Effect of Radiotherapy on Antioxidant Vitamin E in Patients with Carcinoma Uterine Cervix- a Pilot Study

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**ABSTRACT**

Vitamin E, a fat-soluble antioxidant, stops the production of Reactive Oxygen Species (ROS) during the process of fat oxidation. It is used to prevent and repair normal tissue damage and to increase the susceptibility of tumor tissue to radiation treatment. In this study, we analyzed the serum antioxidant vitamin E, before, midway and at the completion of radiotherapy and monthly follow-up for six months and monitored the respective responses to radiotherapy in 33 patients histopathologically confirmed of carcinoma uterine cervix. Statistically significant low levels of serum vitamin E were observed before radiotherapy. Results indicate that decreased antioxidant vitamin E depicts the progression of carcinoma uterine cervix disease. The serum vitamin E analysis as per the stage of the disease is also in agreement with this and at six months follow-up, the mean serum vitamin E levels were found to be higher in IIA, IIB and III stages of carcinoma uterine cervix.
INTRODUCTION

Cancer of the uterine cervix is the second most common cancer among women worldwide. There have been a number of risk factors; however, the etiology of the disease is not clearly understood.

As far as control of the disease is concerned it rests on early detection of the disease through screening by the Pap smear followed by treatment by radical surgery and/or radiotherapy. Currently, radiation therapy is one of the most standard and effective modalities for the treatment of cervical cancer. Surgery is the choice of treatment for the early stage cervical cancer [1, 2], however, radiotherapy remains the most commonly used modality of treatment of choice for patients unwilling for surgery and inoperable cervical cancers. [3] Further, a better understanding of the response of tumors to ionizing radiation might potentially lead to an improvement in tumor control and patient morbidity [4].

Under normal physiological conditions, cells are capable of counterbalancing the production of reactive oxygen species (ROS) with antioxidants. One of the most important ROS is the hydroxyl radical, which is generated by ionizing radiation either directly by the oxidation of water, or indirectly by the formation of secondary partial ROS [5]. Antioxidant vitamin E thought to protect the body against the destructive effect of these radicals by donating one of their own electrons, ending the electron-stealing reaction [6]. The reaction of Vitamin E with damaging free radicals results in the formation of a tocopheryl radical and gets reduced. Vitamin E themselves do not become free radicals by donating an electron because they are stable in either form. It can get incorporated into cell membranes as it is fat soluble and hence protect the cells from oxidative damage. They may also modulate angiogenesis and cancer cell proliferation, induced apoptosis, up-regulate expression of tumor suppressor genes and downregulate oncogenes [7, 8].

Vitamin E, a potent peroxyl radical scavenger, is a chain-breaking antioxidant that prevents the propagation of free radicals in membranes and in plasma lipoproteins [9, 10]. When peroxyl radicals (ROO•) are formed, these react 1000-times faster with vitamin E (Vit EOH) than with polyunsaturated fatty acids (PUFA) [9, 11]. The hydroxyl group of tocopherol reacts with the peroxyl radical to form the corresponding lipid hydroperoxide and the tocopheryl radical (Vit E-O•). The tocopheryl radical (Vit E-O•) reacts with vitamin C (or other hydrogen donors, AH), thereby oxidizing the latter and returning vitamin E to its reduced state [9, 12].

Free radical scavenging reactions of α-tocopherol take place via the α-tocopheryloxyl radical as an intermediate. If a suitable free radical is present, a non-radical product can be formed from the coupling of the free radical and ∞-tocopherol radical. The lipid peroxidation mechanism by α, γ and δ tocopherols are almost the same [13].

The effects of radiotherapy on antioxidant vitamin and their association with the oxidative stress are known to a much lesser extent. Therefore the improved knowledge on the antioxidant vitamin and the impact of radiotherapy could be useful in managing patients with malignancy. Thus, in the present study, we have assessed an extensive profile of serum antioxidant vitamin E before radiotherapy and monitored and evaluated the change in Vitamin E levels with radiotherapy in carcinoma of the uterine cervix during the treatment and subsequent follow-up.
MATERIALS AND METHODS

The study was conducted on 33 patients with histopathologically confirmed carcinoma of the uterine cervix attending the Radiotherapy Department for radiotherapy. 25 age-matched healthy females who did not have cervical cancer were selected as a control group. The control subjects included in this study were asymptomatic female individuals free from any abnormality on routine examination, not on antioxidant supplementation, and were free from any disease. Blood samples were collected once from these women and serum Vitamin E levels were analyzed. Blood samples were obtained from 33 uterine cervix cancer patients (study group) with an average age of 45.0 years (ranging from 21 to 70 years).

