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ASSOCIATION OF PRE-DIAGNOSTIC INFLAMMATORY MARKERS WITH THE DEVELOPMENT OF OVARIAN CANCER

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ABSTRACT

Ovarian cancer (OC) is the fifth most common cancer of females in industrialized countries. More than 80% of all cases are being diagnosed with post-menopausal women in the age group of 50 years. Epidemiological data suggested that biology and the initiation of ovarian cancer is associated with lifetime exposure of estrogen hormone. There were 100 healthy individuals included as control group and 100 diagnosed ovarian cancer patients were taken as subjects. Level of Malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH), catalase (CAT), nitric oxide (NO), vitamin A and vitamin E were assessed by spectrophotometer. Whereas matrix metalloproteinase-2 (MMP-2), MMP-9, 8-OHdG, IL-2, TNF-α, iNOS, Heat shock protein-70 (HSP-70), HSP-90, estradiol (E_2), progesterone, epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF) were measured by commercially available ELISA kits. Significantly increased levels of MDA were observed in ovarian cancer patients (4.87±1.30 nmoles/mL, p= 0.023) as compared to control groups (0.956±0.016 nmoles/mL). The levels of 8-OHdG, NO and iNOS were remarkably high in ovarian cancer patients (0.88±0.004 pg/mL, 58.66±7.15 µmol/L and 21.16±6.88 IU/mL) in comparison to healthy individuals (0.03±0.001 pg/mL, 21.19±1.33 µmol/L and 8.98±1.88 IU/mL) respectively. Higher levels of IL-2 were significantly observed in ovarian cancer patients as compared to control groups (11.26±1.14 pg/mL vs. 8.29±2.16 pg/mL, p= 0.041). Increased levels of MMP-2 and MMP-9 were recorded in ovarian cancer

patients (59.30±4.42 ng/mL and 63.46±6.95 ng/mL) as compared to controls (38.58±4.18 ng/mL and 56.13±5.78 ng/mL) respectively. Similarly, the levels of HSP-70 and HSP-90 were significantly higher in the diseased group (33.28±3.58 ng/mL and 22.29±2.16 ng/mL) as compared to control group (8.86±1.05 ng/mL and 6.56±1.03 ng/mL) respectively. In contrary, the significant low levels of antioxidants, i.e., CAT (2.08±0.03 µmol/mol, p= 0.016), SOD (0.42±0.03 µg/mL, p= 0.000) and GSH (6.26±1.08 µg/mL, p= 0.018) were present in ovarian cancer patients as compared to controls. Significantly increased levels of Estradiol (E_2) (71.26±6.23 vs 22.05±2.16 pg/mL) and low levels of progesterone (5.16±1.05 vs 25.16±2.16 ng/mL) were measured in ovarian cancer patients as compared to control group respectively. This study shows that due to oxidative stress, different cytokines are released by TAMs (Tumor associated macrophages) that enhance the production of MMPs and degrade the epithelial lining of the ovary. MMP is responsible in the formation of different growth factors to induce the angiogenesis. Oxidative stress is found in ovarian tumors and cytokines are released which are more susceptible for the progression of ovarian cancer. MMP-9 is implicated in early invasion and adhesion that damage extracellular matrix of the epithelial lining of the ovary.

Keywords: Ovarian cancer, Malondialdehyde, Matrix Metalloproteinases-9, Interlukins-2

