Full Length Research Article

ASSESSMENT OF MDA IN ADJUVANT WITH CA-125 IN EARLY OVARIAN CANCER DETECTION

*[1]Aseem Kumar Anshu, [2]Shailendra Kumar, [3]Shreya Sinha, [1]Akhileshwari Nath, [1]Priyanka, [4]Singh, J. K. and [5]Sanjesh Kumar

[1]Reseach Center, Mahavir Cancer Institute and Research Center, Patna, Bihar, India
[2]Department of Biochemistry, Buddha Institute of Dental Sciences & Hospital, Patna
[3]Department of Medicine, AIIMS, Patna
[4]Department of Oncology, SS Hospital and Research Institute, Kankarbagh, Patna
[5]Department of Pharmacy Practice, National Institute of Pharmaceutical Education & Research, Hajipur, India

ARTICLE INFO

ABSTRACT

Ovarian cancer (Oca) alone accounts for 16% of all cancers in the female and is the fifth most common form of cancer in the world. As Oca offers poor prognosis, CA-125 is a sole biomarker for the detection of Oca besides histological techniques. CA-125, also known as MUC16 is a membrane bound glycoprotein which is found in higher concentration in blood of Oca patients. In addition to multiple factor endorsing ovarian carcinoma, oxidative stress has also been inscribed to be critical factor in tumor development. The level of oxidative stress on the cells increases as polyunsaturated fatty acid (PUFA) is degraded to generate Malondialdehyde (MDA). The survival percentage of the cell diminishes as the integrity of the plasma membrane slowly falls, leading to apoptosis. MDA can be adopted as supportive biomarker for ovarian cancer along with CA-125 and to confirm, present investigation has been undertaken. LPO (Lipid Peroxidation) assay was performed to measure the MDA level in blood serum collected from 125 ovarian cancer patients with their consent. CA-125 was estimated by ELISA kit method, RBC count and Hemoglobin level were measured by routine method. Mean±S.D of MDA level of OCa patients was higher than normal women, whereas patients of postmenopausal condition had even higher value than premenopausal patients with P<.001. Mean±S.D of CA-125 in patients of postmenopausal condition is lower than premenopausal patients, however both quite higher than normal women with significance <0.0001. RBC count and hemoglobin levels of OCa patients were lower than normal women with p-value <0.0018 and 0.001 respectively. Oxidative stress is directly related with oxygen carrier components and histological study unravels several substantial aspects of anatomy of types of ovarian cancer tissue. Analysis of MDA could directly reflect the total stress on the body and the advancement of the cancer. In the nutshell, analysis of MDA in addition to CA-125 could produce better prognosis and help decide the survival rate and relevant therapy.

INTRODUCTION

Tumors of the ovary are most common form of neoplasia in women and most primary neoplasm in the ovary fall within tumors of mullerian epithelium which contains three major types of such tumors:- serous, endometrioid and mucinous tumors (Young *et al.*,1994). Around, 80-85% of all ovarian cancer is alone covered by serous tumors and another major portion as much as 10% by endometroid carcinoma, predominantly in industrialized countries (Seidman *et al.*, 2004). Tumors of mullerian epithelium are classified under ovarian surface epithelium (OSE) and its cystic derivatives. All cell types of the ovary may undergo neoplastic transformation but majority (80-90%) of benign and malignant tumors is derived from ovarian surface epithelium (Bell, 1991 and Scully, 1995). The "ovulation" hypothesis describes the causal mechanism of ovarian carcinoma. According to this theory, trauma induced by repeated cycles of ovulation and repairs of surface epithelial cells at the site of ovulation, without pregnancy-induced rest period, might contribute to

*Corresponding author: Aseem Kumar Anshu
Reseach Center, Mahavir Cancer Institute and Research Center, Patna, Bihar, India

development of ovarian carcinoma (Cramer *et al.*, 1983 and Rich 1998). The majority of cases of OCa are sporadic while about 5% to 10% familial. Histopathological studies are still most common technique in cancer research other than several biomarkers used for prognosis and to identify the cancer, worldwide. Histopathological classification is based upon characteristics seen under light microscopy of biopsy specimens. On gross examination, the risk of malignancy increases as a function of the amount of palpable solid epithelial growth, including papillary projections of soft tumor, thickened tumor lining the cyst spaces or solid necrotic friable tissue depicting necrosis. In addition, because many of these ovarian neoplasms cannot be detected early in their development, they include a disproportionate number of fatal cancers, being responsible for almost half of death from human genital tract cancers. Cancer antigen-125 (MUC16) was first identified (Bast *et.al.,* 1981) in 1981 with the help of a monoclonal antibody (OCA125) that had been development from mice immunized with an OCa cell line. Although CA-125 antigen was first detected over 20 years ago but still scientific and medical communities have gathered very little information about it.

CA-125 is a high molecular mass glycoprotein, its size ranging from 200-2000 KDa with smaller "subunits" (Davis *et al.*, 1986, Matsukoa *et al.*, 1987, Nagata *et al.*, 1991, De los Frailes *et al.*, 1993, Kobayashi *et al.,* 1993). It has been indicated that CA-125 is a typical mucin with a high carbohydrate content and a preponderance of serine and threonine (*O*-linked) glycan chains (Lloyd *et al.*, 1997 and Lloyd *et al.,* 2001). MUC16 is also reported to stimulate the metastasis of tumor cells. MUC16 specifically binds with mesothelin, a glycoprotein normally expressed by mesothelial cells of the peritoneum (Rump *et al.*, 2004). Such an interaction of MUC16 and mesothelin is thought to promote further advancement in tumor cell invasion of peritoneum (Gubbels *et al.*, 2006). Oxygen free radicals (OFR) and Reactive oxygen species are by-product of normal cellular metabolism. The role of ROS in human diseases, toxicology and deterioration of food has drawn attention of many scientists especially oncologists, who are trying to find out deleterious effect of free radicals in the cancerous tissue. The aerobes like humans use oxygen for their metabolic function during which the free radicals are produced which can be used during phagocytosis of foreign particles (Root *et al.*, 1981), and can produce DNA damage (Von Sonntag, 1987) or lipid peroxidation (Henning and Chow, 1988).

