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

A. F. Abdel-Aziz[1*], A. A. El-Refaeey[2], A. Negm[1] and Hanan M. A. El-Taweel[1]

[1]Biochemistry Division, Department of Chemistry, Faculty of Science, Mansoura University, Mansoura, Egypt.
[2]Department of Obstetrics and Gynecology, Faculty of Medicine, Mansoura University, Mansoura, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: To determine the role of human beta defensin-2 and oxidative stress by measuring serum human beta defensin-2 and antioxidant parameters in ovarian cancer patients.
Study Design: Serum human beta defensin-2 (HBD-2), and the levels of antioxidants such as serum superoxide dismutase (SOD) and catalase (CAT), as well as blood reduced glutathione (GSH) and serum total antioxidant capacity (TAC) and serum malondialdhyde (MDA) were estimated in the circulation of 29 ovarian cancer patients and 15 of age-matched normal subjects as control.
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 free from diseases (age range 22-65 years)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

**Corresponding author: Email: aFaziz2012@hotmail.com;*

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 human beta defensin-2 (.85 +/- .26 ng/ml, *P*=0.001), catalase activities (504.98 +/- 107.65 U/L, *P*=0.001), reduced glutathione levels (7.24 +/- 5.36 mg/dl, *P*=0.001) and total antioxidant capacity levels (1.53 +/- .24 mmol/L, *P*=0.001) compared with controls (2.74 +/- .92 ng/ml, 717.57 +/- 67.32 U/L, 14.79 +/- 4.29 mg/dl and 2.10 +/- .27 mmol/L respectively). On the other hand, highly significant increased in the concentration of malondialdhyde (9.36 +/- 3.30 mmol/ml, *P*=0.001) and significantly increased of superoxide dismutase % inhibition (56.70 +/- 9.23 %inhibition, *P*=0.044) were observed in ovarian cancer patients as compared with controls (6.00 +/- 2.06 mmol/ml and 49.69 +/- 16.83 %inhibition respectively).
Conclusion: The results would suggest that lower human beta defensin-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 malignancy of the female reproductive system and the 5th cause of cancer death in women. It is estimated that 21,880 women will be diagnosed and 13,850 deaths will be attributed to the disease in 2011 alone. The five-year survival rate at Stage I is 93.5% but drops to 27.6% at Stage IV, where a majority of cases are diagnosed due to a lack of symptoms at the earlier stages [1]. Causes of ovarian cancer may be due to gene mutations, which lead to dysfunctional gene products, put their carriers at high risk [2]. The etiology of this pathology is poorly understood and most risk factors are related to hormonal exposure and reproduction. Two major hypotheses have been formulated in order to explain the etiology of ovarian cancer. The first is that of “incessant ovulation” in which cell proliferation is stimulated and malignant transformation of the ovarian epithelium occurs [3]. The second is the “gonadotropin hypothesis” which implicates the role of hormonal stimulation on ovarian epithelial cells. The combinations of current diagnoses and therapies are less than adequate because the cancers’ origins, growth and metastases may remain non-symptomatic and hard to detect within abdominal cavity for a long time prior to diagnosis [4].

At the time of diagnosis, cancers spread beyond the ovaries in more than 68% of patients, reaching stage III – advanced invasion of the neighboring tissues and/or stage IV – distant metastases according to the WHO classification. Hence, ovarian cancer is often termed a “silent killer”; detection strategies include transvaginal ultrasound and carbohydrate antigen 125 (CA-125) levels. With ultrasound, cancer could be mistaken for functional cysts in pre-menopausal women due to the dynamic nature of the ovarian surface [5]. CA-125 has a high false positive rate [5] and is often not detectable in early stage ovarian cancer [6]. 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 [7]. both EC and clear carcinomas (CC) may arise in the context of ovarian endometriosis, although the behavior of CC is aggressive [8,9].

Innate immunity, which is the first line of defense against pathogens, is attracting interest consequent to increasing antibiotic resistance. Short antimicrobial peptides, including

defensins, are key components of the innate immune system [10]. Human beta defensins (HBDs) are cationic cysteine-rich molecules with a three-dimensional structure stabilized by three disulfide bridges [11,12]. They exert a broad spectrum of antimicrobial activity against Gram-positive and Gram-negative bacteria, fungi, and enveloped viruses [13]. Although the role played by HBDs in antimicrobial reactions has been well established [14], the intracellular molecular mechanisms have not been fully elucidated.HBD analogs that differ in structural characteristics from the wild-type peptides appear to preserve their antimicrobial activities [15,16].This finding prompted a race to design HBD analogs potentially endowed with therapeutic properties [17].

