Oxidative stress-induced DNA damage and homocysteine accumulation may be involved in ovarian cancer progression in both young and old patients

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Background/aim: Biochemical, environmental, and genetic factors such as oxidative stress-induced DNA damage and homocysteine (Hcy) accumulation in the blood are involved in the development and progression of ovarian cancer. This study measured some biomarkers closely linked to the progression of ovarian cancer and also found their correlates.

Materials and methods: Thirty patients were diagnosed with ovarian cancer using pelvic examination, transvaginal ultrasound, and cancer antibody (CA-125) measurement. Total oxidative stress (TOS), DNA damage, Hcy, malondialdehyde (MDA), total antioxidant status (TAS), and other biochemical parameters were determined.

Results: TOS and DNA damage were positively and significantly correlated between themselves and were involved in causation of tumors as reflected by significantly (P < 0.001) higher CA-125, erythrocyte sedimentation rate (ESR), creatinine, and C-reactive protein (CRP) in both young and old patients. Both were significantly correlated with Hcy, LDL-cholesterol, alanine aminotransferase, aspartate aminotransferase, CRP, MDA, and CA-125. However, they were negatively correlated with TAS. Thus, excessive inflammation and oxidative stress caused an increase in DNA damage and enhanced Hcy content, leading to development of ovarian cancer.

Conclusion: This study suggests the use of antioxidants as drugs to reduce oxidative stress, DNA damage, and other causes of cancer development.

Key words: Antioxidants, DNA damage, CA-125, C-reactive protein, malondialdehyde, total antioxidant status, total oxidative stress

1. Introduction

Ovarian cancer is the third most common fatal gynecological disease amongst Pakistani women and needs attention in order to have a healthy society. There are numerous risk factors involved in the development of this cancer. These include enhanced production of steroid hormones, inflammation, infertility, excessive ovulations, and nulliparity (3).

Ovarian cancer is a silent killer, affecting a number of women in both developed and developing countries every year. To date there is no reliable way to screen women for ovarian cancer in the developing world. Pelvic examinations, transvaginal ultrasound, and blood tests (CA-125) are the main ways used to diagnose ovarian cancer (1). Diagnosis of this disease is not sought at its early stage of development as the majority of women (>85%) seek help when the disease has progressed to stage III or stage IV as diagnosed by lymphatic, intraperitoneal, or advanced stage of spreading in stage III, in the far sites of the ovaries (stage IV), or in the metastatic phase. This happens because of the lack of visible symptoms of this disease in affected women. There are no noninvasive techniques developed so far for early stage diagnosis (2).
were discontinued from the study.

Patients who experienced complications only, while in 15 others the cancer had distant metastasis. Confirmations were made by magnetic resonance imaging. Fifteen patients had stage III cancer, in which the cancer was confined to ovaries and transvaginal ultrasound. Color flow Doppler tests were checked for CA-125 antibody, lactate dehydrogenase, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are elevated in patients with ovarian cancer, and particularly in stage IV cancer patients. Ovarian cancer causes severe inflammation. C-reactive protein (CRP) is considered a test marker of tumor development. It is produced in the liver in response to inflammation (8). The association of many of these parameters is not currently clear in the Pakistani population. Therefore, this study was designed to evaluate these risk factors in general Pakistani women. This study also measured known biomarkers, closely linked to the progression of ovarian cancer, and computed their correlation.

2. Materials and methods

2.1. Subjects

The fasting blood samples of 30 ovarian cancer patients and 30 healthy volunteers (of the same age and genetic background) were collected. The participants were recruited from Punjab Institute of Nuclear Medicine Allied Hospital, Faisalabad. Informed consent was obtained from all participants. These patients visited the hospitals for routine check-ups and were picked randomly from in and around Faisalabad. All participants were of middle socioeconomic background. Patients suffering from pelvic pressure and having excessive vaginal bleeding were checked for CA-125 antibody, lactate dehydrogenase, and transvaginal ultrasound. Color flow Doppler tests and biopsies were also performed to confirm ovarian cancer in patients. Malignant cancer was confirmed by magnetic resonance imaging. Fifteen patients had stage III cancer, in which the cancer was confined to ovaries only, while in 15 others the cancer had distant metastasis and migrated to the liver. Confirmations were made by gynecologists and oncologists. Body mass index (BMI, kg/m²), blood pressure, pulse rate, and body temperature were recorded. Patients who experienced complications were discontinued from the study.

