RESEARCH ARTICLE

Status of Oxidative Stress and Antioxidant Levels in Smokers with Breast Cancer from Western Nepal

T Nagamma1*, Jalaj Baxi2, PP Singh3

Abstract

**Background:** Research indicates that oxidative stress induced by smoking plays a role in breast cancer. In view of these reports, we aimed to study the relationship between smoking and oxidative stress in breast cancer patients from the western region of Nepal. **Materials and Methods:** The study included a control group of 42 females (non-smoking healthy women) and a test group subdivided into Group I consisting of 46 female breast cancer patients who were smokers and Group II consisting of 42 non-smoking breast cancer patients. Detailed history of the patients was collected with the help of pre-test proforma. Plasma levels of malondialdehyde (MDA), total antioxidant activity (TAA) which represents total dietary antioxidants, vitamin C and α-tocopherol were estimated by standard methods. Statistical analysis was done using SPSS version 16. **Results:** The plasma MDA, TAA, vitamin C and α-tocopherol were 1±1.4nmol/ml, 918±207µmol/L, 1±0.24mg/dL and 0.94±0.31mg/dL in controls, 5±1.2nmol/ml, 458±166µmol/L, 0.64±0.32mg/dL and 0.5±0.3mg/dL in Group-I and 2.56±1.2nmol/ml, 663±178µmol/L, 0.78±0.2mg/dL and 0.77±0.2mg/dL in Group-II, respectively. Vitamin C, α-tocopherol and TAA (p=0.001) were significantly reduced whereas MDA (p=0.001) was significantly raised in Group-I when compared to controls and Group-II. **Conclusions:** We observed a significant rise in oxidative stress and low levels of antioxidants in breast cancer patients with smoking habit. It is well known that free radicals facilitate the progression of breast cancer, possibly increasing the risk of progression to the next stage.

**Keywords:** Oxidative stress - antioxidants - breast cancer - women smokers - Western Nepal

Introduction

Breast cancer is the second most common cancer in Nepal. The major risk factors contributing to breast cancer are menarche at early age, having first pregnancy at late age, obesity, lifestyle changes and family history. Smoking is one of the major lifestyle risk factor. The tobacco leaf contains about 7000 toxic chemical species, 20 of which are established as mammary carcinogens (Reynolds et al., 2009). According to World Bank report 2011, 36% male and 29% female smokers in Nepal. The government spends 16 billion annually for patients with smoke related diseases. Many studies have reported that smoking is associated with increased risk of breast cancer (Band et al., 2002; Li et al., 2005; Reynolds et al., 2009; Luo et al., 2011). Smokers are more exposed to free radicals. These free radicals cause oxidative damage to lipids, proteins and DNA that can result in cancer. Oxidative stress is raised when the antioxidant network is weak. Several workers have also reported that tobacco users tend to consume mostly a diet relatively poor in antioxidants (Ayaori et al., 2000; Ching et al., 2001). Dietary antioxidants have protective role against breast cancer by decreasing oxidative stress and DNA damage produced by free radicals (Ambrosone, 2000; Poljak, 2011).

There is evidence that antioxidant may induce apoptosis in cancer cells and inhibit cell proliferation (Prakash et al., 2000; Borek, 2004). There are a number of studies which proves that carcinogens present in smoke are the major risk factor in the development of breast cancer (Band et al., 2002; Johnson et al., 2011; Gao et al., 2013). Another study suggested that smoke increased the risk of breast cancer in former smokers (Ilic MC et al., 2013).

The present study aimed to study the oxidative stress and antioxidant levels in smokers with breast cancer from Western Nepal.

**Materials and Methods**

This study was conducted in Manipal Teaching Hospital, Pokhara, Nepal. The study was approved by institutional research committee. This study included newly diagnosed breast cancer patients with and without smoking habit. They were divided in two groups.

Group-I: Included 46 newly diagnosed female breast cancer patients who were smokers and Group II consisting of 42 non-smoking breast cancer patients.

