

Research paper

Role of Human Beta Defensin -2 and Oxidative Stress Parameters in Ovarian Cancer Patients

ABSTRACT

Aims: To determine the role of human beta defensin-2 and oxidative stress by measuring serum human beta defensin-2 (HBD-2) and antioxidant parameters in ovarian cancer patients.

Study design:

Place and Duration of Study: Department of Gynecology, Mansoura University Hospital, between May 2011 and November 2012.

Methodology: We included 29 patients (all women; age range 20-76 years) with ovarian cancer and the control group comprised 15 age-matched women (age range 22-65 years) free from diseases and was recruited from the gynecology outpatient clinic, Mansoura University. Exclusion criteria were lack of informed consent, patients with associated gynecologic malignancies like cervical, uterine, breast cancers, preexisting immunological as rheumatoid arthritis, psoriasis, Crohn's disease, smoking, and other associated malignancies as colonic, lung carcinoma. Also, patients with any concomitant illness such as obvious systemic infection, diabetes mellitus, hypertension, and renal diseases prior chemo- or radiotherapy, or using corticosteroid therapy were excluded.

Results: A highly significant lowered levels of HBD-2 (.85 +/- .26, p=.001), catalase (504.98 +/- 107.65, p=.001), reduced glutathione (7.24 +/- 5.36, p=0.001) and total antioxidant capacity (1.53 +/- .24, p=.001) compared with controls (2.74 +/- .92, 717.57 +/- 67.32, 14.79 +/- 4.29 and 2.10 +/- .27 respectively). On the other hand, highly significant increased in the concentration of malondialdehyde (9.36 +/- 3.30, p=.001) and significantly increased of superoxide dismutase % inhibition (56.70 +/- 9.23, p=.044) were observed in ovarian cancer patients as compared with controls (6.00 +/- 2.06 and 49.69 +/- 16.83 respectively).

Conclusion: the results would suggest that lower HBD-2 as well as oxidative stress may be putative factors in the pathogenesis of ovarian cancer.

Keywords: Ovarian cancer, HBD-2, oxidative stress, antioxidants.

1. INTRODUCTION

Ovarian cancer is the most lethal gynecologic malignancy with epithelial ovarian neoplasms comprising ovarian tumors in adult women and can spread (metastasize) to other parts of the body. Approximately two women with epithelial ovarian cancer are diagnosed with advanced-stage disease, contributing to a poor overall survival (1). Epithelial ovarian neoplasms sub classified histologically into serous, mucinous, endometrioid, clear cell, transitional (Brenner), squamous and undifferentiated subtypes. Serous carcinomas (SC) is

the most common histology, accounting for about thirds of ovarian carcinomas while endometrioid ovarian carcinoma (EC) is the next most common subtype representing 15% of cases (2). Both EC and clear carcinomas (CC) may arise in the context of ovarian endometriosis, although the behavior of CC is aggressive (3 and 4). Staging is very important because ovarian cancers have a different prognosis at different stages and are treated differently. The accuracy of the staging may determine whether or not a patient will be cured. There are four stages which are I, II, III and IV (which is the late stage). Risk factors for ovarian carcinoma include inflammation, excessive number of life time ovulations, increases in steroid hormone levels, heredity, infertility, oral contraceptive pills, age, asbestos, talc and reproductive factors such as null parity (5). Ovarian cancer at an early stage is asymptomatic, but later the main symptoms include abdominal swelling, bloating, pain and pressure (6).