2.2. Design

The blood pressure of young and old participants was measured by using sphygmomanometer after the individual had rested for 10 min in a quiet room. The readings were recorded as systolic and diastolic blood pressure. The weight of each individual was taken with the help of a weighing machine. Height in meters was recorded with the help of a measuring tape when subjected were barefoot. Cut-off values were from published clinical values. Efforts were made to match the patients and the controls for any potential confounding factors, namely age and socioeconomic condition. A total of 60 blood samples were collected between 0800 and 0900 hours in a precooled test tube from normal and cancerous patients. Tubes were centrifuged at 769× g for 15 min. Serum was separated and stored in small aliquots at –20 °C till analysis. Another sample with anticoagulant using heparin (1%) was also taken for packed cell volume, erythrocyte sedimentation rate (ESR), and hemoglobin.

2.3. Analytical methods

All tests were performed in Punjab Institute of Nuclear Medicine Clinical Laboratories and Government College University Faisalabad Laboratories using their standard protocols. The concentration of hemoglobin was determined using colorimetric assay kit (Cayman Chemical Company, item # 700540) following the manufacturer’s instructions. The concentrations of cholesterol and triglycerides were determined using enzymatic kits (Randox Laboratory) on a Hitachi 704 analyzer using standard methods. The precision limits for determination of total cholesterol and total triglycerides varied between 1.3% and 2.4% and between 1.9% and 3.5% per run, respectively.

Serum HDL-cholesterol level was determined using commercially available kit (Cat No. D1H20-400, Diasis Diagnostik) following the instruction provided by the suppliers. This kit works on the principle of blocking apoB containing proteins by blocking the agent present in the kit. In this way only HDL-cholesterol is detected under the assay working conditions. HDL-cholesterol in serum was precipitated with phosphotungstic acid in the presence of magnesium ions. The concentration of LDL-cholesterol was calculated using the Friedewald formula.

Serum glucose test was performed using Human kit (Human, GmbH 65205) and a spectrophotometer at 545 nm. The plasma total antioxidant status (TAS) level was measured using a commercial kit (Cell Biolab, cat no STA-360). In this assay, free radicals are produced by hydroxyl radicals and the antioxidative effect of the sample against them is measured. We followed this method according to the manufacturer’s instructions and all assays showed excellent precision values (lower than 3%). The results were expressed as mmol Trolox equiv.L⁻¹ as mentioned by above suppliers.

Serum total oxidative stress (TOS) levels were determined using an automated measurement method

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Serum total oxidative stress (TOS) levels were determined using an automated measurement method
developed by Erel (9). Oxidants present in the test or the standard sample oxidize the ferrous ions-o-dianisidine complex to ferric ions, which make a colored complex with xylenol orange in the acidic medium. The color intensity is directly related to the total amount of oxidants present in the sample or standard. The assay was calibrated with hydrogen peroxide, and the results were expressed in terms of μM hydrogen peroxide equivalent per liter (μmol H₂O₂ equiv. L⁻¹).

Plasma MDA levels were quantified spectrophotometer at 532 nm as described by the suppliers of commercial kit (Cayman Chemical Company, item no. 10009055). One milliliter of working reagent was taken in tubes marked as blank, sample, and standard, respectively. Then 10 µL of sample or standard was added to the respective tubes, mixed well, and incubated at 25 °C for 30 min. Lipid peroxides in plasma were estimated colorimetrically. The absorbance of clear supernatant was measured against reference blank at 535 nm spectrophotometrically, employing the molar absorption coefficient of 1.56 × 10⁵ M⁻¹ cm⁻¹.

Plasma Hcy was measured by Hcy microtiter plate assay (7) from control and ovarian cancer patients using a commercial kit (Diazyme) as per manufacturer’s instructions. Briefly, plasma samples were prepared in polyethylene culture tubes with a reducing agents, tris (2-carboxyethyl) phosphine hydrochloride, to reduce the protein bound Hcy to free Hcy that was subsequently converted to S-adenosyl-L-Hcy (SAH) by SAH-hydrolyase and quantified by horseradish peroxidase (HRP-SAH) competitive assay.