Defensins are synthesized as inactive preproproteins that become post-translational activated. They are produced in the respiratory, gastrointestinal and genitourinary tracts, the skin, and by circulating blood cells [18]. Defensins are considered a first line of defense against invading pathogens. Of all defensins, the ß-defensins comprise the largest group, with around 40 members encoded in the human genome. Most of the genes are located in defensin (DEF) clusters on chromosomes 8 and 20 [18]. HBDs can enhance adaptive immunity by acting as adjuvant and chemoattracting T cells, B cells, neutrophils, and macrophages [19]. 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-1β and tumor necrosis factor-α) [20]. β-defensinsmay play a complex and poorly understood role either promoting or suppressing tumor cell growth [21].

The β-defensins are expressed in most epithelial cells and are found to be impaired in many inflammatory diseases, including Crohn's disease, psoriasis, pulmonary inflammation, and periodontal disease [22,23]. β- defensin expression is associated with some chronic lung diseases, including chronic obstructive pulmonary disease [24,25]. HBD-2 is expressed in epithelium, including skin, lung, vagina, and oral mucosa, and exhibits potent antimicrobial activity against Gram-negative bacteria and fungi [26]. HBD-2 is typically produced by epithelial tissues after stimulation with microorganisms and pro-inflammatory mediators [27], and contributes to initial defense in innate immune response. HBD-2 is expressed in the gingival epithelium in periodontal diseases in human biopsy samples (in vivo) and in vitro studies [28].In addition, studies have demonstrated that hBD-2 is up regulated in the inflamed mucosa of patients with ulcerative colitis [29]. These studies indicated that HBD-2 is regulated by Toll-like receptor (TLR) 2 and TLR4 signaling in human intestinal epithelial cells. Elevated level of HBD-2 was detected in patients with vulval and cervical carcinomas [30] and independently on their histological type express is one in lung tumor samples [31]. Up to date there are scarce data about the role of HBD-2 in ovarian cancer patients and their roles in the pathogenesis.

Tumor growth results in oxidative stress, accompanied by an increase in reactive oxygen species (ROS). ROS not only present as beneficial substances such as in chemotherapy and cancer apoptosis [32,33], but have also proven their role in carcinogenesis [34]. They are either formed via enzymatic reactions (respiratory chain, cytochrome P450 system and phagocytosis), or through non-enzymatic reactions such as those offset by ionizing radiation and those involving oxygen with organic compounds [34]. The balance of ROS as a beneficial substance is accomplished by the antioxidant defense system that is composed of enzymatic [superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GRx) and catalase (CAT)] and non-enzymatic [glutathione (GSH) and coenzyme Q10 (CoQ10)] [33]. The imbalance between the pro-oxidants and antioxidants in favour towards the former gives rise to oxidative stress that has been proven to lead to carcinogenesis [35]. Increased ROS formation and decreased efficiency of the antioxidant

defense not only causes the permanent alteration in structures of DNA, proteins, and lipids but also their functions [33].

Mitochondrial dysfunction and free radical-induced damage play a significant role in the pathogenesis of tumors, tumor-growth, metastasis, and cellular and tissue aging [36]. Decline in mitochondrial function most likely leads to cellular energy deficits, especially during situations known to require increased energy demand and in organs or tissues where the energy needs and metabolic demand are particularly high, such as in the brain or fast-growing tumors. As a result of this increased energy demand coupled with hypoxia and oxidative stress. Similarly, defective ATP production and increased generation of reactive oxygen and nitrogen species (ROS and RNS) may induce mitochondrial-dependent cell death as the damaged mitochondria are unable to maintain the energy demands of the cells [37].

However, several studies conducted on tissue as well as blood/serum samples have shown that levels of the antioxidant enzyme Glutathione peroxidase 3 (GPX3) are decreased in a number of human cancers, including breast, gastric, prostrate and colorectal cancer; a seemingly contradictory effect [38,39]. A number of studies in clear cell ovarian cancer tissues conducted by others have identified a higher expression of GPX3 when compared to control cells and in other epithelial ovarian cancer histologies[40]. Some studies found a strong inverse association of selenium from food sources and ovarian cancer risk, while selenium supplement intake was associated with increased risk. Oxidative stress has been implicated in the early stages of ovarian, cervical and uterine cancer as reported by [41]. Also, it has been considered as a factor in the pathogenesis and or progression of endometriosis [42]. The aim of this study was to evaluate the levels of HBD-2 and oxidative stress parameters in women with ovarian cancer.