Defensins, a family of epithelial cell-derived cationic peptides (4 –5 kDa), exhibit both a direct antimicrobial and immunomodulatory properties. The genes encoding for defensins are located on chromosome 8. According to their molecular structure: 6 α -defensins and 4 β -defensins have been identified in humans (7). β - defensins are involved in innate immunity, they are chemotactic for immature dendritic cells and memory T cells, regulate cytokine production and adhesion-molecule expression. Moreover, they stimulate epithelial cell and fibroblast proliferation, and promote histamine release from mast cells. These effects occur at defensins concentrations much lower than those required for antimicrobial activity (8). In addition, HBDs can enhance adaptive immunity by acting as adjuvant and chemoattracting T cells, B cells, neutrophils, and macrophages (8). Defensins are activated by a direct response to bacterial components mediated by signaling pathways involving Toll-like receptors, and by cytokine-driven induction (e.g., by interleukin-1 β and tumor necrosis factor- α) (9). Beta-defensins may play a complex and poorly understood role either promoting or suppressing tumor cell growth (10). Elevated level of HBD-2 was detected in patients with vulval and cervical carcinomas (11) and independently on their histological type express is one in lung tumor samples (12). Up to date there are scarce data about the role of HBD-2 in ovarian cancer patients and their roles in the pathogenesis.

Oxidative stress (OS) caused by increased free radical generation and/or decreased antioxidant level in the target cells and tissues has been suggested to play an important role in carcinogenesis (13). Free radicals are capable of altering all major classes of biomolecules, such as lipids, nucleic acids and proteins, with changes in their structure and function (14). Prime targets of free radicals are the polyunsaturated fatty acids in cell membranes and their interaction results in lipid peroxidation. The levels of free radical molecules are controlled by various cellular defense mechanisms, consisting of enzymatic and non-enzymatic components (15). Enzymatic antioxidants such as superoxide dismutase and catalase form the first line of the antioxidant defense mechanism, they reduce hydrogen peroxide, prevent oxidative damage from spreading e.g. by interrupting the radical chain reaction of lipid peroxidation and protect the organism from reactive oxygen species (ROS) mediated oxidative damage (16). Oxidative stress has been implicated in the early stages of ovarian, cervical and uterine cancer as reported by (17). Also, it has been considered as a factor in the pathogenesis and or progression of endometriosis (18). Up to date there are scarce data about the role of oxidative stress in patients with ovarian cancer and their roles in the pathogenesis. The aim of this study was to evaluate the levels of HBD-2 and oxidative stress parameters in women with ovarian cancer.

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

The present study was done during the period from May 2011 till November 2012; patients were recruited from the gynecology department, Mansoura University. Patients were divided into two groups: The study group comprised 29 women with surgically resected and histologically proven ovarian cancer, it included 11 cases of papillary serous cyst

adenocarcinoma, 8 cases of mucinous cyst adenocarcinoma, 6 cases of serous adenocarcinoma, 2 cases of endometrioid adenocarcinoma, 1 cases of granulosa cell tumor and 1 cases of undifferentiated ovarian tumor. Also from histopathology we found three stages, it included 6 cases of stage I, 8 cases of stage II and 15 cases of stage III. The histopathology was reviewed by expert pathologist. The control group comprised 15 age-matched women free from diseases and was recruited from the gynecology outpatient clinic, Mansoura University. All participants provided an informed written consent to perform the study, and the research was approved by Ethical Board of Mansoura University. Exclusion criteria were lack of informed consent, patients with associated gynecologic malignancies like cervical, uterine, breast cancers, preexisting immunological as rheumatoid arthritis, psoriasis, Crohn's disease, smoking, and other associated malignancies as colonic, lung carcinoma. Also, patients with any concomitant illness such as obvious systemic infection, diabetes mellitus, hypertension, and renal diseases prior chemo- or radiotherapy, or using corticosteroid therapy were excluded.