The major site for free radical production is mitochondria, while electron transport chain is under process. Even under ideal conditions, some electrons "leak" from electron transport chain and interact with oxygen to produce superoxide radicals (Salvador *et al.*, 2001). A mutation in SDHC gene results in overproduction of superoxide anion (O2) from mitochondria (Ishii *et al.*, 2005) and other component involved in ROS generation is cytochrome P450 which performs detoxification of toxic compounds in the cell. Moreover, monoxygenase system found in Endoplasmic reticulum oxidizes the foreign compounds in order to produce superoxide radicals due to leakage of electrons (Butler and Hoey, 1993). However, organisms have developed system to protect themselves form superoxid radicals by antioxidant enzymes viz-superoxide dismutase, catalase and peroxidases. These enzymatic antioxidants help removing superoxide radicals. Other more favorable options take place when free radical is captured by a scavenger, such as: Ascorbate, tocopherol, CoQH2 (reduced coenzyme Q10), urate and glutathione. In addition to that, ascorbate and tocopherol function together to eliminate lipid peroxides from cell (Buetner, 1993). Polyunsaturated fatty acids (PUFA) is attacked by ROS to degrade it to generate Malondialdehyde (MDA) which results in reduction of fluidity of the plasma membrane and hence the resistivity of the cell.

More than 30% of patients of epithelial ovarian cancer suffer from anemia at the time of initial stages and such cancer related anaemia further aggrandize to from even more aggressive tumors (Obermir *et al.*, 1998). Abundant iron is amassed in bone marrow but iron reutilization efficiency is vitiated as pointed by increased ferritin levels but decreased serum iron levels and iron binding capacity (Bron *et al.*, 2001). In cancer related anemia, erythroid progenitor cells respond normally to erythropoietin, but erythropoietin secreted by renal cells in kidney does not respond optimally (Nowrousian *et al.*, 1996). It has been demonstrated that proinflammatory cytokines which are highly produced in malignant tumors and intensified oxidative stress directly result in both development of anemia and resistance to recombinant erythropoietin (Means, 2000). In this study, an attempt has been made to correlate MDA with erythrocyte count and hemoglobin levels in patients of Oca.

MATERIALS AND METHODS

An intravenous blood was taken from 125 patients suffering from ovarian cancer and 80 normal women and stored at $18^{0}C$. Ovarian cancer patients were categorized as two groups premenopausal Oca and postmenopausal Oca. Tissues of 23 patients out of 125 OCa patients and 11 non-cancerous were also collected for histopathological study. OCa patients were further bifurcated as cohort A and cohort B.

Cohort A- 70 ovarian cancer patients were categorized under premenopausal condition, and

Cohort B- 55 ovarian cancer patients were categorized under postmenopausal.

The group of normal women contained both conditions (pre and post menopause).

a. LPO Assay

The whole method was followed according to modified protocol of Ohkawa *et al.*, 1979 for lipid per oxidation in serum. 2.5ml of 10% TCA was added in each 0.5 ml of test serum. The reaction mixture was incubated at $95^{0}C$ for 15 minutes followed by centrifugation at 3000 rpm for 10 minutes. The supernatant was collected and 1 ml of 0.675% of TBA was added and kept for incubation at $95^{0}C$ for 15 minutes. With the help of spectrophotometer, O.D at 532 nm was taken.

b. CA-125 estimation

100μl of CA-125 standard, test serum into appropriate wells and then, enzyme conjugate Reagent were dispensed into each

well. The solution was mixed well for 30 seconds and incubated at 37^0C for 90 minutes. The reaction mixture I the wells was emptied and washed 5 times with IX was buffer. 100µl of TMB Reagent was dispensed into each well and gently mixed for 10 seconds, then incubated at room temperature in dark for 20 minutes. Reaction was stopped by adding stop solution to each well and waited until blue color changes to yellow color completely. Optical density was read at 450 nm with micro titer plate reader within 15 minutes.

c. Hematological Parameter

RBC count and Haemoglobin level were estimated by standard procedures using Cell Counter (Medonic M Series) in the Department of Haematology, Mahavir Cancer Institute, Patna.

d. Histopathological procedure

Histopathological parameter was studied by collecting tissues of breast cancer patients from operation theatre. Tissues were fixed in 10% formalin and dehydrated in ascending order of alcohol concentration. Tissues were kept in paraffin wax and blocks were prepared. Section was cut and fixed on slide with the help of mayer's solution. Double staining was done and the slides were kept on xylene and hydrated in descending alcohol concentration. The slides were stained with haematoxylin and dehydrated upto 70% alcohol. Again, the slides were stained with eosin and then dehydrated in 90% and absolute alcohol and the slide were mounted with DPX and were seen under microscope.

e. Statistical analysis

Graphical representations, Mean±SD and p-values were obtained using SPSS 16.0 software (statistical package for social sciences, version 16.0). One way analysis of variance between Normal, Premenopausal OCa and Postmenopausal OCa was performed to obtain p-values, p-value<0.05 was considered significant. Moreover, paired sample t-test was also done to evaluate p-value between normal and Cohort A and normal and Cohort B.