2. MATERIALS 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 11 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 HBD-2 levels, SOD activities, CAT activities, MDA levels and 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. [43]. This assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitro blue tetrazolium dye at 560 nm. Serum CAT was determined by the method of Fossati et al.[44]and Aebi[45] by using a commercially available kit (Biodiagnostic Co, Giza, Egypt). Blood GSH was determined by the method of Beutler et al.[46] by using a commercially available kit (Biodiagnostic Co, Giza, Egypt). Serum MDA was determined by the method of Draper and Hadley [47]. Total antioxidant capacity was determined by the method of Koracevic et al.[48] 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 (P=0.001) 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 (P=0.044) in patients with ovarian cancer (56.70 ± 9.23 % inhibition) than in the control (49.69 ± 16.83 % inhibition). On the other hand, CAT and GSH levels were found to be highly significant decreased (P=0.001) 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 (P=0.001) in patients with ovarian cancer (1.53 ± .24 mmol/L) compared with control group (2.10 ± .27 mmol/L). The rise in MDA levels was found to be highly significant (P=0.001) in patients with ovarian cancer (9.36 ± 3.30 nmol/ml) compared with control individuals (6.00 ± 2.06 nmol/ml) as showed in Fig. 1.

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	0.001**
Serum SOD (% inh)			
Mean ± SD	49.69 ± 16.83	56.70 ± 9.23	0.044*
Serum CAT (U/L)			
Mean ± SD	717.57 ± 67.32	504.98 ± 107.65	0.001**
Blood GSH (mg/dl)			
Mean ± SD	14.79 ± 4.29	7.24 ± 5.36	0.001**
Serum MDA (nmol/ml)			
Mean ± SD	6.00 ± 2.06	9.36 ± 3.30	0.001**
Serum TAC (mmol/L)			
Mean ± SD	2.10 ± .27	1.53 ± .24	0.001**

P- Value compared with control group.
** Significant p<0.05*
*** Highly significant p<0.001*

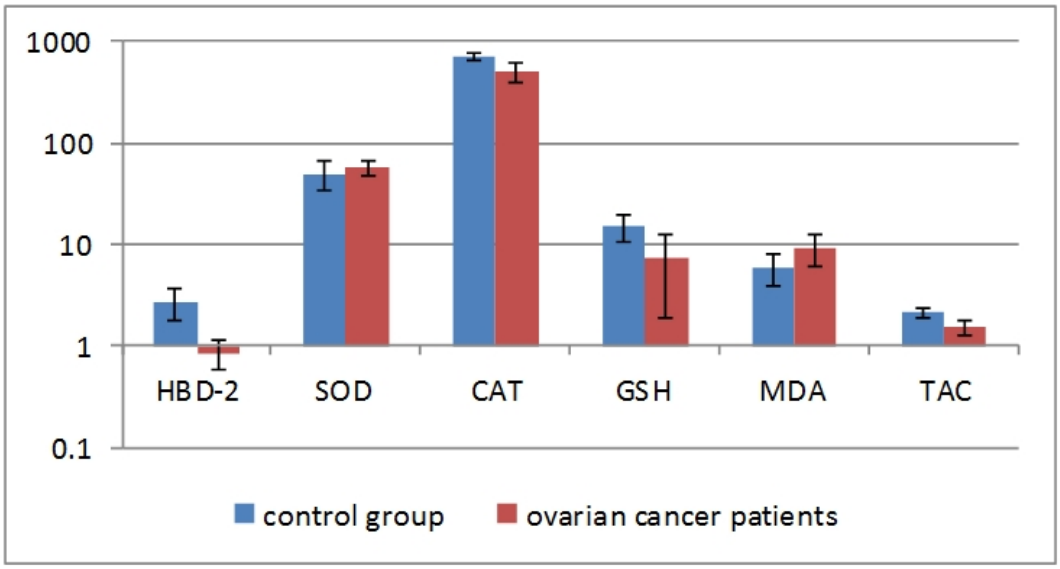

Fig. 1. The levels of serum human beta defensin-2 and oxidative stress parameters in ovarian cancer patients (n=29) compared with control group (n=15)

From Fig. 2, it was clearly that there was a positive correlation (r=.364, significant P= 0.042) between CAT activities and TAC levels in patients with ovarian cancer.