Blood (5 ml) was collected from patients after overnight fasting before operation and also from control group. Blood (1 ml) was collected in EDTA tubes for estimation of reduced glutathione and the rest ml of blood was collected in clean and dry test tubes then allowed to clot. Serum was used for estimation of human beta defensin-2 (HBD-2) levels, superoxide dismutase (SOD) activities, catalase (CAT) activities, malondialdehyde (MDA) levels and total antioxidant capacity (TAC) levels. Serum HBD-2 level was determined by using a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (Glory Science Co., USA) according to manufacture in structure. Serum SOD activity was assayed by the procedure of Nishikime et al., 1972 (19). This assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye at 560 nm. Serum CAT was determined by the method of Fossati et al., 1980 (20) and Aebi 1984 (21) by using a commercially available kit (Biodiagnostic Co, Giza, Egypt). Blood GSH was determined by the method of Beutler et al., 1963 (22) by using a commercially available kit (Biodiagnostic Co, Giza, Egypt). Serum MDA was determined by the method of Draper and Hadley, 1990 (23). Total antioxidant capacity was determined by the method of Koracevic et al., 2001 (24) by using a commercially available kit (Biodiagnostic Co, Giza, Egypt).

Data were analyzed and processed using SPSS PC + for windows version 16. Exploration of data revealed preserved normality. We used mean and standard deviation for description of the central tendency and dispersion. Analysis of differences between two groups as regards quantitative parameters was done using independent t-test with the probability of < 0.05 considering significant. Correlation between two quantitative parameters was assessed using Pearson correlation with r representing the correlation coefficient and its significance was starred if < 0.05 . Sensitivity and specific for the different serologic parameters in diagnosis of the clinical status (normal and cancer) was done using ROC (Receiver Operating Characteristic curve).

3. RESULTS AND DISCUSSION

3.1. RESULTS

The study group comprised 29 women with surgically resected and histologically proven ovarian cancer patients, age ranged from (20-76 years) with the mean 43.33 ± 17.39 years. The control group comprised 15 age-matched controls women free from diseases, age ranged from (22-65 years) with the mean 44.43 ± 10.93 years. There was no significant difference in age between patients and control group ($p = 0.756$).

Table (1) showed that serum HBD-2 was highly significant decreased in ovarian cancer group (.85±.26 ng/ml) as compared with control group (2.74±.92 ng/ml). Also Increased the SOD % inhibition was found to be significantly in patients with ovarian cancer (56.70±9.23 %inh) than in the control (49.69±16.83 %inh). On the other hand, CAT and GSH levels were found to be highly significant decreased in patients with ovarian cancer (504.98±107.65 U/L), (7.24±5.36 mg/dl) compared with control group (717.57±67.32 U/L), (14.79±4.29 mg/dl) respectively. Decreased TAC levels were found to be highly significant in patients with ovarian cancer (1.53±.24 mM/L) compared with control group (2.10±.27 mM/L). The rise in MDA levels was found to be highly significant in patients with ovarian cancer (9.36±3.30 nmol/ml) compared with control individuals (6.00±2.06 nmol/ml).

Table (1): The levels of serum human beta defensin-2 and oxidative stress parameters in ovarian cancer patients (n=29) compared with control group (n=15).

parameters	Control group n=15	Ovarian cancer group n=29	p-value
Serum HBD-2 (ng/ml) Mean ± SD	2.74 ± .92	.85 ± .26	.001**
Serum SOD (% inh) Mean ± SD	49.69 ± 16.83	56.70 ± 9.23	.044*
Serum CAT (U/L) Mean ± SD	717.57 ± 67.32	504.98 ± 107.65	.001**
Blood GSH (mg/dl) Mean ± SD	14.79 ± 4.29	7.24 ± 5.36	.001**
Serum MDA (nmol/ml) Mean ± SD	6.00 ± 2.06	9.36 ± 3.30	.001**
Serum TAC (mM/L) Mean ± SD	2.10 ± .27	1.53 ± .24	.001**

137

138 P: Significant between control group and ovarian cancer group.

139 * Significant p<0.05

140 ** Highly significant p<0.001

141

142 Table (2) showed that there was significantly decreased in serum MDA levels between
143 papillary serous cyst adenocarcinoma group and mucinous cyst adenocarcinoma group.
144 Also there was significantly decreased in serum MDA levels between papillary serous cyst
145 adenocarcinoma group and serous adenocarcinoma group. No correlation between HBD-2
146 and oxidative stress parameters in each types of ovarian cancer were observed.