DNA damage was measured in serum using EIA kit (Cell Biolab, Inc., Cat. STA-320) as described previously (10). Briefly, the quantity of 8-Hydroxy-2’ deoxyguanosine (8-OHdG) in an unknown sample was determined by comparing its absorbance with that of a known 8-OHdG standard curve as per manufacturer’s instructions. The laboratory performed strict external and internal quality control tests. Reagents for calibration of instruments were supplied by their manufacturers.

Serum urea was measured by using commercially available kit (Cat No. UR 222; Randox Laboratories) and following manufacturer’s instructions. Serum AST (Crescent Diagnostics Cat No. CZ 904 C) and ALT (Crescent Diagnostics Cat No. CZ 902 C) were determined by colorimetric method. The serum CRP level was determined by CRP ELISA Human (Cat. No. CYT298, Merck Millipore) kit test. CA-125 was measured by using ovarian cancer antigen CA-125 ELISA kit (Phoenex cat #KA0205). All the work was done in highly sterilized conditions. Strict external and internal quality control tests were performed.

2.4. Statistical and sensitivity analyses
The collected data were subjected to calculations of means ± standard deviation (SD). To calculate the differences between normal and ovarian cancerous patients of young and old age, data were analyzed by two-way ANOVA (11). In case of significant differences, Duncan’s multiple range test was applied (12) using GraphPad 3.0 software. Spearman’s correlation coefficients between different anthropometric data, biochemical profiles, and their effects in term of TOS and oxidative DNA damage were also determined using the above-mentioned software.

3. Results
General anthropometric characteristics of the subjects are shown in Table 1. There was no significant difference in BMI (P = 0.5865) between the controls and patients. Similarly, SBP (P = 0.5088) and DBP (P = 0.1445) also were not significantly different in the patients as compared with the controls. The young and old patients had a mean age of 24 ± 4 and 50 ± 7 years, respectively. In younger patients ovarian cancer was located in the ovaries while in older patients it was found to be in the metastatic phase.

Serum hematological, biochemical, tumor, and health biomarkers of young and old normal and cancerous patients are shown in Table 2. Mean serum ESR was significantly higher (P < 0.0001) in older patients with ovarian cancer, followed by younger patients. All healthy participants had normal values. Hemoglobin

Table 1. Anthropometric parameters of normal and cancerous patients.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal</th>
<th>Ovarian cancer</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Young N = 15</td>
<td>Old N = 15</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.33 ± 1.92</td>
<td>24.83 ± 2.03</td>
</tr>
<tr>
<td>Systolic blood pressure (SBP; mm/Hg)</td>
<td>120.0 ± 2.0</td>
<td>126.0 ± 2.45</td>
</tr>
<tr>
<td>Diastolic blood pressure (DBP; mm/Hg)</td>
<td>82.83 ± 0.70</td>
<td>87.17 ± 0.60</td>
</tr>
<tr>
<td>Age (Years)</td>
<td>24 ± 4</td>
<td>50 ± 7</td>
</tr>
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</table>

N.D. = not determined.
was nonsignificantly lower in older cancer patients (P = 0.6602), followed by younger cancer patients. Healthy participants had normal hemoglobin levels. Mean serum glucose was nonsignificantly higher (P = 0.1201) in cancer patients. Creatinine was significantly higher (P = 0.0001) in older cancer patients, followed by younger patients. The control participants had normal amounts of creatinine. Levels of ALT and AST were significantly higher (P > 0.0001) in cancer patients. Mean serum CRP was extremely high in older cancer patients (P < 0.001) as compared with that of healthy older participants. However, it was not significant in younger cancer patients (P > 0.05) as compared with that of healthy participants. On the whole, the level of CRP was significantly higher in ovarian cancer patients. CA-125 was significantly higher in older individuals with cancer as compared with that of healthy individuals. All cancer patients, irrespective of age, showed a significant increase in CA-125 levels (P = 0.0230). Urea was nonsignificantly higher in ovarian cancer patients (P > 0.05). Control participants had normal levels of urea.

