

Serum vitamin E levels in carcinoma of the cervix: a risk factor

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Abstrak

Penderita dengan kadar α -tocopherol yang rendah mempunyai risiko kanker yang lebih tinggi dibandingkan dengan mereka yang kadarnya lebih tinggi. Telah dilakukan studi kelola untuk mengevaluasi fakta ini pada penderita karsinoma serviks dan mengusulkan pedoman untuk mendesain percobaan lebih lanjut. Dilakukan pemeriksaan kadar vitamin E serum dengan spektrofotometri pada 50 wanita normal dan 50 penderita karsinoma serviks yang telah terbukti secara histologis. Kadar vitamin E serum rata-rata pada penderita karsinoma serviks ialah $9,17 \pm 2,89 \mu\text{g/ml}$. Hal ini lebih rendah secara bermakna ($p < 0,05$) dibandingkan kelola sehat ($11,39 \pm 4,39 \mu\text{g/ml}$). Kadar vitamin E serum yang rendah merupakan faktor risiko yang pasti bagi karsinoma serviks.

Abstract

Individuals with a low level of α -tocopherol are at increased risk of cancer as compared to those with a higher level. We conducted a case control study to evaluate this fact in patients with carcinoma of the cervix and to propose guidelines for designing further trials. Serum vitamin E levels were estimated in 50 normal women and 50 patients of histologically proven carcinoma of the cervix by spectrophotometry. Mean serum vitamin E level in patients with carcinoma of the cervix was $9.17 \pm 2.89 \mu\text{g/ml}$. This was significantly lower ($p < 0.05$) than in healthy controls ($11.39 \pm 4.39 \mu\text{g/ml}$). Reduced serum vitamin E levels are a definite risk factor for carcinoma of the cervix.

Keywords : carcinoma, cervix, tocopherol

INTRODUCTION

Cancer of the uterine cervix is the commonest cancer of an individual organ in India. Over ninety percent of these tumours are squamous cell type. Approximately seven percent to ten percent are classified as adenocarcinomas and one percent to two percent are clear cell, mesonephric type.¹ No definite tumour markers have yet been identified for this tumour.

The association between serum alpha tocopherol levels and the subsequent incidence of cancer was investigated in a longitudinal study of 21,172 men in Finland.² The mean levels of serum alpha tocopherol among cancer cases and controls was $8.02 \mu\text{g/ml}$ and $8.28 \mu\text{g/ml}$ respectively. A high serum alpha tocopherol was thus associated with a reduced risk of cancer. The risk varied for different sites and was strongest for some gastrointestinal cancers and for the combined group of cancers unrelated to smoking.² Vitamin E was also found lower in all patients of leukemia and lymphoma as compared to normal healthy

adults.³ Vitamin E supplementation reduced the rate of tumour growth, improved host survival and elevated serum vitamin E levels.³ Serum vitamin E level in breast cancer patients ($n=39$) was $4.7 \mu\text{g/ml}$ compared to $6.0 \mu\text{g/ml}$ in healthy controls ($n=78$).⁴ The difference was statistically significant ($p < 0.025$). High serum levels of vitamin E were related to low oral cancer risk and there is definite role of vitamin E in the chemoprevention of these malignancies.⁵

We conducted a case control study to evaluate the role of tocopherol as a risk factor for the carcinoma of the cervix in Indian setting.

METHODS

The present study was carried out in a total of 100 females. Fifty of them were normal healthy controls and fifty were histopathologically proved cases of carcinoma of the cervix admitted or attending outdoor at our institute.

Control group

This comprised of 50 healthy, nonsmoking controls. Most of them were attendants accompanying patients

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in the Radiotherapy Department. Effort was made to match the controls with patients of carcinoma of the cervix with respect to age, socioeconomic and nutritional status. Any chronic illness was ruled out in controls.

Blood sample collection

About 7 ml of venous blood was collected from the antecubital vein under aseptic conditions. The blood was allowed to clot and then the serum separated by centrifugation. Vitamin E was estimated within 24 hours.

