently, Dr. Ganguly is abroad, working in the Dept. of Haematology and Oncology of Duke University Medical Center, Durham, North Carolina, USA.