RESULTS

The values for MDA levels in Oca patients were compared with the values of MDA level obtained in normal persons and according to data, ovarian cancer patients have predisposition to produce massive amount of MDA in contrast with normal persons. Furthermore, ovarian cancer patients were grouped under cohort A and cohort B (pre and post menopausal conditions). Mean MDA levels were analyzed according to different menopausal conditions of the patients and an interesting result have been squeezed out. According to figure 1, mean±S.D of MDA in normal women was found to be 23.56±4.3 whereas that of cohort A produced means ±S.D as 34.537±5.25, mean±S.D of MDA for cohort B was calculated to be 38.0533±7.026 (nMol/ml). The p-value obtained for MDA levels in both normal and Oca was P<.001. 2-tailed p-value for normal and Cohort A was <0.0001 and normal and Cohort B <0.0001. CA-125 was estimated in the ovarian cancer patients and classified according to values obtained for lipid peroxidation.

Mean±S.D of CA-125 levels were calculated and graph was plotted against lipid peroxidation. According to calculated data, mean of MDA level in ovarian cancer patients was found to be significantly higher than normal women who had CA-125 below 35 U/ml. The tallest peak of mean value of MDA was witnessed in patients with CA-125 levels greater than 500 U/ml and relatively low peaks in patients with CA-125 within 100-500 U/ml in figure 2A.
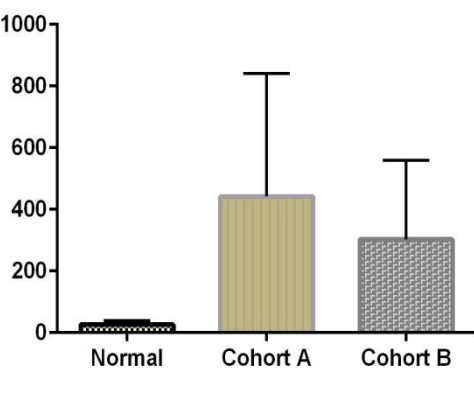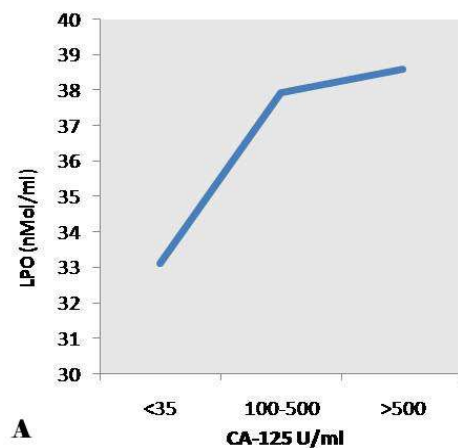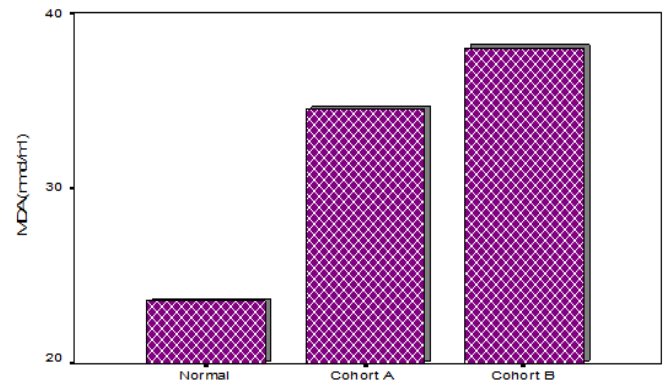

Fig. 02. Levels of MDA in ovarian cancer patients with different range of CA-125 levels (A), Mean CA-125 values in normal persons and ovarian cancer premenopausal and postmenopausal conditions (B)

The CA-125 for normal persons was set standard to be 35U/ml. According to text figure 2 (A), mean±S.D of MDA patients with CA-125 lower than standard normal value i.e. lower than 35 U/ml was calculated to be 30.10±6.65 and higher level of CA-125 i.e. greater than 500 U/ml which is increased up to 38.59±5.15 nMol/ml. It can be observed that

CA-125 level is significantly higher in both conditions of ovarian cancer (fig. 2(B)). The mean±S.D CA-125 level was measured to be 26.05±12.50 in normal women. On the other hand, mean±S.D values of cohort A ovarian cancer were obtained as 442.35±399.095 and cohort B was 302.5±256.5 (U/ml), with the significance p-value<0.0001. In addition, 2-tailed p-value for normal and Cohort A was found to be <0.001 and that of normal and Cohort B was <0.0001. RBC count and haemoglobin level were estimated for the patients of ovarian cancer. The graphs plotted according to data obtained showed an apparent decrease in mean value of RBC count and haemoglobin level which is manifested by cancer related anemia. But somehow, mean RBC count of Oca patients in cohort B is demonstrated to outnumber mean RBC count in patients in cohort B.
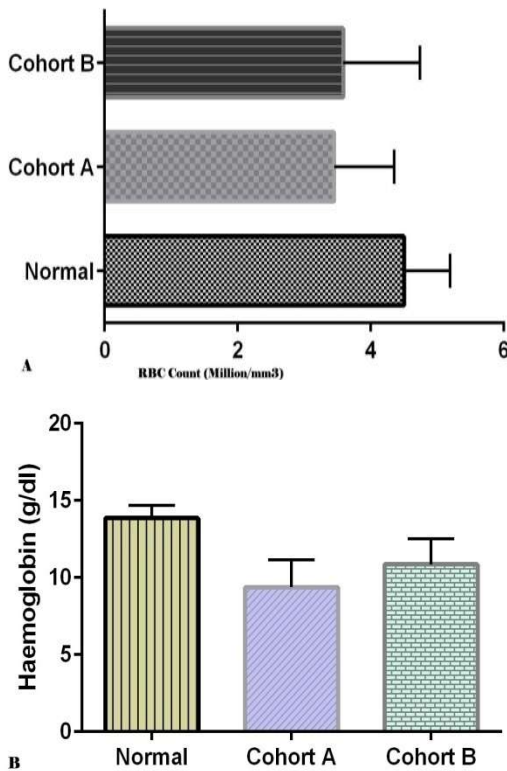