Patients were classified according to the International Federation of Gynecologists and Obstetricians (FIGO) classification for cancer of the uterine cervix. The patients in this study were IIA, IIB and III stages of cervical cancer. All the patients were treated by External Beam Radiation Therapy (EBRT) using cobalt-60 Teletherapy unit with a dose prescription of 50 Gy in 25 fractions by rectangular anterior and posterior fields. Blood samples were collected and analyzed for serum vitamin E (Vitamin E, reference range 0.8-1.2 mg/dl) by using bathophenanthroline (Baker and Frank, 1968; modified by Frbianek et al, 1968) in all the patients before EBRT (BT), mid-way of EBRT (MT) (30 Gy tumor dose), at completion of EBRT (CT) (60-70 Gy tumor dose) and during subsequent monthly follow-up to 6 months.

All values are expressed as mean values with standard deviation (SD). The observation was compared with Student’s t-test and P values <0.05 were considered to indicate statistical significance.

RESULTS

In the present study, the mean serum vitamin E levels were found to be 1.09 ± 0.29 mg/dl in control subjects. The mean serum vitamin E level in study group was 0.55 ± 0.23 mg/dl at BT, which increased to 0.76 ± 0.22 mg/dl at CT and 0.89 ± 0.17 mg/dl at 6 months follow-up (table 1). The levels of vitamin E were found to be significantly low at BT in the study group as compared to the control group. The mean level of serum vitamin E in control and study group is depicted in fig.1.

Further table 2 shows the comparison of vitamin E levels in carcinoma uterine cervix according to the stage of the disease. The maximum rise of vitamin E level at 6 months follow-up was seen in stage IIA disease of study group as compared to the control group. In stage IIA the level of vitamin E increased by 42.4% as compared to BT, followed by 39.3% in stage III and 29.9% in stage IIB group and the rise in stage III was 39.3 which is statistically significant(p<0.001).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>BT</th>
<th>MT</th>
<th>CT</th>
<th>1M</th>
<th>2M</th>
<th>3M</th>
<th>4M</th>
<th>5M</th>
<th>6M</th>
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<tr>
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<td>0.55</td>
<td>0.68</td>
<td>0.76</td>
<td>0.65</td>
<td>0.68</td>
<td>0.72</td>
<td>0.75</td>
<td>0.80</td>
<td>0.89</td>
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<tr>
<td>SD</td>
<td>0.29</td>
<td>0.23</td>
<td>0.24</td>
<td>0.22</td>
<td>0.17</td>
<td>0.15</td>
<td>0.18</td>
<td>0.14</td>
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<tr>
<td>T</td>
<td>7.90</td>
<td>5.87</td>
<td>4.88</td>
<td>4.88</td>
<td>7.13</td>
<td>6.98</td>
<td>5.73</td>
<td>5.00</td>
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<td>P</td>
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<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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[Table 1: Statistical analysis of mean serum vitamin 'E' levels [mg/dl] in carcinoma uterine cervix]
[Table 2]

<table>
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<tr>
<th></th>
<th>BT II A</th>
<th>BT II B</th>
<th>BT III</th>
<th>6 Month Follow Up</th>
<th>6 Month Follow Up</th>
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<td>Mean</td>
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<td>0.68</td>
<td>0.51</td>
<td>0.92</td>
<td>0.97</td>
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<tr>
<td>SD</td>
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<td>0.22</td>
<td>0.17</td>
<td>0.18</td>
<td>0.14</td>
</tr>
<tr>
<td>t</td>
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<td></td>
<td></td>
<td>2.28</td>
<td>2.67</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>% (↑/↓)</td>
<td>42.4↑</td>
<td>29.9↑</td>
<td>39.3↑</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[Table-2: Comparison of vitamin ‘E’ levels [mg/dl] in carcinoma uterine cervix according to stage of disease]

[Figure 1]

[Fig.1: Vitamin E level [mg/dl] in the patients of carcinoma uterine cervix before therapy (BT), mid-therapy (MT), completion of therapy (CT) and at monthly follow-up for six months (Values of Vitamin E represents mean).]
DISCUSSION

Vitamin E consists of at least four tocopherols. Further, it has been reported that Vitamin E has been shown to decrease radiation–induced chromosome damage in human tumor cells but not in normal cells and has an inhibitory effect on a variety of cancer cells \cite{15,16}.