147

148 Table (2): Serum HBD-2 levels and oxidative stress parameters among different types of
149 ovarian cancer patients.

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parameters	Papillary serous cyst adenocarcinoma n=11	Mucinous cyst adenocarcinoma n=8	Serous adenocarcinoma n = 6
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Serum HBD-2 (ng/ml)			
Mean $\pm$ SD	.65 $\pm$.38	.87 $\pm$.48	.78 $\pm$.47
P1	-	.327	-
P2	-	-	.593
P3	-	-	.723
Serum SOD (% inh)			
Mean $\pm$ SD	57.17 $\pm$ 10.92	54.55 $\pm$ 8.38	65.60 $\pm$ 3.40
P1	-	.561	-
P2	-	-	.875
P3	-	-	.545
Serum CAT (U/L)			
Mean $\pm$ SD	522.60 $\pm$ 129.42	489.76 $\pm$ 109.56	527.31 $\pm$ 103.39
P1	-	.558	-
P2	-	-	.936
P3	-	-	.525
Blood GSH (mg/dl)			
Mean $\pm$ SD	5.56 $\pm$ 2.23	6.44 $\pm$ 3.16	9.58 $\pm$ 8.75
P1	-	.511	-
P2	-	-	.316
P3	-	-	.434
Serum MDA (nmol/ml)			
Mean $\pm$ SD	11.61 $\pm$ 3.58	8.03 $\pm$ 2.05	6.90 $\pm$ 2.57
P1	-	.014*	-
P2	-	-	.008*
P3	-	-	.396
Serum TAC (mM/L)			
Mean $\pm$ SD	1.53 $\pm$.24	1.50 $\pm$.26	1.65 $\pm$.29
P1	-	.842	-
P2	-	-	.415
P3	-	-	.361

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152

153 P1: Significant difference between papillary serous cyst adenocarcinoma group and

154 mucinous cyst adenocarcinoma group and also serous adenocarcinoma group.

155 P2: Significant difference between papillary serous cyst adenocarcinoma group and

156 serous adenocarcinoma group.

157 P3: Significant difference between mucinous cyst adenocarcinoma group and serous

158 adenocarcinoma group.

159 **Notes:** Granulosa cell tumor group was one patient and undifferentiated ovarian tumor

160 group was one patient and endometrium adenocarcinoma group was two patients so cannot

161 make statistical analysis for them.

162 * Significant $p < 0.05$

163 ** Highly significant $p < 0.001$

164

165 From table (3), it was clearly that only a significantly increased in serum MDA were observed

166 between stage I and stage II and between stage II and stage III of ovarian cancer patients.

167 Also there was a highly significant increased were observed between stage I and stage III of

168 ovarian cancer patients.

169 Table (3): Serum HBD-2 levels and oxidative stress parameters in different stages of ovarian cancer patients.

parameters	Stage I group n=6	Stage II group n=8	Stage III group n=15
Serum HBD-2 (ng/ml)			
Mean $\pm$ SD	.79 $\pm$.53	.61 $\pm$.34	1.03 $\pm$.61
P1	-	.235	-
P2	-	-	.540
P3	-	-	.495
Serum SOD (% inh)			
Mean $\pm$ SD	55.13 $\pm$ 6.07	57.36 $\pm$ 5.49	56.97 $\pm$ 10.9
P1	-	.927	-
P2	-	-	.630
P3	-	-	.581
Serum CAT (U/L)			
Mean $\pm$ SD	531.35 $\pm$ 128.75	500.70 $\pm$ 99.08	496.71 $\pm$ 109.38
P1	-	.930	-
P2	-	-	.577
P3	-	-	.639
Blood GSH (mg/dl)			
Mean $\pm$ SD	11.50 $\pm$ 7.83	5.09 $\pm$ 2.87	6.68 $\pm$ 4.55
P1	-	.317	-
P2	-	-	.204
P3	-	-	.105
Serum MDA (nmol/ml)			
Mean $\pm$ SD	6.34 $\pm$.69	8.46 $\pm$ 2.21	11.05 $\pm$ 3.43
P1	-	.040*	-
P2	-	-	.001**
P3	-	-	.032*
Serum TAC (mM/L)			
Mean $\pm$ SD	1.52 $\pm$.11	1.43 $\pm$.31	1.59 $\pm$ 0.19
P1	-	.219	-
P2	-	-	.573
P3	-	-	.590

