RESEARCH ARTICLE

Significance of the Plasma Lipid Profile in Cases of Carcinoma of Cervix: A Tertiary Hospital Based Study

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Abstract

Aims: To study alterations of plasma lipid profiles in carcinoma cervix and to assess significance compared with controls in different histological grades and stages. Materials and Methods: Totals of 99 histopathologically diagnosed cases and 35 controls from a tertiary hospital situated in the southern part of India which caters the rural and semi-urban populations were considered for the study. Fasting blood samples were taken to analyze total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C) and low density lipoproteins cholesterol (LDL-C), for comparison of cases, grouped according to histological grades and stages, and controls. One way ANOVA was used for multiple group comparisons and the Student’s t test (unpaired) for group wise comparisons. For all tests a ‘p’ value of 0.05 or less was considered as significant. Results: Out of 99 cases, most (n=39) were seen in the 40-49 year age group followed by 60-69 years (n=22). Serum TG significantly differed between cases and controls but without any relation to differentiation grade. The lipid profile parameters in various grades of cervical cancer were not statistically significant. Statistically significant increase of TC and LDL-C values was observed with increase in stage of the disease. Conclusions: The study showed TG is elevated in cervical cancer, and that TC and LDL-C are proportional to the spread of cancer as it increases from stage I to stage IV. An in-depth study of molecular changes in lipid metabolism in cervical cancer patients, enzymes/genes responsible and alterations in LDL receptors is necessary to provide information to decide whether the lipid profile has any diagnostic/prognostic role in cervical cancer.

Keywords: Carcinoma cervix - lipid profile - differentiation grade - stage - Karnataka - India

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Introduction

Cervical cancer is one of the leading cancer related death among women worldwide (Satija, 2013). Cervical cancer is the third most common cancer in women worldwide accounting for 9% of all female cancer and 9% death in females due to cervical cancer. Worldwide it is seventh cancer with estimated 530,000 new cases in 2008 accounting for 4% of cancer worldwide. More than 85% of the global burden is seen in developing countries compared to western countries and it accounts for 13% of all female cancers. In India, 134,000 were detected to have cervical cancer, out of which 72,825 women died of cervical cancer in 2008 (Globocan, 2008; Misra et al., 2009). At our institute cervical cancer accounts for about 17.5% of total cancers in females (Kalyani et al., 2010).

Lipids are high energy yielding molecules and include fats and oils, waxes, phospholipids, steroids and other related compounds. Fats and oils are made from two kinds of molecules, one glycerol and three fatty acids bounded together by dehydration synthesis, known as triglycerides (TG), which are the major form of energy storage. These are major cell membrane components essential for various biological functions including energy production, signaling, growth and division of normal and malignant cells, maintenance of the structural and functional integrity of all biological membranes, activity of membrane-bound enzymes and stabilization of DNA helix. Usefulness of variations in lipid parameters (total cholesterol (TC), high density lipoproteins-cholesterol (HDL-C), TG, low density lipoproteins-cholesterol (LDL-C) in diagnosis and treatment of various diseases has been studied by several workers (Nayak et al., 2000; Patel et al., 2004).

The relation of cancer and lipid profile parameters is controversial. The potential clinical utility of lipid parameters to predict cancer risk or prognosis is still questionable (Dabrowa et al., 2011). Many studies have reported the correlation of lipid profile and cancer i.e
cancer of oral cavity, breast, colorectal, ovary and prostate (Nayak et al., 2000; Patel et al., 2004; Laisupasin et al., 2013). Only a few studies have documented relation between carcinoma cervix and lipid profile. One study has shown that profiles of lipid in cancer and normal cervical tissue differ and reveal information about cancer development and progression (Preetha et al., 2005). Hence we have taken up this study to assess the significance of serum lipid parameters in cervical cancer.

Materials and Methods

The objective of this study was to study the alterations of plasma lipid profiles in carcinoma cervix and to derive its significance. The ethical clearance was obtained from institutional ethical committee. The study group consisted of 134 subjects from inpatient department of obstetrics and gynecology, in a tertiary hospital, which was grouped as; group 1 consisting of controls (n=35) and group 2 consisting of cases (n=99). Control group consisted of age matched individuals without any systemic or genital malignancies. Cases consisted of clinically and histopathologically confirmed cases of carcinoma cervix constituting of 93 new cases in which the treatment was not started, four cases treated with hysterectomy, one advanced case treated with chemotherapy and one case with recurrence.