INTRODUCTION

Ovaries are a pair of major organs which are the major part of the female reproductive system. Reproductive system is the set of organs which is functional to produce offsprings. In females, the reproductive system comprises of fallopian tubes, cervix, ovaries and vagina. The size and shape of ovary is about to grape which is located in the pelvis (the area lowers the abdomen between the bones of hip). One of the ovaries is located on left side and second one is present on right side of the uterus. Both ovaries are linked along with uterus using thin and long tube known as fallopian tube. The ovaries are responsible to produce eggs for reproduction and also synthesize hormones that influence menstrual cycle, body shape and breast growth. In women, atleast one ovary and uterus are required for the menstrual cycle to become pregnant. The term cancer is a disease of cells, which is the building block to form tissues in the body **[1]**. All cells are coded by instructions that control and form new cells and these instructions are known as genes. The aberrant genetic expression may turn normal ovarian cells into cancerous cells. The normal cells grow and divide into new cells that are formed as the body required to removes injured cells **[2]**. The cancerous cells have the capability to form new cells begin to grow and multiply quickly, creating a mass

of abnormal cells. Once, the ovarian cancer cells mature and divide adequately, then resultantly produce a mass known as a tumor. Female ovaries are made up of three major types of cells: germ cells, stromal cells and epithelial cells. Ovarian cancer can originate from each type of cell. Therefore, there is more than one type of ovarian cancer in the female reproductive system **[3]**. Majority of ovarian cancers originate from epithelial cells that are identified as epithelial ovarian cancer (EOP). There are approximately 90% of ovarian cancers are epithelial ovarian cancer due to its common type in the female ovaries. This cancer is frequently spread to the fallopian tube and uterus. This process is known as metastasis **[4]**. In addition, ovarian cancer cells shed from the primary tumor to generate new tumors on the surface layer of adjacent tissues and organs. These are known as "seed" or "implants" Reactive oxygen species (ROS) are contributed in the progression and development of various types of cancers that trigger DNA damage, genetic instability, protumorigenic signaling, tumor cell proliferation and survival **[5]**. The term oxidative stress stated that the imbalance between free radicals and cellular antioxidative defense system which lead to the disturbance of redox signalling. ROS interrelates with macromolecular structures such as lipids, proteins and nucleic acids. These essential macromolecular structures are involved in tumor metabolism and ROS may cooperate with normal physiological activities **[6]**. Thereby, high production of ROS has been observed in various types of cancer, but the main etiology in tumor progression and carcinogenesis has not been fully cleared. Increased levels of reactive species are responsible for tumorigenesis, genetic instability and DNA damage. They may also act as signaling molecules that lead tocell proliferation, abnormal cell development, cell invasion, cell metastasis and apoptosis **[7]**. In addition, ROS is also involved in angiogenesis through signaling of angiopoietin and vascular endothelial growth factor, activation of endothelial progenitor and perivascular cells **[8]**.

The aim of the current study was to establish the role of oxidative stress and its association with inflammatory cytokines and matrix metalloproteinases in the patients with ovarian cancer. It has also been analysed that the presence of tumor in the ovarian cancer patients can increase the synthesis of ROS, inflammatory cytokines and MMPs which correspond to the synchronization with the treatment strategy.

MATERIAL AND METHOD

In the present study, fifty (n=50) working females with ovarian cancer and fifty (n=50) healthy individuals were added. The clinical diagnosis and history of the patients were collected from INMOL Hospital Lahore Pakistan. The current study was approved by the ethical committee of Molecular Biology and Biotechnology (IMBB), The University of Lahore. Females with clinically diagnosed ovarian cancer and their ages from 20-50 years were included in this study. On the other hand, the individuals with the history of taking different drugs, including alcohol, cigarette and medicine were excluded from this study. In addition, none of the control persons were included which were on any medications, malnutrition syndrome, metabolicdys function (including cancer, diabetes and hypertension) and depression. Half of the drawn blood sample (2 mL) was taken in EDTA tube (for anticoagulated whole blood) and other half (4 mL) in gel tube (for serum). Gel tubes were centrifuged at 4000 rpm to separate serum. The isolated specimens were used to measure the levels of physiological and biochemical parameters. Whole blood was used to analyze the Complete blood count (CBC) by Beckman Coulter. Inc. (automated analyzer). The serum samples were used to determine the levels of MDA and NO with the help of spectrophotometer by the method of Ohkawa *et al* **[9]** and Moshage *et al* **[10]** respectively. The serum level of antioxidants such as CAT (Aeibi *et al*) **[11]**, SOD (Kakkar *et al*) **[12]** and GSH (Moron *et al*) **[13]** were also estimated by their respective spectrophotometric methods. MMP-9, TNF-α and IL-2 were measured by commercially available ELISA kits (Abcam). Statistical analysis was accomplished by SPSS version 17.0 and tests were executed by independent sample T-test and Pearson correlation coefficient. The P-value was measured by using one way ANOVA.