Fig. 03. Showing (A) mean RBC count in million per mm³ for normal person and pre and postmenopausal conditions of Oca patients and (B) mean hemoglobin level in g/ml for normal persons and pre and postmenopausal conditions of Oca patients

Table 1. Mean±S.D of MDA, CA-125, RBC count and Haemoglobin level

	Normal persons	Cohort A	Cohort B	p-value
MDA (nMol/ml)	23.56±4.3	34.537±5.25	38.0533±7.026	<0.001
CA-125 (U/ml)	26.05±12.50	442.35±399.095	302.5±256.5	<0.0001
RBC Count (x10⁶/mm³)	4.5±0.68	3.129±0.7	3.863±0.429	<0.0018
Haemoglobin (g/dl)	13.85±2.15	9.38±1.41	10.866±1.72	<0.0001

RBC count and Haemoglobin level exhibit similar kind of pattern in text figure 3 as both are directly correlated and act mainly as oxygen carrier. In fig 3 (A), Mean ±S.D of RBC count in normal persons obtained the value 4.5±0.68

(million/mm³) whereas in cohort A ovarian cancer patients, value was obtained to be 3.129±0.7 but in cohort B patients, it was 3.863±0.429 (x10⁶/mm³) with p-value<0.0018. While analyzing fig. 3 (B), it becomes clear that hemoglobin level wanes in ovarian cancer patients of both cohort A and B when compared with normal range. After calculating mean±S.D of hemoglobin level of normal persons, it was found to be 13.85±2.15 and that of cohort A ovarian cancer was 9.38±1.41 whereas cohort B ovarian cancer patients remained at 10.866±1.72 (g/dl) with significance value <0.0001. The p-value of t-test between normal and Cohort A was also <0.0001 and that of between normal and Cohort B was <0.0001. Serous carcinoma (fig. 4, E & F) depicts a wide range of Histopathological appearances, which are unequivocally distinct from normal ovarian section (fig. 4, A & B). Nuclei are intermediate and uniform in size.

Papillary and micropapillary architecture is quite evident in most of the serous carcinoma with apparent slot-like spaces, at least focally. Serous carcinoma possesses columnar cells with pinkish cytoplasm as demonstrated clearly in fig. 4 E&F. The papillae outgrows from surface of ovary into lumen. Complex papillary and some cyst formations can also be observed. Although, papillae with smaller branches in low grade serous tumors conspicuously unravels fibrovascular cores. Columnar cells are piled up many layers as to gorge papillae. Nuclear pleomorphism of low grade is common and mitotic count shoots up and scattered in entire area (fig. 4 E & F). Endometroid ovarian carcinoma resembles quite closely with its uterine counterpart. Anatomical pattern demonstrates tubule, solid, sheet like growth and papillary outlining can easily help us identify endometroid carcinoma as seen in fig. 4 (C & D). Lack of nuclei and fusiform structure of cytoplasm are prominent. As depicted in microphotograph of fig. 4 (C & D), such endometroid carcinoma shows blood vessels at some sites.