Detailed clinical history of the cases was recorded in semi-structured proforma. After obtaining informed consent, the local and systematic examination of patients was done. In clinically suspected cases of cervical carcinoma and in patients with cervical intraepithelial lesion in Pap smears, colposcopy guided cervical biopsy was obtained and subjected to histological examination. After the histopathological confirmation of cervical carcinoma, the blood samples were collected from patients with a minimum of 12 hours of fasting. About 5ml of fasting blood sample was collected under aseptic precautions with minimal trauma into plain vacutainer tube. The blood was allowed to clot and then was centrifuged at 3000rpm for 15 mints. Within 30 mints of sample collection, serum was separated and the estimation of lipid profile was done within 6 hours of the separation, which included estimation of serum TC, TG, HDL-C and LDL-C.

Serum TC, TG, HDL-C was estimated by using Accucare cholesterol kits in autoanalyser. Serum TC was estimated by CHOD-PAP method. Five μl of serum sample which was mixed with 500μl of working reagent that contained cholesterol oxidase, cholesterol esterase, peroxidase, 4-amino phenazone, surfactant, phenol, buffer, preservatives and stabilizer. The mixture was incubated at 37°C for 10 minutes and absorbance was read at 505nm (Young et al., 1973; Patel et al., 2004). Serum HDL-C was estimated by GPO-PAP method. Ten μl serum was mixed with 1000μl of triglycerides assay reagent containing goods buffer, lipase, 4-chlorophenol, magnesium ion, ATP, peroxidase, glycerol kinase, sodium azide, 4-amino antipyrene, glycerol-3-phosphate oxidase and detergents. The mixture was then incubated for 10 minutes at 37°C and absorbance was read at 505nm (Denahcherp et al., 1775; Patel et al., 2004). LDL-C levels was calculated by Friedwald formula after considering its limitations as, LDL-C=Total Cholesterol minus Triglycerides/5 minus HDL-C (Patel et al., 2004).

The cases were divided into different grades histologically as: well, moderate and poorly differentiated squamous cell carcinoma. The cases were also divided into different stages (Greene et al., 2006). Mean and Standard Deviation (SD) was calculated for each group. One way ANOVA was used for multiple group comparison and student’s t test (unpaired) for group wise comparison. Diagnostic validity tests were performed using lipid values as biomarkers to differentiate different disease groups with healthy controls. For all the tests ‘p’ value of 0.05 or less was considered as statistical significant.

Results

In the present study, all the patients were of low socioeconomic status, were having poor nutritional status and had various grades of squamous cell carcinoma. Maximum cases (n-39) were seen between 40-49 years of age group followed by 60-69 years (n-22) (Table 1). All the four parameters of lipid profile were compared for both controls and cases (Table 2, Figure 1). The serum TG for cases and controls were statistically significant. Of the total 99 cases, 32 cases (32.3%) were well differentiated squamous cell carcinoma (WDSCC) and 52 cases (52.5%) were moderately differentiated squamous cell carcinoma (MDSCC). The age range in these two groups was of 30-70 years. The poorly differentiated squamous cell carcinoma was 15 cases (15.2%), in which the age range was 40-70 years. The mean, standard deviation and p value of all four

<table>
<thead>
<tr>
<th>Age groups in years</th>
<th>No of cases</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-39</td>
<td>7</td>
<td>7.14</td>
</tr>
<tr>
<td>40-49</td>
<td>39</td>
<td>39.79</td>
</tr>
<tr>
<td>50-59</td>
<td>21</td>
<td>21.42</td>
</tr>
<tr>
<td>60-69</td>
<td>22</td>
<td>22.44</td>
</tr>
<tr>
<td>70-79</td>
<td>7</td>
<td>7.14</td>
</tr>
<tr>
<td>80-89</td>
<td>2</td>
<td>2.04</td>
</tr>
<tr>
<td>Total</td>
<td>98</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 1. Shows Age Distribution of Cases

<table>
<thead>
<tr>
<th>Groups</th>
<th>No.of subjects</th>
<th>TC Mean±SD</th>
<th>HDL-C</th>
<th>LDL-C</th>
<th>TG Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>155.1±23.8</td>
<td>38.0±5.9</td>
<td>87.9±27.1</td>
<td>131.8±26.4</td>
<td></td>
</tr>
<tr>
<td>p value</td>
<td>0.0797</td>
<td>0.3552</td>
<td>0.1199</td>
<td>0.0241</td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>166.4±88.1</td>
<td>166.3±34.7</td>
<td>36.6±8.2</td>
<td>97.1±30.8</td>
<td></td>
</tr>
<tr>
<td>t value</td>
<td>1.7662</td>
<td>0.9277</td>
<td>1.5651</td>
<td>2.2825</td>
<td></td>
</tr>
<tr>
<td>p value</td>
<td>0.0497</td>
<td>0.3552</td>
<td>0.1199</td>
<td>0.0241</td>
<td></td>
</tr>
</tbody>
</table>