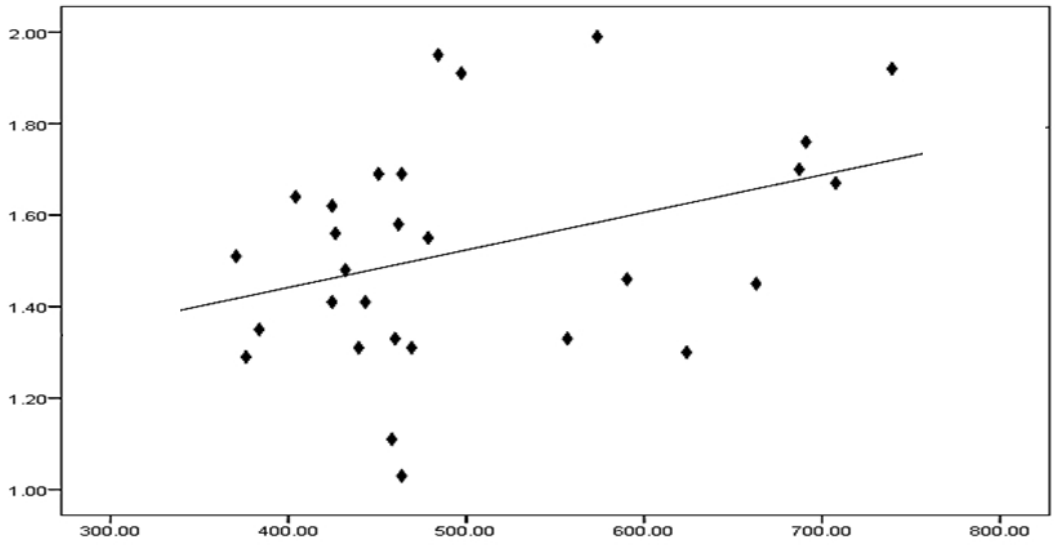

Fig. 2. Linear Pearson correlation between CAT activities and TAC levels in ovarian cancer group

P = 0.42 (<0.05)

Fig. 3 showed that there was negative correlation (r= -.219, non-significant*P*= 0.272) between SOD activities and HBD-2levels in ovarian cancer group.

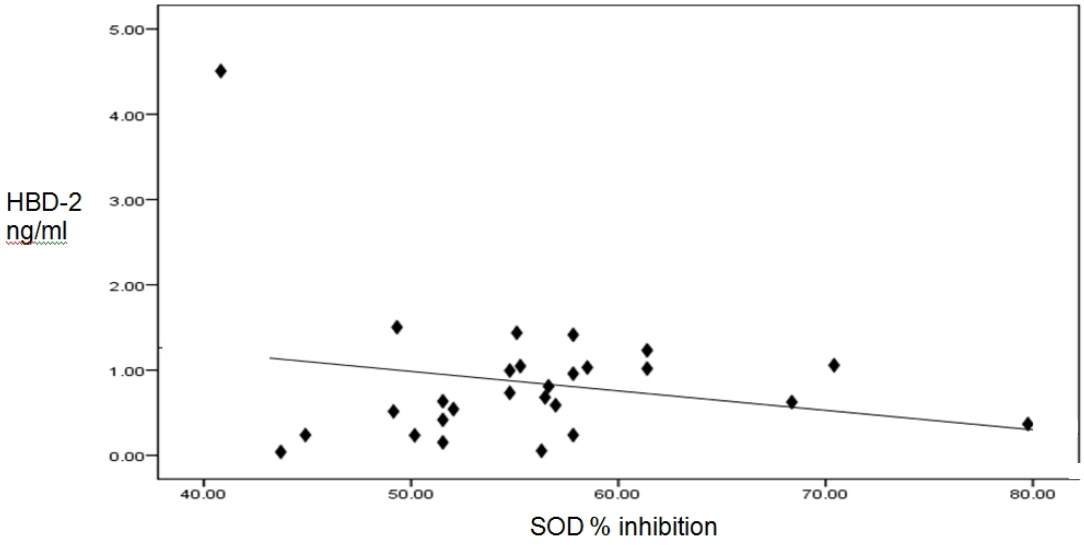

Fig. 3. Linear Pearson correlation between SOD activities and HBD-2 levels in ovarian cancer group

P = 0.272 (non-significant)

Table 2 showed that there was significantly decreased in serum MDA levels between papillary serous cyst adenocarcinoma group and mucinous cyst adenocarcinoma group.