DISCUSSION

The spectrophotometric reading of samples interpreted higher MDA level in ovarian cancer than normal person (Fig. 1); similar results have been proclaimed in polysystic ovarian syndrome (Sabuncu *et al.*, 2001, Mohan and Vishne, 2009). The for increment in MDA level is attributed to higher oxidative stress bore by ovarian cancer patients as increased concentration of ROS and free redicals work out to degrade cellular components and diminish antioxidant concentration (Sanjyotoi and Melinker, 2011, Weinstein *et al.*, 2000). MDA possesses intensified action of cytotoxic and inhibitory effect on several protective enzymes and provoke carcinogenicity and tumor development (Manimaran and Rajneesh, 2009). Patients with postmenopausal status delivered higher TBARS level in blood serum due to morbidity of ovarian cancer when compared with premenopausal patients. The reason behind why cohort B patients suffered greater oxidative stress could be major deterioration of cellular components and their functionality that characterizes the process of aging (Yu, 1993). It has been proposed that aging is provoked by increase in oxidative damage to bimolecular like lipid, protein or DNA including hassle in development process governed by gene regulation (Yu, 1993, Sohal, 1993, Lora ER and Hornbrook, 1997). Sex-dependent differences in TBARS level in tissue
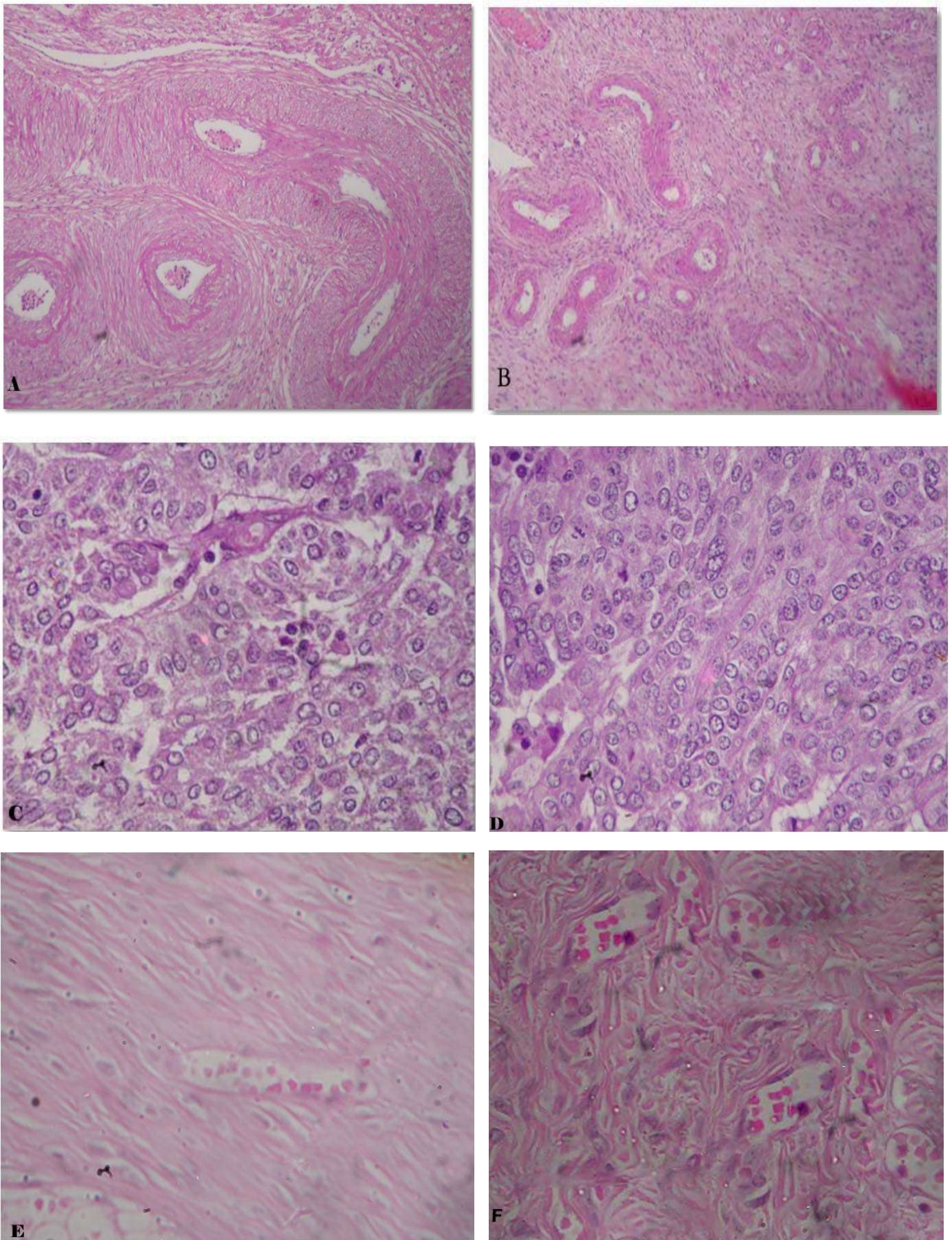
Fig. 4. Photomicrograph (X400) of (A) & (B) Normal ovarian tissue section, (C) & (D) Endometroid carcinoma with papillary change and (E) & (F) Serous cyst adenoma with papillary growth
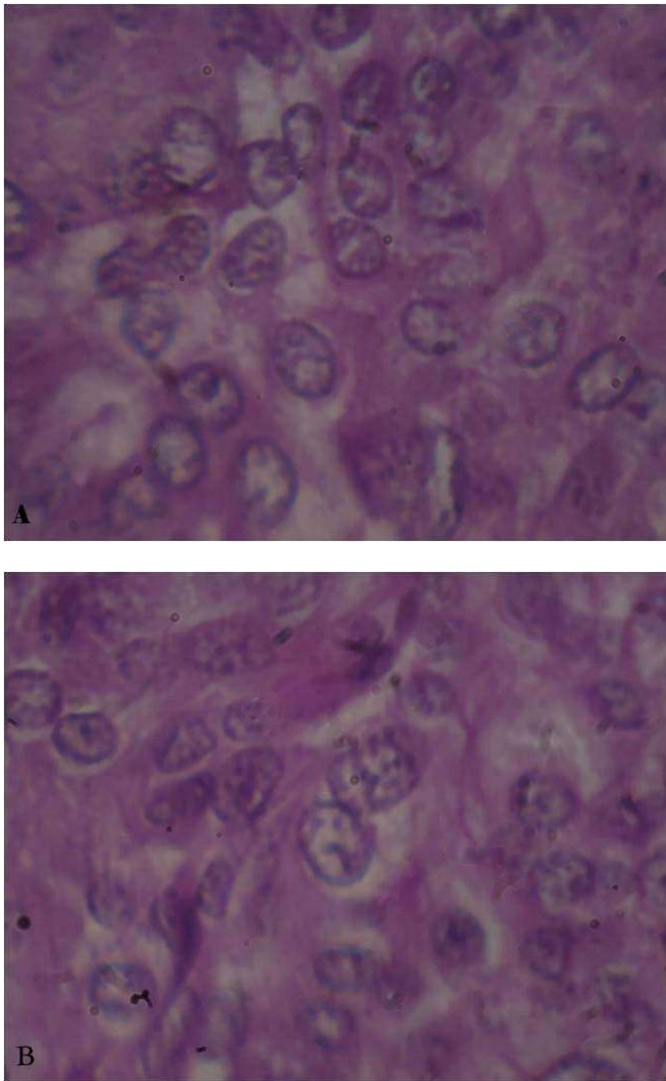
Fig. 5. Photomicrograph (X1000) of serous carcinoma (A) adhesion of nuclei (arrow), diffusion of cytoplasm all over the tumor area (arrow), (B) blebbing cytoplasm (arrow) and perichromatic nuclei can be seen