*TC; total cholesterol, HDL-C; high density lipoproteins-cholesterol, LDL-C; low density lipoproteins-cholesterol, TG; triglycerides, SD; standard deviation
controls

Values of Parameters of Lipid Profile in Cases and in Controls

Figure 1. Shows Comparison Between Mean Serum Values of Parameters of Lipid Profile in Cases and in Controls

Figure 2. Shows Comparison Between Mean Serum Values of Parameters of Lipid Profile in Different Histological Grades of Cervical Cancer

Table 3. Shows Mean, Standard Deviation and p value for Varying Grades of Squamous Cell Carcinoma

Table 4. Shows Mean Values of Lipid Profiles in Various Stages, Treated Cases and Cases with Recurrence in Cervical Cancer

Discussion

Cholesterol and triglycerides are the important lipid constituents of cell and are essential to carry out several vital physiological functions. Cholesterol is essential for maintenance of the structural and functional integrity of all biological membranes. It is also involved in the activity of membrane bound enzymes and is important for stabilization of DNA helix. Cellular uptake and regulation of cholesterol is mediated by lipoprotein receptors especially located on the surface of the cells. For transport in plasma, triglycerides and cholesterol are packed into lipoproteins, which are then taken up and degraded by cells to fulfill demands for cellular functions. LDL-C is involved in transport of 75% of cholesterol in blood, of which 80% is removed from blood by LDL-C receptors and 20% is deposited in arterial wall. HDL-C is associated with taking cholesterol out of blood and with cholesterol levels in the risk of cancer and provided a base for further epidemiological research. Since then, conflicting hypotheses have been put forward by many workers. Several authors propose that hypercholesterolemia is a predisposing factor for cancer development. The alterations in the circulatory cholesterol levels have been found to be associated with oral, breast, colorectal, ovary and prostate cancer. In some malignant diseases, blood cholesterol undergoes early and significant changes. Low levels of cholesterol in the proliferating tissues and in blood compartments could be due to the process of carcinogenesis (Patel et al., 2004, Mehrotra et al., 2009; Chawda et al., 2011; Singh et al., 2013).

Although, lipids have prime role in pathogenesis of coronary heart disease, researchers have reported association of plasma/serum lipids and lipoproteins with various cancers. The question whether hypolipidemia at diagnosis of cancer is the causative factor or result of cancer has remained unanswered (Singh et al., 2013). Studies have reported an inverse association between blood cholesterol level and the risk of cancer and provided a base for further epidemiological research. Since then, conflicting hypotheses have been put forward by many workers. Several authors propose that hypercholesterolemia is a predisposing factor for cancer development. The alterations in the circulatory cholesterol levels have been found to be associated with oral, breast, colorectal, ovary and prostate cancer. In some malignant diseases, blood cholesterol undergoes early and significant changes. Low levels of cholesterol in the proliferating tissues and in blood compartments could be due to the process of carcinogenesis (Patel et al., 2004, Mehrotra et al., 2009; Chawda et al., 2011; Singh et al., 2013).

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Alteration in cholesterol metabolism, increased synthesis of cholesterol, increased accumulation of cholesterol in tumour tissue with decreased serum levels of cholesterol esters are reported. In addition hypocholesterolemia is suggested to be a possible prognostic factor in malignancies (Naik et al., 2006). However, little data is available regarding cervical cancer and lipid parameters.