RESULT

Hematological Profile of Patients with Ovarian Cancer (Stage-III)

The data compiled in **Table 1** and **Figure 1** suggested the hematological and demographic profile in ovarian cancer patients as compared to control group. The mean body weight and age of the patients were 78.26±1.17 kg and 45.26±6.23 years, while the mean body weight and age of control individuals were 81.26±1.26 kg and 47.26±4.26 years respectively. The low levels of red blood cells (RBC), Hematocrit (Hct) and Hemoglobin (Hb) were observed in ovarian cancer patients ($3.36\pm0.18 \times 10^{12}$/L, 44.19±3.09% and 12.31±2.09 g/dL) in comparison to control individuals

(4.11±1.09x10^{12}/L, 55.06±4.16% and 14.32±1.09 g/dL) correspondingly. While the mean values of white blood cells (WBC) and neutrophil counts were significantly increased in ovarian cancer patients (10.32±2.16x10^9/L and 9.16±1.01x10^9/L) as compared to control individuals (6.95±2.16x10^9/L and 6.17±2.06x10^9/L).

Expression of Prognostic Variables in the Patients with Ovarian Cancer (Stage-III)

The data presented in **Table 2** and **Figure 2** shows the prognostic variables of medical importance and their impulsive interplay in the development of ovarian cancer. Malondialdehyde (MDA) which is the end product of lipid peroxidation, has significant difference between the values of control and patients with ovarian cancer. The mean value of MDA was significantly increased in ovarian cancer patients (5.16±1.33 nmol/mL) as compared to control individuals (0.84±0.45nmol/mL). The mean value of NO was also to be raised in ovarian cancer subjects (49.36±8.16 µmol/L) in comparison to control persons (19.65±2.18 µmol/L). On the other hand, the levels of anti-oxidants were significantly decreased in ovarian cancer subjects as compared to control persons. Mean value of SOD, GSH and CAT were significantly reduced in the patients with ovarian cancer (0.431±0.061µg/mL, 4.09±2.01 µg/mL and 3.954±0.656 µmol/mol) as compared to control individuals (0.61±0.026 µg/mL, 8.16±1.01 µg/mL and 6.55±1.52 µmol/mol) respectively. Significantly increased levels of IL-2, TNF-α and MMP-9 were observed in ovarian cancer patients (16.35±2.39 pg/mL, 37.26±4.26 pg/mL and 66.35±3.88 ng/mL) as compared to control (21.26±2.01pg/mL, 16.35±3.88pg/mL and 42.16±8.16 ng/mL).

Table 1: Hematological Profile of Patients Ovarian Cancer (Stage-III)

VARIABLES	CONTROL (n=50)	SUBJECTS (n=50)	*P*-VALUE (0.05)
B. Weight kg	81.26±1.26	78.26±1.17s	0.133
Age years	47.26±4.26	45.26±6.23	0.214
RBCs (x10^{12}/L)	4.11±0.4	3.36±0.18	0.031
WBCs (x10^9/L)	6.95±1.16	10.32±2.16	0.041
Hct %	55.06±4.16	44.19±3.09	0.000
Neutrophil (x10^9/L)	6.17±1.06	9.16±1.01	0.021
Hb g/dL	14.32±1.09	12.31±2.09	0.041

Table 2: Expression of Prognostic Variables of Medical Importance and Their Impulsive interplay to Develop Ovarian Cancer (Stage-III