due to the effect of aging has been reported in liver and brain of Winstar rats (Chen and Yu, 1994, Rikans *et al.*, 1991, Łukaszewicz-Hussai *et al.*, 2007, Nuria Sanz *et al.*, 1996, Lee *et al.*, 2008, Pieri *et al.*, 1992, Pulla Reddyetal *et al.*, 1994). Moreover, estrogen effect acts as carcinogen and reinforces severity of Oca and has direct relationship with menopausal conditions (Shuk-Mei, 2003, Issa *et al.,* 2009). However, study on estrogen effect and oxidative stress related to aging and cancer could unveil a lot more information. Since CA-125 is detected in serum which gets detached from plasma membrane, lipid peroxidation might pay a pivotal role in liberation of this membrane bound glycoprotein. Higher MDA level signifies enervated plasma membrane and loss of PUFA content, which allows departure of CA-125 from plasma membrane and loss of PUFA content, which allows departure of CA-125 from plasma membrane as manifested by fig 2A. As MDA level goes higher, CA-125 simulates its progression. However, it is significant to note that the cohort B Oca produce lower CA-125 than cohort A patients. But the same is not valid when fig. 1 and fig. 2b are correlated in case of cohort A and cohort B Oca patients. Mean value of CA-125 in

cohort A is higher than the cohort B Oca patients unlike MDA levels in these groups. A report claims, re-oxygenation damages cells due to increased oxygen free radicals generation from endothelial cells, parenchyma cells and infiltrating leukocytes (Anaya-prado *et. al*., 2002, Thiagarajan, *et al*., 1997) and according to fig. 3A & B, RBC count and hemoglobin level are pretty higher in cohort B than cohort A, which means more oxygen supply to tissue in cohort B than in cohort A, this is possible reason why postmenopausal ovarian cancer patients suffers great deal of oxidative stress which is due to incomplete electron transfer or reduction of oxygen by damaged mitochondria (Kaminski, *et.al.,* 2002) and cellular antioxidant defense mechanism is also vitiated. Bone marrow stem cells are self-renewing and can maintain normal hemoglobin level for a lifetime. Committed stem cells that differentiate and multiply through different erythroblast stages are responsible for production of RBCs. Nephron senses hypoxia, and the kidney responds to it by producing erythropoietin.

A tumor oxygenation or erythropoietin dose of regular schedule which is associated with improved with improved survival in patients with various malignancies has been used to treat anemia in cancer (Albain *et al.*, 1991, Bookemeyer *et al.*, 2002, Dunst *et al.,* 2003, Brian *et al.,* 2005). According to a report, Breast cancer erythropoietin survival Trial (BEST) has been devised to determine the effect of Hemoglobin (Brian *et al.,* 2005). RBC count and hemoglobin levels show similar pattern in figure 3, which explict that RBC count and hemoglobin are indubitably correlated. As depicted in fig. 4, endometroid carcinoma (fig. 4C & D) and serous cyst-adenoma (fig. 4E & F) contain increased number of nuclei with clearly visible nucleolus. Mitotic count can be heeded to be significantly elevated in number and hence outnumbering normal cell mitotic count. When fig. 5A & B scrutinized, it was observed that there is a heavy diffusion and irregularities of cytoplasm which answers most of our questions. Due to attack of ROS, PUFA gets degenerated and plasma membrane become weakened and can be easily ruptured. In microscopic study of tissue on X100 magnification, spewing cytoplasm indicated that plasma membrane no longer provides support and integrity. Moreover, other organelles are damaged too.

Conclusion

As discussed previously, MDA level in ovarian cancer patients, in either pre or postmenopausal condition, are analyzed to be higher than normal and similarly overall CA-125 level is also shown to be higher than normal. Re-oxygenation has been shown to generate more ROS from damaged mitochondria. Histopathological study has revealed the action of ROS and loss of plasma membrane ultimately. It would not be unwise to conclude that when MDA level analyzed along with CA-125 can have capability to produce better prognosis than CA-125 alone. Moreover the solemnity of the ovarian cancer in the patients can only be established with the help of analysis of MDA along with CA-125 and histological studies. Moreover, elevated MDA also signifies the advancement of the cancer and helps clinicians decide the therapy accordingly.

REFERENCES

Albain, KS., Crowley, JJ., LeBlanc, M. *et al*. 1991. Survival determinants in extensive-stage non-small-cell lung cancer: The Southwest Oncology Group experience. *J. Clin Oncol*; 9:1618-1626.

Anaya-prado, R. *et al*. 2002. Ischemia/Reperfusion injury. *J Surg Res*; 105:248

Bast, RC., Feeney, M., Lazarus, H., Nadler, LM., Colvin, RC. and Knapp, RC. 1981. Reactivity of a monoclonal antibody with human ovarian carcinoma. *J. Clin. Invest.*; 68:1331-1337.