170 P1: Significant difference between stage I group and stage II.

171 P2: Significant difference between stage I group and stage III.

172 P3: Significant difference between stage II and stage III.

173 * Significant $p < 0.05$

174 ** Highly significant $p < 0.001$

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178 From figure (1), it was clearly that there was a positive correlation ($r = .364$, significant $P =$
179 $.042$) between CAT activities and TAC levels in patients with ovarian cancer.

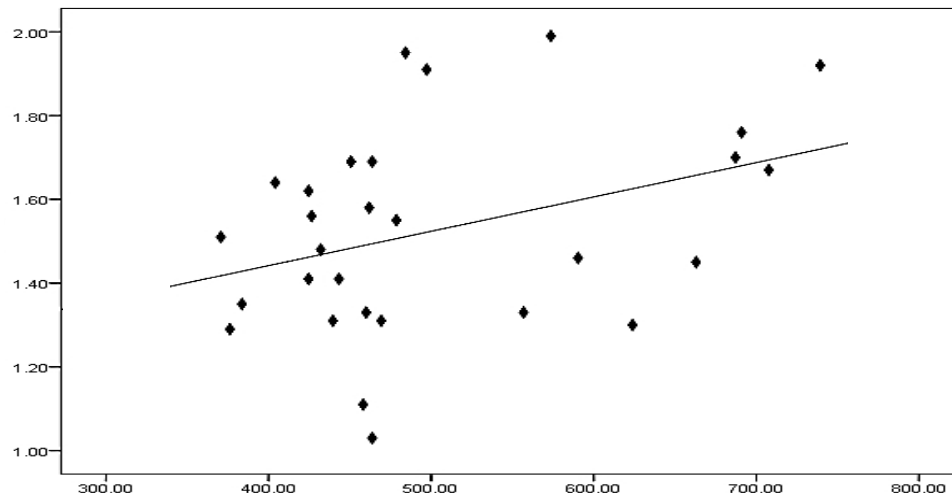


Fig (1): Linear Pearson correlation between CAT activities and TAC levels in ovarian cancer group.

There were significant positive correlation ($r=.725$, $P=.042$) between SOD activities and HBD-2 levels in stage II malignant ovarian tumor patients as showed in figure (2).