VARIABLES	CONTROL (n=50)	SUBJECT (n=50)	*P*-VALUE (0.05)
MDA nmol/mL	0.84±0.45	5.16±1.33	0.015
SOD µg/mL	0.61±0.026	0.431±0.061	0.001
GSH µg/mL	8.16±1.01	4.09±2.01	0.031
CAT µmol/mol of protein	6.55±1.52	3.954±0.656	0.041
IL-2 (pg/mL)	21.26±2.01	16.35±2.39	0.000
TNF-α (pg/mL)	16.35±3.88	37.26±4.26	0.021
MPP-9 (ng/mL)	42.16±8.16	66.35±3.88	0.019
NO (µmol/L)	19.65±2.18	49.36±8.16	0.008

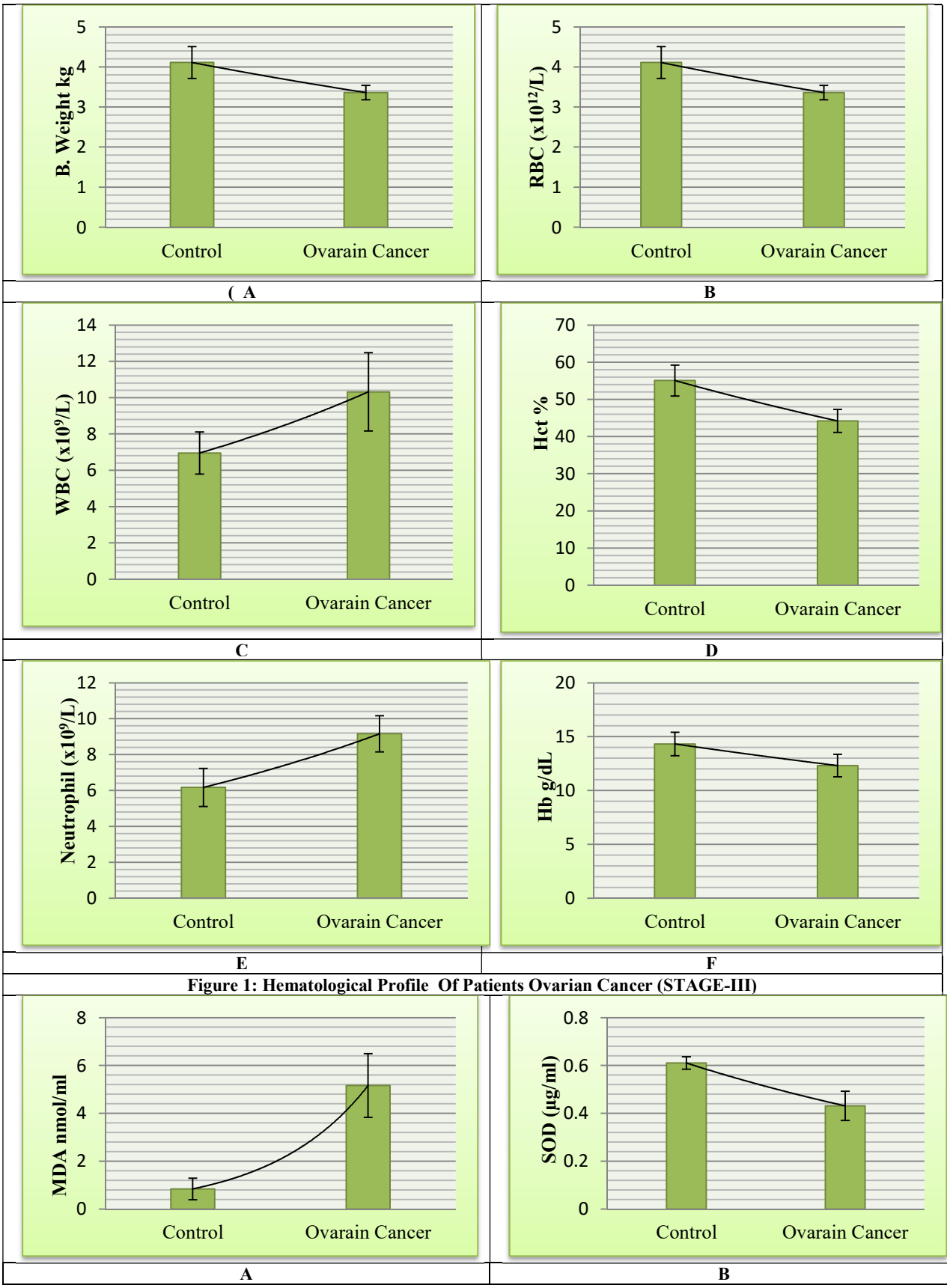

Figure 1: Hematological Profile Of Patients Ovarian Cancer (STAGE-III)