In the present study, statistically significant low level of serum vitamin E (0.55 ± 0.23 mg/dl, p<0.001) was observed at the start of therapy as compared to control group (1.09 ± 0.29mg/dl). The pretreatment low levels of vitamin E in cervix cancer patients were also reported by various workers, \cite{17} have studied the circulating lipid peroxide, antioxidant component and the activities of defense enzymes in patients with carcinoma, uterine cervix (before and after radiotherapy and radiotherapy combined with chemotherapy) and compared with controls. They found that some of the antioxidant components such as glutathione, vitamin E and selenium are reduced in cancer cervix patients. Further, they observed that reduced levels of vitamin E and glutathione normalized after the completion of treatment. These findings are in support of the present study with regard to vitamin E levels and the decreased antioxidants may be one of the factors responsible for the progression of carcinoma uterine cervix.

Ahmed M et al (1999) \cite{17} have reported that the serum vitamin E was diminished in carcinoma uterine cervix patients. They suggest the oxidative stress in the form of elevated levels of lipid peroxides together with impaired antioxidant defense mechanism may play a role in the etiology and progression of cervical carcinoma. Gitanjali G et al (1999) \cite{19} have observed the reduced serum vitamin E in cancer cervix patients in comparison to control. Further, they found that serum vitamin E levels in the patients did not correlate with oral supplementation of vitamin E. Palan P R et al (1996) \cite{20} observed that alpha-tocopherols and gamma-tocopherols were significantly lower in patients with various grades of cervical intraepithelial neoplasia and carcinoma uterine cervix. Ismail MS et al (2010) \cite{23} evaluated the prophylactic effect of a mixture of antioxidants and observed that the antioxidant group showed significantly lower levels of apoptosis and lipid peroxides as compared to patients who did not receive antioxidant supplementation. Karamali M et al (2015) \cite{24} studied the effects of long-term Selenium administration on the regression and metabolic status of patients with cervical intraepithelial neoplasia grade 1 and favorable results were observed.

There are few studies on FIGO stage wise measurement of vitamin E levels in carcinoma uterine cervix patients (Manoharan S et al (2004) \cite{21}; Ahmed M et al (1999) \cite{17}; Manju V et al (2002) \cite{22}). In the present study the mean serum vitamin E levels in stage IIA, IIB, and III group were 0.53 ± 0.31mg/dl, 0.68 ± 0.22mg/dl and 0.51 ±0.17mg/dl respectively at the start of therapy. The levels of vitamin E increased by 42.4 % in stage IIA group, by 39.3 % in stage III group and by 29.9 % in stage IIB group at 6 months follow-up. The results indicate that the maximum rise in the level of vitamin E was observed in stage IIA. The rise in mean serum E concentration in carcinoma uterine cervix patients after therapy can be attributed to the decrease in oxidative stress due to improvement in disease. Ahmed M et al (1999) \cite{17} observed vitamin E level in membranes of carcinoma uterine cervix patients. In stage I patients, membrane vitamin E remained unaltered, however, the vitamin showed a progressive decrease in stage II, III and IV.
CONCLUSION

It is concluded that the rise in mean serum vitamin E concentration in carcinoma uterine cervix patients after therapy can be because of decrease in the oxidative stress due to improvement in disease. The vitamin E deficiency in carcinoma uterine cervix patients could have occurred by the increased requirement of this antioxidant to combat the disease conditions and thus supplementation of these antioxidant vitamins may help in improved outcome following treatment of malignancy. An elaborate monitoring of carcinoma uterine cervix cases incorporating more number of patients will enable better statistical analysis.

DECLARATION OF CONFLICTING INTEREST

The authors have no conflicts of interest (political, personal, religious, ideological, academic, intellectual, commercial or any other) to declare in relation to this manuscript.

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REFERENCES