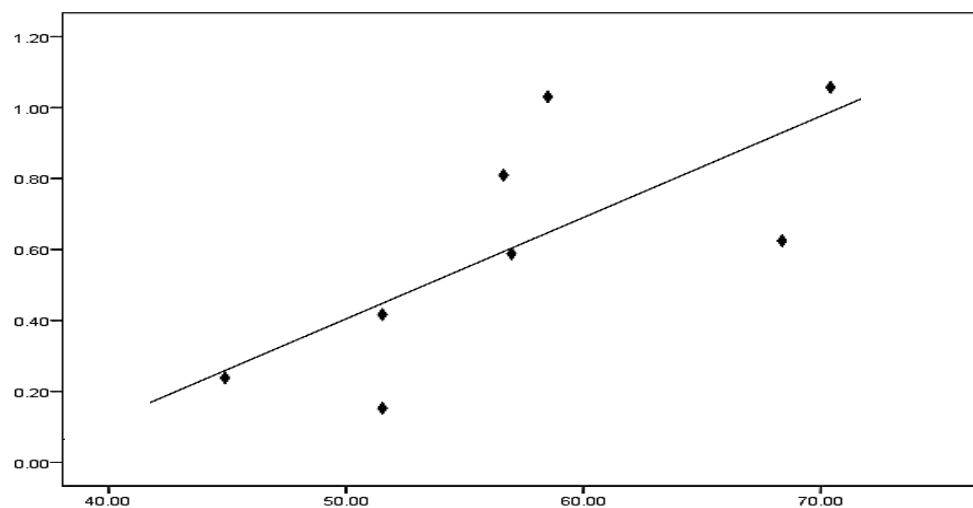


Fig (2): Linear Pearson correlation between SOD activities and HBD-2 levels in stage II of ovarian cancer patients.

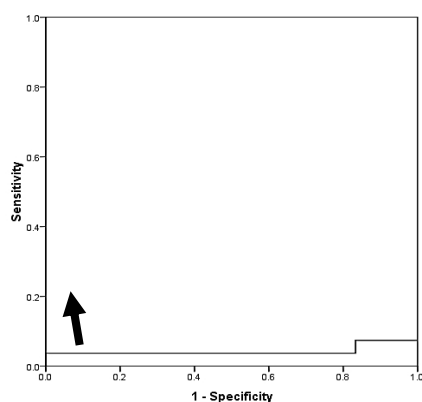
Values of HBD-2, SOD, CAT, GSH, MDA and TAC in 29 ovarian cancer patients and in 15 control group were used to construct ROC curve as showed in figure (3) to determine cut off

value for optimal sensitivity and specificity in ovarian cancer patients as showed in table (4). The cut off value of HBD-2 was 4.39ng/ml which give 3.70% sensitivity, 0.00% specificity, 3.60% PPV and 0.00% NPV. The cut off value of SOD was 49.24 % inhibitions which give 86.2% sensitivity, 67.90% specificity, 72.87% PPV and 83.11% NPV. It may be a good test due to high sensitivity and specificity. The cut off value of CAT was 689.01 U/L which give 10.30% sensitivity, 28.60% specificity, 12.61% PPV and 24.18% NPV. The cut off value of GSH was 22.13 mg/dl which give 6.90% sensitivity, 96.40% specificity, 65.71% PPV and 59.47% NPV. The cut off value of MDA was 6.69nmol/ml which give 79.30% sensitivity, 71.40% specificity, 73.49% PPV and 77.52% NPV. It may be added as a good test due to have a high sensitivity and specificity. The cut off value of TAC was 1.63 mM/L which give 34.50% sensitivity, 3.60% specificity, 26.36% PPV and 5.21% NPV.

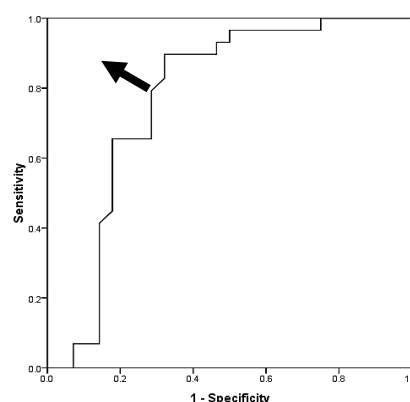
Table (4): Sensitivity, Specificity, PPV and NPV at cut off values for HBD-2 and oxidative stress parameters in ovarian cancer patients.

Parameters	Serum HBD-2	Serum SOD	Serum CAT	Blood GSH	Serum MDA	Serum TAC
Area under the curve	.043	.772	.066	.104	.841	.051
cutoff value	4.39 ng/ml	49.24 % inh	689.01 U/L	22.13 mg/dl	6.69 nmol/ml	1.63 mM/L
Sensitivity	3.70%	86.2%	10.30%	6.90%	79.30%	34.50%
specificity	.00%	67.90%	28.60%	96.40%	71.40%	3.60%
PPV	3.60%	72.87%	12.61%	65.71%	73.49%	26.36%
NPV	.00%	83.11%	24.18%	59.47%	77.52%	5.21%

PPV: positive predictive value.
NPV: negative predictive value.