The cholesterol did not follow any linked trend. The cholesterol was observed significantly lower (P < 0.05) in young patients suffering from ovarian cancer. The triglyceride levels were significantly higher in younger cancer patients (P < 0.001) as compared with those in healthy young participants. Levels of HDL-cholesterol were extremely significantly lower in all patients (P < 0.0001), while LDL-cholesterol was significantly higher (P < 0.0001) in ovarian cancer patients. Levels of homocysteine and total oxidant status were significantly higher in all patients (P < 0.0001). MDA levels were significantly higher (P < 0.0001) in cancer patients as compared with those of healthy participants. Total antioxidant status was significantly higher in all control participants than in cancer patients (P < 0.0001). DNA damage was significantly higher in older cancer patients. DNA damage was also significantly higher in younger cancer patients (P < 0.05).

We performed the correlation analysis between TOS and all studied parameters (Table 3) in healthy individuals and cancer patients. The results showed that TOS was significantly and positively correlated with LDL-cholesterol (P < 0.001), ALT (P < 0.003), CRP (P < 0.0051), DNA damage (P < 0.003), MDA (P < 0.004), Hcy (P < 0.004), and CA-125 (P < 0.001). However, it was significantly and negatively correlated with TAS (P < 0.002). TOS was not significantly correlated with any of the studied parameters in control participants.

The correlation of DNA damage with all studied parameters is shown in Table 4. DNA damage (%) was positively and significantly correlated with homocysteine (P < 0.0001), LDL-cholesterol (P < 0.001), ALT (P < 0.0001), and CRP (P < 0.0001). However, it was not significantly correlated with any of the studied parameters in control participants.
However, it was negatively significantly correlated with TAS (P < 0.001). All other studied parameters did not show any significant correlation with DNA damage (P > 0.05) in normal subjects.

4. Discussion
Ovarian cancer, one of the most common malignant ailments in women, is a major public health problem in both developing and developed countries (13). Oxidative stress has become a focus of intense interest since it is involved in the causation and progression of many deadly cancers throughout the world (5). Quality of life is a new and undervalued issue in Pakistan with relatively little attention being paid to measuring the subjective health status of Pakistan’s general population. BMI was normal in ovarian cancer patients, similar to that of healthy individuals and was not a risk factor for ovarian cancer. Likewise, systolic and diastolic blood pressure was normal in individuals diagnosed with ovarian cancer. Some studies also reported an increased risk of ovarian cancer among obese women (14,15). The results from this study are in support of Greggi et al. (15) who have found no association between obesity and ovarian cancer. Socioeconomic and environmental factors, as well as psychosocial stressors could be responsible for these differences.

Hemoglobin was lower in older cancer patients, but the low levels were not significantly (P = 0.6602) different; low levels of hemoglobin can be a side effect of chemotherapy. The associations of high glucose levels with ovarian cancer risk are not currently clear in the Pakistani population. The current study did not show any positive association between glucose levels and ovarian cancer. The results of this study supported the results of Lambe et al. (16), who found no associations between glucose levels and ovarian cancer risk.

Creatinine level was significantly higher (P = 0.0001) in older cancer patients as compared with that in healthy individuals. The higher creatinine levels observed in patients may be due to the drugs used by ovarian cancer patients as treatment. Serum urea levels were normal in in

<table>
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<tr>
<th>Parameters</th>
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<th>Patients P-value</th>
</tr>
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<tbody>
<tr>
<td>LDL- cholesterol (mg/dL)</td>
<td>0.133 NS</td>
<td>0.921 0.001</td>
</tr>
<tr>
<td>Alanine aminotransferase (ALT; U/L)</td>
<td>0.121 NS</td>
<td>0.612 0.005</td>
</tr>
<tr>
<td>Aspartate aminotransferase (AST; U/L)</td>
<td>0.672 NS</td>
<td>0.711 0.003</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>0.795 NS</td>
<td>0.691 0.005</td>
</tr>
<tr>
<td>DNA damage</td>
<td>0.321 NS</td>
<td>0.886 0.003</td>
</tr>
<tr>
<td>Malondialdehyde (MDA; µmol/L)</td>
<td>0.320 NS</td>
<td>0.792 0.004</td>
</tr>
<tr>
<td>Homocysteine (µmol/L)</td>
<td>0.089 NS</td>
<td>0.687 0.004</td>
</tr>
<tr>
<td>CA-125 (U/mL)</td>
<td>0.130 NS</td>
<td>0.990 0.001</td>
</tr>
<tr>
<td>Total antioxidant status (TAS; mmol/Trolox)</td>
<td>0.018 NS</td>
<td>–0.979 0.002</td>
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NS is for nonsignificant (P > 0.05).