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cancer patients with smoking habit, smoking 3-6 (either cigarette or bidi) per day with mean duration of smoking was equal to 12±2 years.

Group-II: Forty two newly diagnosed non-smoking female breast cancer patients.

Controls: Forty two healthy female individuals without any smoking habit were included.

Detailed history of the patients was collected with the help of pre-test proforma. The proforma included, name, age, dietary habit (vegetarian/non-vegetarian), family history of disease, smoking habit, drinking habit, socio-economic status, community and occupation. BMI was calculated by using formula (BMI=Weight/Height in m²). Breast cancer patients who were having tobacco chewing habit, alcohol intake, antioxidant supplementation were excluded from the study.

Sample collection and biochemical analysis

Prior verbal consent was taken from all the subjects. Six ml of blood was collected from each subject by venipuncture with standard blood collection technique. Sample was centrifuged for 10 minutes at 3000rpm. Plasma was transferred to another labeled vial for immediate analysis or stored in deep freezie for analysis within 24 hours. The plasma was used for the estimation of thiobarbituric acid reacting substances (TBARS) (Buege and Aust, 1978), Total antioxidant activity (Benzie and Strain, 1996), Vitamin C (McCormic et al., 1999) and α-tocopherol (Baker and Frank, 1968) by standard methods.

Statistical analysis

Computer software program SPSS version 16 was used for the statistical analysis of different biochemical parameters for the interpretation of the result and reported as mean±SD. Data were processed for obtaining ‘p’ value of Independent Sample t-test and p<0.05 was considered statistically significant.

Results

Characteristics of the breast cancer patients (Group I and Group II) recorded in table. Patients in both groups were identical with reference to age and BMI. About 44 women presented themselves with 2nd stage of the disease. In Nepal breast cancer is the disease of middle, low socio-economic class population and postmenopausal women with smoking habit. In both groups most of the patients had their first child between 16-25y.

In Figure 1 the plasma TBARS level was significantly raised (p<0.001) in group I patients when compared with controls and group II. In group I vitamin C and α-tocopherol levels were significantly lower than controls (p<0.001) and group II (p<0.05).

In Figure 2 the TAA level in group I patients was significantly lower than the controls (p<0.001) and group-II patients (p<0.05).

Discussion

In spite of innumerable studies on breast cancer, causes of breast cancer is still unclear. In Table 1 BMI did not differ significantly in group I when compared to group II.

The most important factor which determines the best outcome is the early diagnosis and treatment. In our study 44 cases were diagnosed in 2nd stage. Health awareness and screening test help in early diagnosis of breast cancer. Our data shows that middle and low socio-economic status has high risk for breast cancer, due to lack of health awareness neither literacy nor health education, nor screening facilities. Pregnancy after 30 years of age is known to be a risk factor for breast cancer. In our study 28 patients of group I and 29 patients of group II thought they had their first child between 16-25 years of age. It shows that age at first child birth is not a risk factor in Neplai population. Another study suggests that there is a

<table>
<thead>
<tr>
<th>Table 1. Characteristics of the Breast Cancer Patients</th>
</tr>
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<tbody>
<tr>
<td>Cases</td>
</tr>
<tr>
<td>Smokers (n= 46)</td>
</tr>
<tr>
<td>Nonsmokers (n=42)</td>
</tr>
<tr>
<td>Age (y)</td>
</tr>
<tr>
<td>47.5±5.1</td>
</tr>
<tr>
<td>43.7±5.8</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
</tr>
<tr>
<td>20.2±3.1</td>
</tr>
<tr>
<td>22.4±3.1</td>
</tr>
<tr>
<td>Tumor in Left breast</td>
</tr>
<tr>
<td>15</td>
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<td>34</td>
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<tr>
<td>Stage</td>
</tr>
<tr>
<td>I 2</td>
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<tr>
<td>II 44</td>
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<tr>
<td>Socio-economic status</td>
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<tr>
<td>High 3</td>
</tr>
<tr>
<td>Middle 18</td>
</tr>
<tr>
<td>Very poor 25</td>
</tr>
<tr>
<td>Family history of breast cancer</td>
</tr>
<tr>
<td>Yes 16</td>
</tr>
<tr>
<td>No 30</td>
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<tr>
<td>Age at firstchild birth</td>
</tr>
<tr>
<td>16-25 y</td>
</tr>
<tr>
<td>28</td>
</tr>
<tr>
<td>Premenopausal</td>
</tr>
<tr>
<td>18</td>
</tr>
<tr>
<td>Postmenopausal</td>
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<td>28</td>
</tr>
</tbody>
</table>