HBD-2



SOD

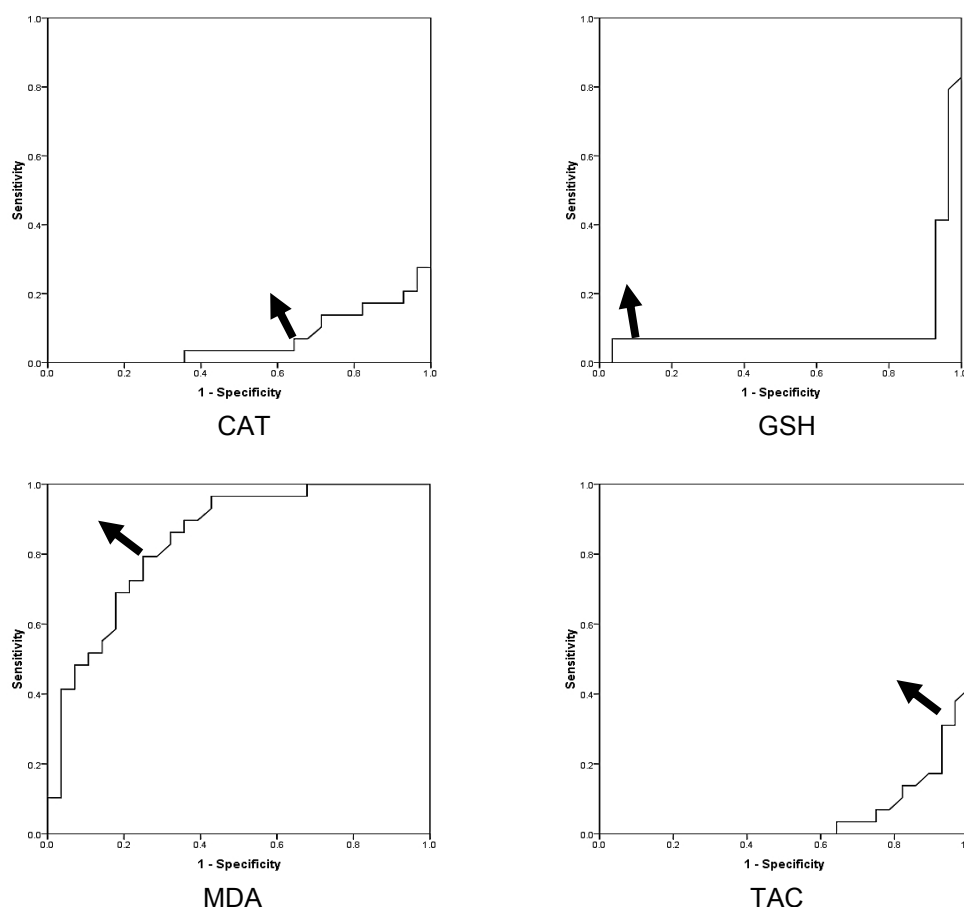


Fig (3): Receiver operating characteristic (ROC) curve constructed to determine the cut off value for optimal sensitivity and specificity for each test.

(The arrow: the best cut off value is usually denoted by the point nearer to upper left corner of graph taking in consideration that sensitivity is represented on the Y axis and reversed specificity (1- specificity) on the X axis).

3.2. DISCUSSION

3.2.1. HBD-2 Profile in Women with Ovarian Cancer Patients

In the present study, we found that a highly significant decrease of HBD-2 level in women with ovarian cancer as compared with control. The exact causes of this decrease are unknown; it may be due to environmental pollution, genetic abnormalities, or immunological dysfunction in these patients, or due to lack of stimulatory effects to the HBD-2 gene as IL-1 β and tumor necrosis factor - α (25).