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all participants. The concentrations of AST and ALT are used for detecting hepatocellular injury and may help in monitoring the status of the liver. In the current study, ALT and AST were significantly higher in some cancer patients. Both enzymes tend to increase in many hepatic diseases and have limited value in providing a differential diagnosis. However, aminotransferases are considered useful in differentiating hepatocellular from cholestatic forms of liver injury. AST activity is related to damage of cells in the kidney, heart, pancreas, and erythrocytes (3). Chronic inflammation plays a role in ovarian carcinogenesis (18). In the current study, CRP levels were significantly (P < 0.0001) elevated in cancer patients as compared with those of healthy individuals. ESR is a nonspecific measure of inflammation. The levels of ESR were significantly higher (P < 0.0001) in older cancer patients. Thus, the current study supports the role of inflammation in ovarian cancer. CA-125 is best known as a marker for ovarian cancer (19). Its levels are elevated in ovarian cancer or in cases of inflammation. The current study shows a significantly higher (P = 0.0230) increase in its levels in cancer patients as compared with healthy individuals.

Overall mean serum LDL increased, while cholesterol, triglycerides and HDL were significantly lower in cancer patients. The current study supports that low levels of HDL can be linked with the incidence of ovarian cancer. Memon et al. (20) also found an inverse relation of cholesterol with increased incidence of cancer.

In the current study, the levels of Hcy were significantly higher (P < 0.0001) in ovarian cancer patients. High levels of Hcy are believed to promote the formation of oxidation products, which have the capacity to damage endothelial cells. It was also reported by Corona et al. (7) that higher total Hcy concentration was significantly higher in ovarian carcinoma patients.

In the present study, TOS was significantly higher (P < 0.0001) in cancer patients as compared with that of healthy individuals. Senthil et al. (6) also found increased levels of oxidative stress in ovarian cancer patients. MDA levels were significantly higher (P < 0.0001) in cancer patients as compared with those of healthy individuals. Thus, both parameters of oxidative stress increased in cancer patients. Klaunig and Kamendululis (5) also provided evidence of cellular oxidant involvement in the carcinogenesis process.

8-OHdG is a marker of oxidative DNA damage. The current study shows a highly significant increase (P < 0.0001) in DNA damage of ovarian cancer patients as compared with that of healthy individuals. This DNA damage can be due to oxidative stress. The current study supports the findings reported in Bandebuche and Melinkeri (13) that DNA damage plays a major role in ovarian cancer initiation and progression.

The current study shows a highly significant (P < 0.0001) decrease in TAS, which may be induced by imbalance between TOS and body’s defense mechanisms. The low levels of antioxidants in the plasma of ovarian cancer patients were also found by Senthil et al. (6), and may be due to their increased utilization to inactivate lipid peroxides as well as their sequestration by tumor cells. This evidence suggests that TAS can be monitored as a marker in order to understand progression of ovarian cancer.

The correlation of TOS and DNA damage with other parameters shows that they both were positively correlated with homocysteine, LDL-cholesterol, ALT, AST, CRP, MDA, and CA-125. However, both were negatively correlated with TAS. These results show that excessive inflammation, as indicated by significantly higher levels of CRP, CA-125, creatinine, tissue damage, and oxidative stress, and low levels of antioxidants cause an increase in DNA damage. The study also shows that there is a direct relationship between oxidative stress and tumor markers (CA-125 and CRP). In healthy participants these were not related to the above parameters.

In conclusion, this study shows that increase in oxidative stress enhances DNA damage, elevates homocysteine levels, and reduces total antioxidant status in the blood samples of ovarian cancer patients. These indices may be considered as causative agents in ovarian cancer in both younger and older patients. The detection of Hcy, CA-125, CRP, and transaminases in patients’ blood samples gave information about the presence of tumors, but they are inconclusive in detecting a particular cancer. Further work is needed to enhance our ability for its detection in the early stages. More comprehensive study of the relationship between ROS and the body defense mechanism in carcinogenesis may greatly improve our ability to develop interventions to decrease oxidative stress in cancer patients. Defining the ovarian cancer risk profiles may help public health authorities to better understand and target the disease. Extensive research in this field would enable the medical community to develop new drugs to more effectively treat ovarian cancer patients.

Acknowledgments
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References