Bell, DA. 1991. Ovarian surface epithelial-stromal tumors. *Hum Pathol.*; 22:750-762.

Bookemeyer, C., Oechsle, K., Hartmann, JT. *et. al*. 2002. Treatment-induced anemia and its potential clinical impact in patients receiving sequential high dose chemotherapy for metastatic testicular cancer. *Br J Cancer*; 87:1066-1071.

Brian, LJ., Vladimir, S., Marek, P. *et al*. 2005. Maintaining normal hemoglobin Levels with epoetin alfa in mainly nonanemic patients with metastatic Breast cancer receiving first line chemotheraphy: A survival study. *J of Clin*; 23:5962-5972.

Bron, D., Meuleman, N. and Mascaux, C. 2001. Biological basis of anemia. *Semin Oncol.*; 28:1-6.

Buetner, GR. 1993. The pecking order of free radicals and antioxidants: lipid peroxidation, α-Tocopherol, and Ascorbate. *Arch. Biochem. Biophys.*; 300:535-543.

Butler, J. and Hoey, BM. 1993. The one – electron reduction potential of several substrates can be related to their reduction rate by cytochrome-p450 reductase. *Biochem, Biophys Acta*; 1161:73-78.

Chen, JJ. and Yu, BP. 1994. Alterations in mitochondrial membrane fluidity by lipid peroxidation products. *Free Radic. Biol. Med.*; 17:411-418.

Cramer, DW., Hutchison, GB., Welch, WR., Scully, RE. and Ryan, KJ. 1983. Determinants of ovarian cancer risk. in Reproductive experiences and family history. *Natl Cancer Inst.*; 71:711-716.

Davis, HM., Zurawski, VR., Bast, RC. Jr. and Klug, TL. 1986. Characterization of the CA 125 antigen associated with human epithelial ovarian carcinomas. *Cancer Res.*; 46:6143-6148.

De los Frailes, MT., Stark, S., Jaeger, W., Hoerauf, A. and Wildt, L. 1993. Purification and characterization of the CA 125 tumor-associated antigen from human ascites. *Tumour Biol.*; 14:18-29.

Dunst, J., Kuhnt, T., Strauss, HG. *et al*. 2003. Anemia in cervical cancers: Impact on survival, pattern of relapse, and association with hypoxia and angiogenesis. *Int J Radiat Oncol Biol Phys*; 56:778-787.

Gubbels, JA., Belisle, J., Onda, M., Rancourt, C., Migneault, M., Ho, M., Bera, TK., Connor, J., Sathyanarayana, BK., Lee, B., Pastan, I. and Parankar, MS. 2006. Mesothelin-MUC16 binding is a high affinity, N-glycan dependent interaction that facilitates peritoneal metastasis of ovarian tumor. *Mol. Cancer*; 5: 50. Doi:10.1186/1476-4598-5-50

Henning, B. and Chow, CK. 1988. Lipid peroxidation and endothelial cell injury: implications in atherosclerosis. *Free Radiation Biol. Med.*; 4:99-106

Ishii, T., Yasuda, K., Akatsuka, A., Hino, O., Philip, S. and Hartman, Ishii, N. 2005. A Mutation in the SDHC Gene of Complex II Increases Oxidative stress, resulting in Apoptosis and Tumorigenesis. *Cancer Res.*; 65:203-209.

Issa, RM., Lebean, A., Grob, T. and Holest, F. 2009. Estrogen receptor gene amplification occurs rarely in ovarian cancer. *Modern Pathology*; 22(2):191-196.

Kaminski, KA. *et al*. 2002. Oxidatives stress and neutrophil activation-the two keystone of Ischemia/reperfusion injury. *Int J Cardiol*; 86:41.

Kobayashi, H., Ida, W., Terao, T. and Kawashima, Y. 1993. Molecular characteristics of the CA125 antigen produced by human endometrial epithelial cells: comparison between eutopic and heterotopic epithelial cells. *Am. J. Obstet. Gynecol.*; 169:725-730.

Lee, LM., Noor, AAH. and Yasmin, AM. Y. 2008. Effects of Palmvitee on Status of Superoxide Dismutase and Glutathione Peroxidase in Rat Liver during Aging. *Malaysian Journal of Biochemistry and Molecular Biology*; 11:114-117.

Lloyd, KO. and Yin, BWT. 2001. Synthesis and secretion of the ovarian cancer antigen CA 125 by the human cancer cell line NIH: OVCAR-3. *Tumor Biol.*; 22:77-82.

Lloyd, KO., Yin, BWT. and Kudryashov, V. 1997. Isolation and characterization of ovarian cancer antigen CA125 using a new monoclonal antibody (VK-8): identification as a mucin-type molecule. *Int. J. Cancer*; 71:842-850.

Lora, ER. and Hornbrook, KR.1997. Lipid peroxidation, antioxidant protection and aging. Biochimica et Biophysica Acta (BBA) Molecular Basis of Disease; 1362:3116–127.

Lukaszewicz-Hussain, A., Moniuszko-Jakoniuk, J. and Rogalska, 2007. Assessment of Lipid Peroxidation in Rat Tissues in Subacute Chlorfenvinphos Administration. *Polish J. of Environ. Stud.*; 16:233-236.

Manimaran, A. and Rajneesh, CP. 2009. Activities of Antioxidant Enzyme and Lipid Peroxidation in Ovarian Cancer Patients. *Academic Journal of Cancer Research*, 2:68-72.