To our knowledge, this study is among the first ones where serum beta-defensin-2 has studied in patients with ovarian cancer. It is well known that HBD-2 may play a complex and poorly understood role either promoting or suppressing tumor cell growth depending on their concentration, at a lower concentrations they stimulate adhesion molecule expression, cytokine production, influence signal transduction, and stimulate cell proliferation, while at a higher one it has an antitumor activity and lead to cell lysis (26). Thus we can speculate that

decreased serum levels of HBD-2 in women with ovarian cancer may be one of the initiating factors for the occurrence of ovarian cancer.

3.2.2. Oxidative Stress and Antioxidant Status in Patients of Ovarian Cancer

In our studies, we observed a significant increase in activities of SOD and a highly significant decreased in CAT activities in ovarian cancer patients as compared with control group. A decrease in the activity of CAT could be due to increase in the lipid peroxidation product, malondialdehyde which can form cross links, thereby inactivating several membrane bound enzymes (27). The increase in circulating lipid peroxides may be related to a deficiency of SOD in tumor tissues. The highly significant decrease in blood glutathione of ovarian cancer patients as compared with control group is another finding in our study. It is well known that NADPH is necessary for reducing GSSG (oxidized glutathione) to GSH by glutathione reductase in the red cell (28). Therefore the reduced level of GSH may be due to either a decrease in availability substrate (amino acid) for glutathione synthesis (29), or decreased activity of glutathione reductase (28). The reason for these acquired enzyme deficiency is not clear but it may be suggested that biochemical abnormalities in the red cell precursor in cancer conditions may reduce the production and action of these antiperoxidative enzymes thereby debilitating the system to an inefficient state to manage free radical damage. By assessing the status of these enzymes we could indicate the oxidative damage in the cell at stage I of the disease along with clinical manifestations.

Low levels of TAC as observed in ovarian cancer could be due to the increased its utilization in scavenging the excessive lipid peroxides production as well as sequestration by neoplastic cells (30). In present study, increased levels of MDA, as index of cell membrane lipid peroxidation, in patients with ovarian cancer can be attributed to increase in oxidative stress due to exhaustion of the antioxidant mechanism. MDA has a high cytotoxic and inhibitory action on protective enzymes and was suggested to act as a tumor promoter and a co-carcinogenic agent (31) so there was a highly significantly decreased of TAC levels in ovarian cancer patients as compared with control group. There was a positive correlation between CAT activities and TAC activities. In cancer patients, the decreased in catalase activities due to increase the lipid peroxidation causing decrease the total antioxidant capacity levels (antioxidant defense mechanism).

On the other hand, there was no statistically significant difference between each type in serum HBD-2 and all oxidative stress parameters; this means that each parameter couldn't affect different types of ovarian cancer. There were statistically significant decreased in serum MDA levels between papillary serous cyst adenocarcinoma and mucinous cyst adenocarcinoma and between papillary serous cyst adenocarcinoma and serous cyst adenocarcinoma. The cause for decreased in MDA not clear until now and should make further studies. Since MDA is an index of lipid peroxidation. In present study, increased levels of MDA in the circulation of ovarian cancer patients can be attributed to increase in oxidative stress due to the deficiency of antioxidant mechanism. The result showed that the rise in MDA levels was found to be highly significant in stage III ovarian cancer patients than stage II ovarian cancer patients than stage I ovarian cancer patients. In stage II ovarian cancer patients, there was significant positive correlation between HBD-2 and SOD but the reason was not clear until now and should make further studies.

3.2.3. Sensitivity and Specificity Test

The receiver operating characteristic curve for ovarian cancer showed that SOD and MDA may be a good test due to high sensitivity and specificity so we could be used this test for diagnosis of ovarian cancer patients.

4. CONCLUSION

In conclusion, the decreased of HBD-2 levels in women with ovarian cancer may be one of the initiating factors for the occurrence of ovarian cancer. Also there was positive correlation between HBD-2 and antioxidant (SOD) so it is recommended that assessment of human beta defensin -2 and antioxidant parameters should be done for early diagnosis of the ovarian cancer in women.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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