Also there was significantly decreased in serum MDA levels between papillary serous cyst adenocarcinoma group and serous adenocarcinoma group. No correlation between HBD-2 and oxidative stress parameters in each types of ovarian cancer were observed.

Table 2.Serum HBD-2 levels and oxidative stress parameters among different types of ovarian cancer patients

parameters	Papillary serous cyst adenocarcinoma n=11	Mucinous cyst adenocarcinoma n=8	Serous adenocarcinoma n = 6
Serum HBD-2 (ng/ml)			
Mean ± SD	.65 ± .38	.87 ± .48	.78 ± .47
P1		0.327	
P2			0.593
P3			0.723
Serum SOD (% inh)			
Mean ± SD	57.17 ± 10.92	54.55 ± 8.38	65.60 ± 3.40
P1		0.561	
P2			0.875
P3			0.545
Serum CAT (U/L)			
Mean ± SD	522.60 ± 129.42	489.76 ± 109.56	527.31 ± 103.39
P1		0.558	
P2			0.936
P3			0.525
Blood GSH (mg/dl)			
Mean ± SD	5.56 ± 2.23	6.44 ± 3.16	9.58 ± 8.75
P1		0.511	
P2			0.316
P3			0.434
Serum MDA (nmol/ml)			
Mean ± SD	11.61 ± 3.58	8.03 ± 2.05	6.90 ± 2.57
P1		0.014*	
P2			0.008*
P3			0.396
Serum TAC (mmol/L)			
Mean ± SD	1.53 ± .24	1.50 ± .26	1.65 ± .29
P1		0.842	
P2			0.415
P3			0.361

P1: probability value between papillary serous cyst adenocarcinoma group and mucinous cyst adenocarcinoma group.
P2: probability value between papillary serous cyst adenocarcinoma group and serous adenocarcinoma group.
P3: probability value between mucinous cyst adenocarcinoma group and serous adenocarcinoma group.
***Notes:** Granulosa cell tumor group was one patient and undifferentiated ovarian tumor group was one patient and endometrium adenocarcinoma group was two patients so cannot make statistical analysis for them.*
** Significant p<0.05; ** Highly significant p<0.001*

From Table 3, it was clearly that only a significantly increased in serum MDA were observed between stage I and stage II and between stage II and stage III of ovarian cancer patients. Also there was a highly significant increased were observed between stage I and stage III of ovarian cancer patients.

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 ± SD	.79 ± .53	.61 ± .34	1.03 ±.61
P1		0.235	
P2			0.540
P3			0.495
Serum SOD (% inh)			
Mean ± SD	55.13 ± 6.07	57.36 ± 5.49	56.97 ± 10.9
P1		0.927	
P2			0.630
P3			0.581
Serum CAT (U/L)			
Mean ± SD	531.35 ±	500.70 ± 99.08	496.71 ± 109.38
P1	128.75	0.930	
P2			0.577
P3			0.639
Blood GSH (mg/dl)			
Mean ± SD	11.50 ± 7.83	5.09 ± 2.87	6.68 ± 4.55
P1		0.317	
P2			0.204
P3			0.105
Serum MDA (nmol/ml)			
Mean ± SD	6.34 ± .69	8.46 ± 2.21	11.05 ± 3.43
P1		0.040*	
P2			0.001**
P3			0.032*
Serum TAC (mmol/L)			
Mean ± SD	1.52 ± .11	1.43 ± .31	1.59 ± 0.19
P1		0.219	
P2			0.573
P3			0.590

P1: probability value between stage I group and stage II.
P2: probability value between stage I group and stage III.
P3: probability value between stage II and stage III.
** Significant p<0.05;*
*** Highly significant p<0.001*

There were significant positive correlation (r=.725, *P=0.042)* between SOD activities and HBD-2 levels in stage II malignant ovarian tumor patients as showed in Fig. 4.