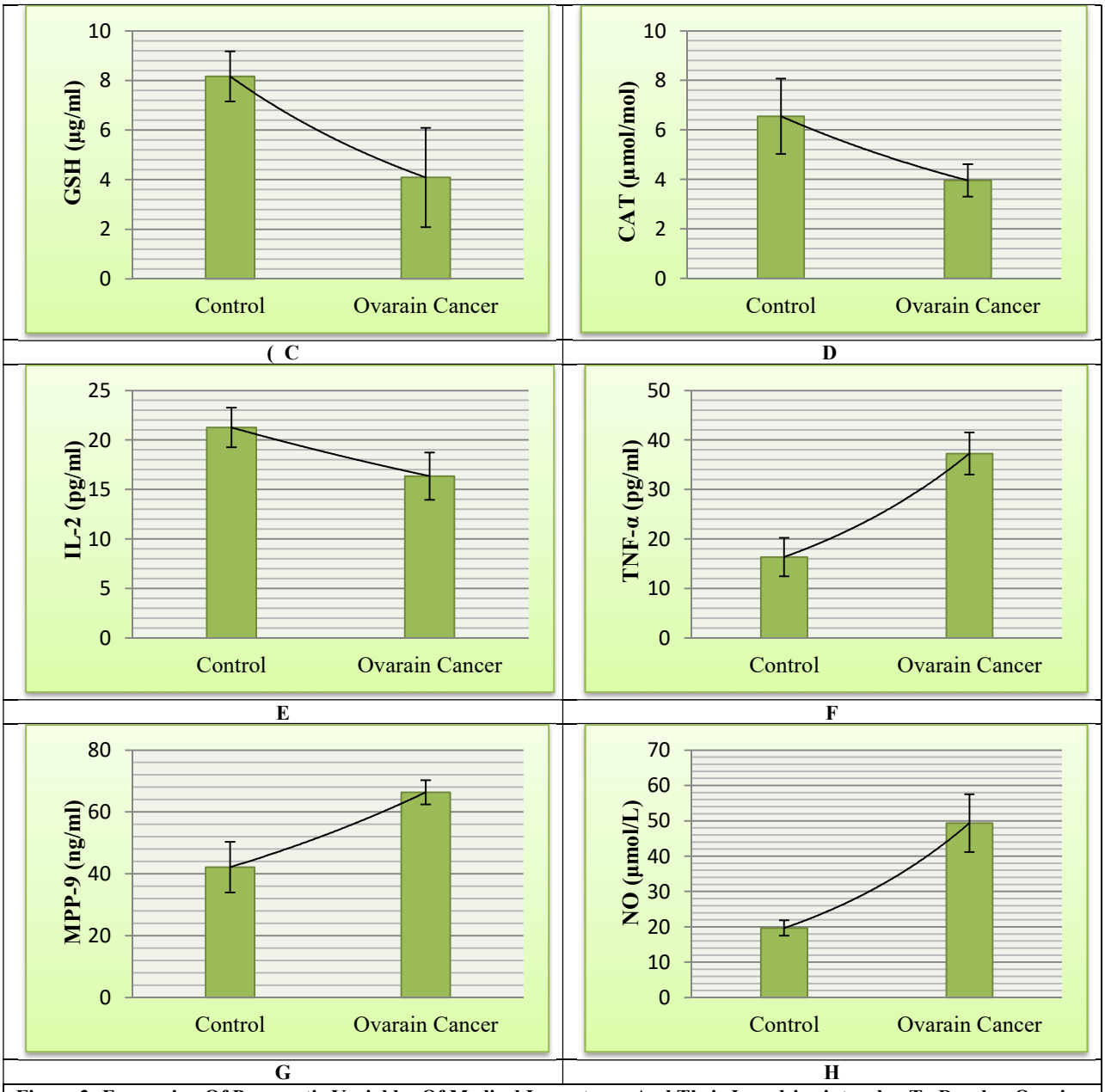

Figure 2: Expression Of Prognostic Variables Of Medical Importance And Their Impulsive interplay To Develop Ovarian Cancer (Stage-III)

DISCUSSION

An accumulating body of evidence suggested that various factors such as pelvic inflammatory diseases, endometriosis and ovulation are strongly linked to inflammation of ovarian surface epithelium (OSE) and high risk of epithelial ovarian cancer. Specially, different cytokines and inflammatory markers are expressed by innate immune cells, including IL-6, TNF-α and IL-1β, which have been strongly involved to stimulate ovarian cancer growth, progression and angiogenesis **[14]**. However, one of the most significant hypotheses regarding to

ovarian cancer carcinogenesis was the ovulation theory. This theory is based on the mechanism of inflammation, which is accomplished by incessant ovulation **[15]**. As a result of incessant ovulation, the ovarian surface epithelium is exposed to oxidative stress and inflammatory mediators, which consequently lead to malignant transformation. On the other hand, the ovulatory process works with the repair steps after the secretion of the ovum under physiological conditions. This phenomenon is distinguished by the formation of huge amount of chemokines, cytokines, and matrix remodeling enzymes, such as matrix metalloproteinases, collagenases, prosta-glandin, plasminogen activator, interleukins, growth factors and bioactive eicosanoids **[16]**. The recent research work suggested that clear cell cancer, endometroid cancer and high grade serous ovarian cancer originate from fallopian tube epithelium. Consequently, retrograde menstruation occurs which is derived from iron induced oxidative stress. Intriguingly, fimbriae of fallopian tube which is floating in peritoneal fluid may exposed to catalytic action of iron and ROS that is synthesized by