Matsukoa, Y., Nakashima, T., Endo, K., Yoshisa, T., Kunimatsu, M., Sakahara, H., Koizumi, M., Nakagawa, T., Yamaguchi, N. and Torizuka, K. 1987. Recognition of ovarian cancer antigen CA125 by murine monoclonal antibody produced by immunization of lung cancer cells. *Cancer Res.*; 47:6335-6340.

Means, RT Jr. 2000. The anemia of infection. *Baillieres Best Pract Res Clin Haematol*; 13:151-162.

Mohan, SK. and Vishne, PV. 2009. Lipid peroxidation, glutathione, Ascorbic acid, vitamin E, antioxidant enzyme and serum homocysteine status in patients with polycystic ovary syndrome. *Biology and Medicine*; 1: 44-49.

Nagata, A., Hirota, N., Sakai, T., Fujimoto, M. and Komoda, T. 1991. Molecular nature and possible presence of a membranous glycan-phosphatidylinositol anchor of CA125 antigen. *Tumour Biol.*; 12:279-286.

Nowrousian, MR., Kasper, C. and Oberhoff, C. 1996. Pathophysiology of cancer –related anemia. In: Smythe J, ed. RHu Erythropoietin in Cancer Supportive Treatment. New York, NY: Marcel Dekker; 13-34.

Nuria Sanz, Carmen Diez-Fernfindez and Marfa Cascales, 1996. Variations of hepatic antioxidant systems and D N A ploidy in rats aged 2 to 8 months, *Biochimica et Biophysica Acta*; 1315:123-130.

Obermir, A., Handisurya, A., Kaider, A. *et al.* 1998. The relationship of pretreatment serum hemoglobin level to the survival of epithelial ovarian carcinoma patients: a prospective review. *Cancer*; 15:726-731.

Ohkawa, H., Ohishi, N. and Yagi, K. 1979. Assay for lipid peroxidates in animal tissues by thiobarbituric acid reaction. *Anal Biochem*; 95:351-8.

Pieri, C**.**, Falasca, M**.**, Marcheselli, F**.**, Moroni, F**.**, Recchioni, R., Marmocchi, F. and Lupidi, G. 1992. Food restriction in female Wistar rats: V. Lipid peroxidation and antioxidant enzymes in the liver. Arch Gerontol Geriatr.; 14:93-9.

Pulla Reddy, AC. and Lokesh**,** BR. 1994. Effect of dietary turmeric (curcuma longa) on iron-induced lipid peroxidation in the rat liver. Food and Chemical Toxicology, 32:279–283.

Rich, HA. 1998. Hormonal etiology of epithelial ovarian cancer, with a hypothesis concerning the role of androgens and progesterone. *Natl Cancer Inst.*; 90:1774-1786.

Rikans, LE., Moore, DR. and Snowden, CD. 1991. Sex-dependent differences in the effects of aging on antioxidant defense mechanisms of rat liver. *Biochim. Biophys. Acta*, 1074:195-200.

Root, RK. and Cohen, MS. 1981. The microbicidal mechanisms of human neutrophils and eosinophils. *Rev. Infect. Dis.*; 3:565-598.

Rump, A., Morikawa, Y., Tanaka, M., Minami, S., Umesaki, N., Takeuchi, M. and Miyajima, A. 2004. Binding of ovarian cancer antigen CA125/MUC16 to mesothelin mediates cell adhesion. *J. Biol. Chem.*; 279: 9190-8.

Sabuncu, T., Vural, H., Harma, M. and Harma, M. 2001. Oxidative stress in polycystic ovary syndrome and its contribution to the risk of cardiovascular disease. *Clinical Biochemistry Elsevier*; 34:407-413.

Salvador, A., Sousa, J. and Pinto, RE. 2001. Hydroperoxyl, superoxide and PH gradients in the mitochondrial matrix : A theoretical assessment. *Free Rad Biol Med*; 31: 1208-1215.

Sanjyotoi, B. and Melinker, R. 2011. Oxidative stress and antioxidant status in patient of ovarian cancer. *Biomedical Research*; 22:193-197.

Scully, RE. 1995. Pathology ovarian cancer precursors. *Cell Biochem Suppl.*; 23:208-218.

Seidman, JD., Horkayne-Szakaly, I. and Haiba, M. 2004. The histologic type and stage distribution of ovarian carcinomas of surface epithelial origin. *Int J Gynecol Pathol.*; 23:41-4.

Shuk-Mei, HO. 2003. Estrogen, progesterone and epitheial ovarian cancer. *Reproductive Biology and Endocrinology*; 1:1-18

Sohal, RS. 1993. The free radical hypothesis of aging: An appraisal of the current status ageing, *clinical and experimental research* 1993; 5:3-17.

Thiagarajan, RR. *et al*. 1997. The role of lecukocyte and endothelial adhesion molecules in ischemia-reperfusion injury. *Thromb Haemost* 1997; 78:310.

Von Sonnttag, C. 1987. The chemical basic of Radiation Biology, Taylor and Francis, London.

Weinstein, T., Changanac, A., Korteza, A., Booz, M., Malachi, T. and Gafter, U. 2000. Hemolysis in hemodialysis patient: against oxidative stress. *Nephrol. Dial. Transplant*; 15:883-887.

Young, RH. *et al*. 1994. The ovary in Sternberg SS, *et al* (eds): Diagnostic surgical pathology. New York: Raven press: 2195.

Yu, BP. 1993. Free radicals in aging, CRC Press, Boca Raton; 57-88.

****