Estimation of serum vitamin E

Serum was tested for vitamin E by the method described by Duggan.⁶ *Principle:* after precipitation of serum proteins with ethanol, the vitamin is extracted from the serum into hexane. Free α -tocopherol is exposed to ultraviolet light at 295 nm and fluorescence is measured at 330 nm. *Reagents used:* 1) hexane (distilled): - one litre of hexane and one litre of concentrated H_2SO_4 (sulphuric acid) were mixed in a separating funnel. Hexane was separated and treated with one litre of alkali (NaOH: sodium hydroxide) and mixed. Hexane was again separated and treated with one litre of distilled water. Distilled 1 litre of reagents grade solvent discarding the first 25 ml and the last 100 ml at 60°C. (2) ethanol (distilled):- distill 1 litre over 20 mg of KOH discarding the first 25 ml and the last 100 ml. (3) water (double distilled): - distill deionised water or redistill water in a glass distillation apparatus. (4) stock vitamin E standard 2500 $\mu\text{g/ml}$: - dissolve 375 mg/dl α -tocopherol in 5 ml of distilled ethanol and dilute to 10 ml/dl α -tocopherol was obtained from Sigma-Chemicals (1 gm contained 670 mg). So stock has $37.5 \times 0.67 = 25 \text{ mg}/10 \text{ ml} = 2500 \text{ mg/ml}$ concentration. It is stable for 6 months at 4°C. (5) working vitamin E standard 25 $\mu\text{g/ml}$: - dilute 1 ml stock standard to 100 ml with distilled ethanol. This standard is stable for 1 month in a refrigerator. *Procedure:* - three glass stoppered 10 ml centrifuge tubes were set up and labelled as T (test), S (standard) and B (blank) (Table 1).

Hexane layer was transferred to quartz cuvette Spectrophotofluorometer was set with the activation wavelength at 295 nm and emission wavelength at 330 nm. The relative fluorescence of the blank (Fb), standard (Fs) and unknown (Ft) were read and calculated as

$$\mu\text{g free vitamin E/ml} = \frac{F_t - F_b}{F_s - F_b} \times 25$$

Table 1. Estimation of vitamin E

	B	S	T
Distilled water	0.2 ml	-	-
Working standard	-	0.2 ml	-
Serum	-	-	0.2 ml
Distilled water	1 ml	1.2 ml	1 ml
	(mixed all tubes manually for 30 secs.)		
Distilled ethanol	2 ml	1.8 ml	2 ml
	(mixed all tubes manually for 30 secs.)		
Distilled hexane	5 ml	5 ml	5 ml
	(mixed all tubes manually for 30 secs.)		

B=blank, S=standard, T=test

Precautions: all glassware used for the test were soaked at least for one hour in 8N nitric acid, rinsed thoroughly with double distilled water and dried.

The results were statistically evaluated and differences in the two groups were tested by student's t-test for statistical significance.

RESULTS

The present study was carried out in a total of 100 persons. Fifty of them were normal healthy controls, rest fifty were histopathologically proved cases of carcinoma of the cervix.

Age distribution

- i) Maximum patients were in the age range of 40-49 years: 19 (38%) patients. Healthy controls were matched for age with the patients of both the groups as shown in Table 2.

Table 2. Age distribution

Age (years)	Control group	Patient group
30-39	6 (12%)	4 (8%)
40-49	18 (36%)	19 (38%)
50-59	12 (24%)	12 (24%)
60-69	10 (20%)	10 (20%)
70-79	4 (8%)	5 (10%)
Total	50	50

Stage distribution

Eighteen (36%) patients presented in stage IIb and 19 (38%) presented in stage IIIb. On the other hand, only 7 (14%) patients presented in IIa, 3 (6%) patients presented in IIIa, and only 1 (2%) patient presented in

stage IV disease. In all, 37 (74%) presented in stage IIb and IIIb taken together. Only 13 (26%) presented in the other stages as shown in Table 3.

Table 3. Stagewise distribution

Stage	Patient group
Ia	
Ib	2 (4%)
IIa	7 (14%)
IIb	18 (36%)
IIIa	3 (6%)
IIIb	19 (38%)
IV	1 (2%)
Total	50

Investigations

Investigations done in all the patients are shown in Table 4 including haemoglobin, TLC, serum cholesterol and serum calcium.