Fundamentally the development of a malignancy requires the uncontrolled and excessive proliferation of cells. These newly forming cells would need many basic components well above the normal limits, used in physiological process. One such component is lipids which form major cell membrane components essential for various biological functions including cell division and growth of normal and malignant tissues. The increased requirement of lipids to fulfil the needs of these new cells will diminish the lipid stores. In general carcinogens induce generation of free radicals and reactive oxygen species, which are responsible for high rate of oxidation and peroxidation of polyunsaturated fatty acids. This peroxidation further release peroxide radicals. This affects essential constituents of the cell membrane leading to greater utilization of lipids including total cholesterol, lipoproteins and triglycerides for new membrane biogenesis. Cells fulfill these requirements either from circulation, by synthesis through the metabolism or from degradation of major lipoprotein fractions like VLDL, LDL or HDL (Patel et al., 2004; Mehrrota et al., 2009; Gupta et al., 2012; Singh et al., 2013). The antioxidant vitamins A and E have protective effects against lipid peroxidation (Patel et al., 2004). LDL-C is more susceptible for oxidation, whereas HDL-C counter balance the oxidative damage of LDL-C on cell membrane, prevent peroxidation and decrease the formation of oxygen free radicals which are carcinogenic. The lipid peroxidation product malondialdehyde cross link DNA on the same and opposite strands via adenosine and cytosine. This may in theory contribute to carcinogenicity in mammalian cells (Dabrowa et al., 2011). One study showed that alpha linolenic acid treated cervical and breast cancer cells increase lipid peroxidation and simultaneous decrease nitric oxide release resulting in apoptosis (Gupta and Singh, 2013). Peterson stated that hypercholesterolemia in cancer patients may be because of increased LDL receptor activity (Naik et al., 2006; Singh et al., 2013). Hypopilipidemia can also be due to secondary malfunction of lipid metabolism (Singh et al., 2013). Cholesterol accumulation and dysregulation in metabolism is reported in many malignancies (Tania et al., 2010). Precursor particles of HDL-C are thought to be derived from lipolysis of TG. Lipoprotein lipase activity is decreased in cancer; hence increased TG may be one of the factors for low HDL-C levels. The pathway of cholesterol synthesis produces various tumorigenic compounds (Dabrowa et al., 2011). Theoretically synthesis of lipoprotein and cholesterol by the liver could be inhibited by tumour metabolites. TC and HDL-C levels are significantly inversely associated with incidence of cancer whereas TG levels significantly elevated in cancer patients (Raste and Naik, 2000).

Many studies have been reported regarding oral cancer and lipid profile. One study has shown significant decrease in TC and HDL-C in head and neck cancer and oral precancerous patients whereas, TG values were decreased in cancer patients than precancerous patients and LDL-C did not show significant difference. HDL-C was considered as an additional predictor of cancer and also consequence of cancer (Patel et al., 2004; Singh et al., 2013). In another study there was significant decrease in LDL, HDL-C in oral precancerous condition and no significant difference in TG levels. An inverse relation of cholesterol level and oral precancerous condition was noted indicating progression of disease. Hence it was concluded that estimation of lipid profile can be a good marker for increased cell turnover (Nayak et al., 2000). In a study of lipid profile and submucosal fibrosis, significant decrease in TC and HDL-C and significant increase in LDL-C and TG was observed (Mehrotra et al., 2009). In another study TC, TG and HDL-C showed significant decrease in oral cancer patients compared with controls with insignificant values of LDL-C. However there was no significant relation between serum cholesterol and histological type/grade of the cancer. But lower serum cholesterol and HDL-C was seen in widespread cancer compared with localised tumour (Chawda et al., 2011; Dabrowa et al., 2011; Singh et al., 2013).

In a study of lipid profile and breast cancer, it was found that serum TG and LDL-C were significantly increased, TC was not significantly increased and HDL-C was not significantly lower compared to control group. HDL-C has been reported to be increased in women with excessive mammographic dysplasia and family history of breast cancer. The higher concentration of TG decrease sex hormone binding globulin resulting in increase in free estradiol levels which increase the risk of breast cancer (Dabrowa et al., 2011; Laisupasin et al., 2013). In another study of breast cancer, there was no change in serum TC level, however TG level decreased compared to controls (Timovska et al., 2011). Hypercholesterolemia is reported to impair angiogenesis by suppressing endothelial and tumoral bFGF and VEGF expression via decrease of tumoral nitric oxide synthetase causing lower microvessel proliferation and therefore lowers the risk of metastases and recurrence in case of invasive breast cancer compared with normo-cholesterolemia and thus determines mortality. Similar features were observed in prostatic cancer. In addition hypercholesterolemia causes endothelial cell dysfunction because of accumulation of toxic lipoprotein degradation products in the arterial wall. The tumour cells express increased LDL receptor levels which lead to low LDL levels. Cholesterol level is low in larger tumour than in smaller one (Ozdemir et al., 2004). The molecular genetic factors and some risk factors of colorectal carcinoma cause increase in serum total cholesterol level and result in adenomatous polyyp and colorectal carcinoma. An inverse relation is also reported. However no such relation exists with serum triglycerides (Herbey et al., 2005). Higher intake of dietary lipids, systemic lipid metabolism malfunction, abnormal serum lipids levels and lower intake of vegetables are related with ovarian cancer. Dietary cholesterol increases the risk...
of ovarian cancer through elevated circulating oestrogen and progesterone. Over expression of some lipid metabolic enzymes are reported in ovarian cancer. Increased serum cholesterol level is a risk factor for ovarian cancer (Tania et al. 2010). Ovarian cancer has shown marked decrease in TG/HDL-C and moderate decrease in TC and LDL-C. In other gynaecologic cancers there was significant decrease in TG, TC, HDL-C and insignificant decrease in LDL-C. HDL-C was decreased in all gynaecologic cancers (Dabrowa et al 2011). Serum TC, HDL-C and LDL-C were reported to be significantly decreased and serum TG significantly elevated in leukaemia and Hodgkin’s disease patients (Naik et al., 2006). Study of lung and haematological cancers with lipid profile showed statistically insignificant value for TG and lower values of TC and LDL-C. Many epidemiological studies have shown increased risk of death in cancer patients with low serum TC levels (Raste and Naik, 2000).