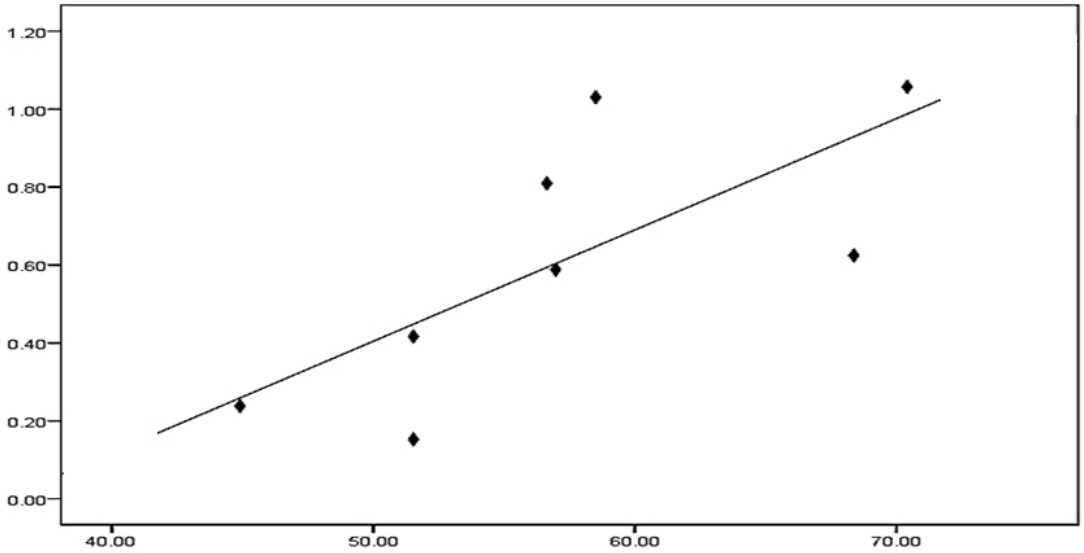

Fig. 4. Linear Pearson correlation between SOD activities and HBD-2 levels in stage II of ovarian cancer patients

P = 0.042 (<0.05)

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 Fig. 5, 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 4.39 ng/ml which give 3.70%sensitivity, 100%specificity, 100 % (positive predictive value) PPV and 50.94 % (negative predictive value) NPV so it may be a good test. 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 so it may be a good test. 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 so it may be a good test. The cut off value of MDA was 6.69 nmol/ml which give 79.30%sensitivity,71.40% specificity, 73.49% PPV and 77.52% NPV so It may be a good test. The cut off value of TAC was 1.63 mmol/L which give 34.50% sensitivity, 3.60% specificity, 26.36 % PPV and 5.21% NPV.