Table 4. Patients investigations (range/percentage)

Sr.No.	Investigations	Patient group
1.	Haemoglobin (gm%)	3.8-12.8
2.	TLC (Cumm)	3600-12400
3.	S.Cholesterol (mg%)	145-280
4.	S.Calcium (mg%)	7.0-11.0
5.	Histopathology	
	(i) MDSCC	32 (64%)
	(ii) SCC	7 (14%)
	(iii) PDSCC	6 (12%)
	(iv) WDSCC	4 (8%)
	(v) PDASCC	1 (2%)

MD = Moderately differentiated

PD = Poorly differentiated

PDA = Poorly differentiated adeno

SCC = Squamous cell carcinoma

TLC = Total leucocyte count

WD = Well differentiated

Comparison of serum vitamin E levels in control group and patients

Mean serum vitamin E level in 50 patients of carcinoma of the cervix at presentation was 9.17 ± 2.89 $\mu\text{g/ml}$. This was statistically significantly lower ($p < 0.05$) as compared to that found in healthy controls (11.39 ± 4.39 $\mu\text{g/ml}$).

Stagewise variation of serum vitamin E levels

Mean serum vitamin E levels in patients 13.87 ± 6.54 $\mu\text{g/ml}$ for stage Ib, 8.78 ± 3.45 $\mu\text{g/ml}$ for stage IIa, 8.64 ± 2.17 $\mu\text{g/ml}$ for stage IIb, 6.23 ± 0.42 $\mu\text{g/ml}$ for stage IIIa and 10.1 ± 2.6 $\mu\text{g/ml}$ for stage IIIb. Range of vitamin E levels in all the 50 patients is given stagewise in Table 5.

DISCUSSION

In our study, the patients were in the age group of 40-49 years as has been reported in the literature.⁷

All the patients were symptomatic at the time of presentation. The most common complaint was bleeding per vaginum associated with the watery discharge. Patients also complain of low back pain (3 i.e. 6%), abdominal pain (4 patients i.e. 8%), retention of urine and decreased amount of urine (1 patient i.e. 2% each). This agrees with the literature observed till date.⁷

Eighteen (36%) patients presented in stage IIb and 19 (38%) presented in stage IIIb. On the other hand, only 7 (14%) patients presented in IIa, 3 (6%) patients presented in IIIa, and only 1 (2%) patient presented in stage IV disease. In all, 37 (74%) presented in stage IIb and IIIb taken together. Only 13 (26%) presented in the other stages.

Haemoglobin, TLC, DLC, serum cholesterol, serum calcium and histopathological type of cancer were recorded for all the patients. The commonest histological type was squamous cell carcinoma i.e. 98% of the

Table 5. Stagewise variation of pre-radiotherapy serum vitamin E levels

number of cases = 50

Serum levels of vitamin E ($\mu\text{g/ml}$)	F180 stage				
	Ib	IIa	IIb	IIIa	IIIb
Mean	13.87 ± 6.54	8.78 ± 3.45	8.64 ± 2.17	6.23 ± 0.42	10.1 ± 2.6
Range	9.25-18.5	4.5-15	5.2-12.5	5.9-6.7	6.4-15

patients, as has been found in the literature.⁶ Only one patient (2%) had a poorly differentiated adeno-squamous carcinoma (Table 4).

Stagewise variations of serum vitamin E and MD levels

Stagewise variation of serum vitamin E levels revealed a decreasing trend from stage Ib to IIIa. However patients of carcinoma of the cervix stage IIIb had higher values than stage IIa, IIb and IIIa patients. We could not find any correlation of serum vitamin E levels with the stage of cancer of the cervix.

The serum vitamin E levels were measured by spectrofluorometry.⁶ The mean serum vitamin E level in the control group was $11.39 \pm 4.39 \mu\text{g/ml}$ by this method. Henson et al reported vitamin E level to be $10.34 \pm 0.36 \mu\text{g/ml}$ in normal healthy population.⁹ Our findings correlate well with their results. We have observed statistically significant lower value of serum vitamin E in all the fifty patients at presentation, i.e. before radical radiotherapy as compared to the healthy control group ($p < 0.05$). The patients of carcinoma of the cervix had a mean serum vitamin E level of $9.17 \pm 2.89 \mu\text{g/ml}$ in our study. Serum vitamin E has thus, emerged as a risk factor for carcinoma of the cervix.

The evidence from this prospective study is significantly strong to justify further studies with antioxidants like vitamin E so that strategies for chemoprevention of cancer may be finalized.

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