Figure 1. Comparison of Level of Antioxidants and TBARS Level between Controls, Group-I and Group-II

Figure 2. Comparison of Level of TAA between Controls, Group-I and Group-II
greater risk of developing breast cancer in postmenopausal women who were smokers (Luo et al., 2011) and our data also supports the same (Table).

We found that TBARS level was significantly increased in Group I when compared with controls and group-II patients. This is due to the presence of potential carcinogens in tobacco smoke. These carcinogens are stored in adipose tissue and the mammary cells of the breast producing more reactive hydroxyl radicals. These radicals irreversibly damage the DNA, which affecting the balance between proliferation and apoptosis; this leading to tumor development. Luo et al had demonstrated that there was a positive correlation between breast cancer and smoking (Luo et al; 2011). There are numerous studies indicating that raised oxidative stress played an important role in breast cancer development and progression (Nagata et al., 2006; Mehta and Singh, 2007; Kedzierska et al., 2010).

Vitamin C plays a multifarious metabolic role in human body and one of the important role is as an antioxidant in aqueous medium inside as well as outside the cell and in vitro. In vivo, it directly accepts an unpaired electron from α- tocopherol present in the cell membrane, there by assisting α- tocopherol in protection of cellular membrane integrity. In our previous studies we did not find any significant difference in vitamin C levels in smokers of cancer patients (Naggama et al., 2011). In other studies we have observed that there was no significant difference in vitamin C and α- tocopherol levels in alcoholic cancer patients, alcoholics with and without smoking habit and also in healthy smokers in Nepali population (Jha et al., 2007; Nagamma et al., 2012). In our present study, statistically decreased levels of vitamin C and α- tocopherol were observed in group-I when compared with controls and group-II (Fig-1). Our study was supported by several other studies which have showed that significant lower levels of α- tocopherol and vitamin C were seen in patients with breast cancer (Sharhar et al., 2008; Shah et al., 2009).

TAA was significantly decreased in breast cancer patients with smoking habit when compared with group-II and healthy controls. The method of Benzie and Strain which we have used in our study, practically measures only dietary antioxidants as it does not measure glutathione and other thiol groups which represents the bulk of endogenous antioxidants. These observations explain the importance of dietary antioxidants in maintaining the oxidative stress. A study by Sener et al revealed that breast cancer patients had lower blood TAA and higher lipid peroxidation levels (Sener et al., 2006). Many studies have reported that total antioxidants status was significantly reduced in cigarette smoking individuals (Mahmood et al., 2007; Gupta et al., 2012). A case-control study revealed that women with high levels of TAA had lesser risk to develop breast cancer (Ching et al., 2001). The decreased TAA levels indicate that more utilization of antioxidants to neutralize the free radicals produced by smoking and also the depending on the nutritional intake of smokers (Figure 2).

In conclusion, Our study proves that there is significant rise in oxidative stress and low levels of dietary antioxidants in breast cancer patients with smoking habit. It is well known that free radicals facilitate the progression of breast cancer, possibly increasing the risk of progression to the next stage. Further studies are required to establish the role of smoking in breast cancer patients in association with oxidative stress and antioxidants in Nepali population before coming to a conclusion.

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