activated macrophages and inflammatory cytokines. Both oxido-reductive fallopian tube destruction and incessant ovulation play key role in inflammatory mechanism that are responsible for the development of ovarian cancer. The increasing trends of evidences explain that genetic factors are significantly involved in the tumor microenvironment and stromal events in the tumor cells **[17]**. Moreover, abberrant expression of peritoneal and stromal cell ovarian cancer along with lymphocytes constituents and inflammatory cytokines are strongly involved in the growth of ovarian malignancy. Formation of cytokines by tumor cells can both stimulate their development and hampers apoptosis Inflammation has a potent role in every step of carcinogenesis, such as promotion, progression and initiation. Contrary to that, tumour cells can synthesize immunogenic proteins that induce anti-neoplastic interaction. The tumor cells utilized these immunological responses to avoid from destruction and recognition by immune cells **[18]**. One of such example is HLA-G secretion to hamper natural killer (NK) cells activity and Fas ligand formation to trigger lymphocyte apoptosis.

CONCLUSION

The findings of the present study indicated that oxidative and nitrosative stress pathways have significant importance in the development of ovarian cancer. The oxidative stress mechanism is responsible for

the lipid peroxidation, DNA damaging and secretion of inflammatory cytokines including interleukin-2 and TNF-α, which cause inflammation in the patients with ovarian cancer. These inflammatory markers may lead to the secretion of matrix metallorproteinase-2 (MMP-2), that causes ECM degradation in the epithelial lining of the ovary. Thereby, it has been concluded that all these prophetic variables have potent role in the progression of ovarian cancer.

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