In a study of cervical cancer and lipid profile, HDL-C was not significantly decreased in stage III and IV cervical cancer; TG and TC was significantly increased in stage II, III and IV cervical cancer. Following radiotherapy/tumour resection/combined therapy altered lipid parameters reverted back (Ravichandran et al., 1996; Preetha et al., 2005). The cholesterol content of cancerous cervical tissue was roughly double that of normal human cervical tissue, altering the surface activity which in turn alters cell adhesion and metastases (Preetha et al., 2005; 2007).

One study of lipid profile and advanced cervical cancer showed a significant decrease in TC, HDLC & TG; however LDL-C was higher in the cases than in the C controls. In addition there was direct relationship between reduced lipid levels and cervical cancer progression which was statistically significant. The lower level of lipid constituents in patients was thought to be due to low levels of antioxidants which are a common finding among cancer patients (Okonkwo et al., 2011). In our study, TG value was higher in cases than in controls and the difference was significant. TC and LDL-C values were higher in cases than in controls, while HDL-C value was lower in cases than in controls; however the difference in both were not significant. These results were comparable to the studies of lipid profile with submucosal fibrosis and breast cancer by Mehrrota et al. (2009); Dabrowa et al. (2011); Laisupasin et al. (2013). Considering varying grades of cervical cancer, TC and LDL-C increased from WDSCC to PDSCC but was not statistically significant. TG and HDL-D values were not significant. Hence in all four parameters, the values were statistically insignificant. This was comparable to studies done on oral cancer and lipid profile by Chawda et al. (2011); Dabrowa et al. (2011); Singh et al. (2013) and Comparing lipid profile parameters in various stages of cervical cancer, TC and LDL-C increased from stage I to stage IV and was statistically significant. HDL-C also increased from stage I to stage IV but was statistically insignificant. TG values were not significant. This was in contrast to results of studies of oral cancer and lipid profile by Chawda et al. (2011); Dabrowa et al. (2011); Singh et al. (2013). However the TC value was comparable and HDL-C value was not comparable to the studies of cervical cancer and lipid profile by Ravichandran et al. (1996); Preetha et al. (2005). The lipid parameters following treatment cannot be concluded as the number of cases were few. There are many studies showing inverse relationship of lipid parameters with malignancy. Variability of the values of plasma lipid profile in cancer patients may be due to multiple reasons, such as age, nutritional status, body mass index, alcohol consumption and exercise habits. The variability in the levels of parameters of lipid profile may also be due to methodological difference (Nayak et al., 2000). It is reported that the apparently conflicting studies are actually consistent because of the trend of decline in the serum cholesterol level gradually ten years preceding the diagnosis of cancer (Raste and Naik, 2000).

In conclusion, in the present study, we have estimated lipid profile in normal healthy age matched controls and patients with cervical cancer. Analysis of data revealed, TG value higher in cases than in controls and the difference was significant. In different histological grades of cervical cancer, all four parameters of lipid profile were statistically insignificant. TC and LDL-C increased from stage I to stage IV and was statistically significant. This study shows that TG is a consequence of cervical cancer, lipid parameters are not related with histological grades of cancer and TC & LDL-C is proportional to the spread of cancer as it increases from stage I to stage IV. However a larger study sample with long term follow up of cases and periodic estimation of lipid profile would be needed to establish this correlation. In addition, an in-depth study of molecular changes in lipid metabolism in cervical cancer patients, enzymes/enzymes responsible and alterations in LDL receptors gives more information and one can decide whether lipid profile has diagnostic/prognostic role in cervical cancer.

References