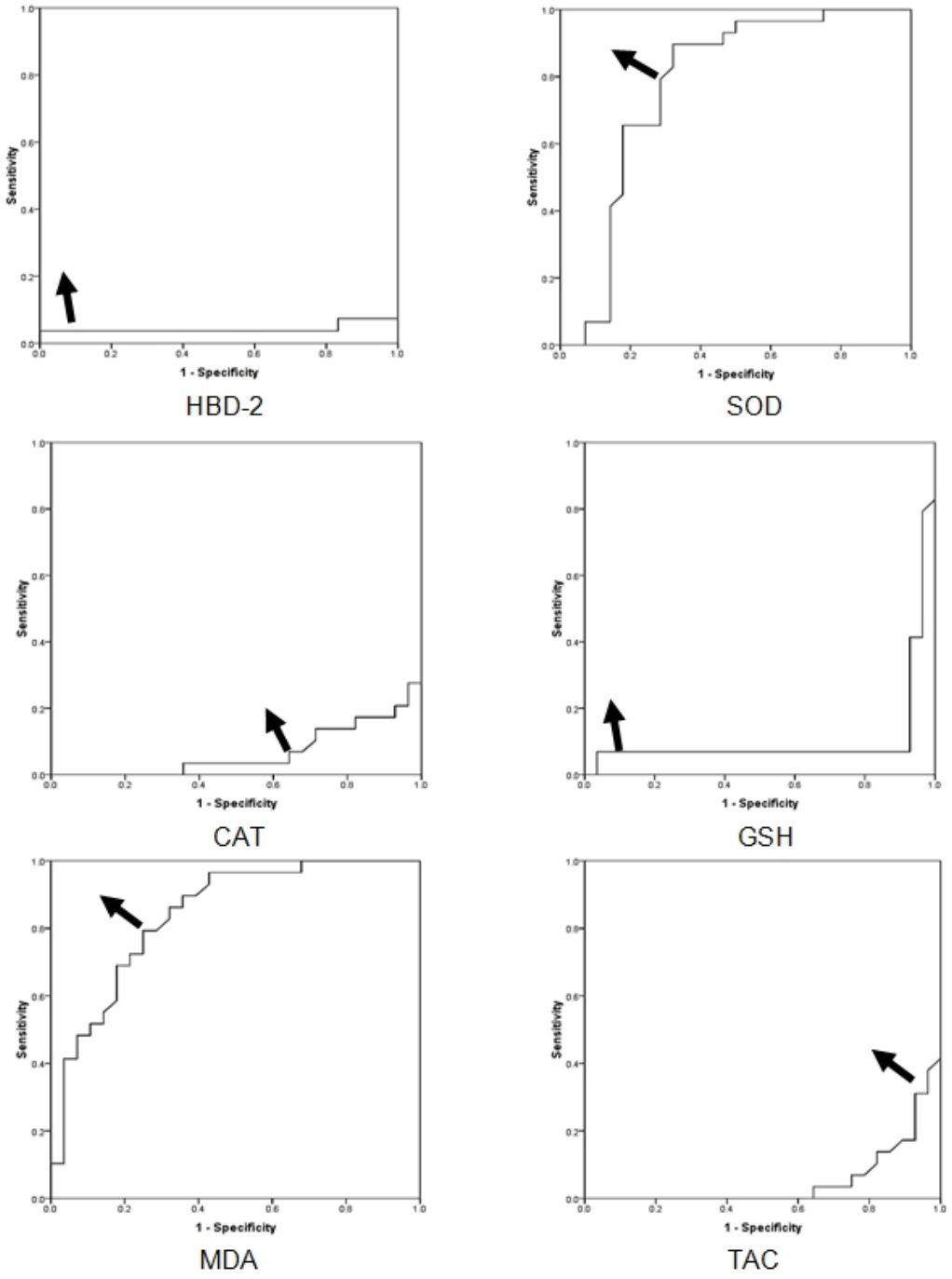

Fig. 5. Receiver operating characteristic (ROC) curve constructed to determine the cut off value for optimal sensitivity and specificity for each test

(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).

Table 4. Sensitivity, specificity, PPV and NPV at cut off values for HBD-2 and other 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 mmol/L
Sensitivity	3.70%	86.2%	10.30%	6.90%	79.30%	34.50%
Specificity	100%	67.90%	28.60%	96.40%	71.40%	3.60%
PPV	100%	72.87%	12.61%	65.71%	73.49%	26.36%
NPV	50.94%	83.11%	24.18%	59.47%	77.52%	5.21%

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

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-1β and tumor necrosis factor -α [49].

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 [50]. Thus we can speculate that decreased serum levels of hBD2 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 [51]. The increase in circulating lipid peroxides may be related to a deficiency of SOD in tumor tissues (increase the % inhibition of SOD).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 [52]. Therefore the reduced level of GSH may be due to either a decrease in availability substrate (amino acid) for glutathione synthesis [53], or decreased activity of glutathione reductase [52]. 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.

In present study, increased levels of MDA, as index of cell membrane lipid peroidation, 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 [54] so that there was a highly significantly decreased of TAC levels in ovarian cancer patients. In ovarian cancer patients, the decreased in catalase activities due to increase the lipid peroxidation causing decrease the total antioxidant capacity levels (antioxidant defense mechanism) so there was significant positive correlation between them.

Our result showed that there was no statistically significant difference between ovarian cancer types except MDA levels. We couldn't find areason for these decreased in serum MDA between papillary serous cyst adenocarcinoma and mucinous cyst adenocarcinoma and also between papillary serous cyst adenocarcinoma and serous cyst adenocarcinoma therefore, we need 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 and we couldn't find a reason therefore, we need further studies.

3.2.3 Sensitivity and specificity test

The receiver operating characteristic curve for ovarian cancer showed that HBD-2, SOD GSH and MDA may be a good test so we could be used these 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. There was Pearson correlation between HBD-2 and antioxidant (SOD). From sensitivity and specificity test it is recommended that assessment of HBD-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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