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Evaluation of Some Biochemical Parameters and Their Use in The Diagnosis and Follow-Up Women Ovary Cancer's Patients

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ABSTRACT

Introduction: The common type of cancer among women is epithelial ovarian carcinoma (EOC); this is because there aren't sufficient early diagnostic indicators. Each tumor marker has a special set of use for screening, diagnosing, evaluating response to therapy, and monitoring for cancer recurrence.

Objective: The aim of the current is to assess role of biochemical markers in diagnosis of ovarian cancer patients, such as serum total sialic acid, Glycoprotein, Ferritin, and Cancer Antigen 125. Additionally, the impacts of oxidative stress in cancer cells that initiate apoptotic pathways will be investigated.

Patients and methods: The participants of this study comprised 36 women with epithelial ovarian cancer patients, and 36 women with benign ovarian mass (pathological controls). Other 36 women were considered normal healthy controls, who attended for treatment in Al-Amal National Cancer Hospital from January 2022 till June 2022.

Results: Epithelial ovarian cancer patients had a mean age of 52.8 (SD 9.7) years, and benign ovarian mass (pathological controls) had a mean age of 49.6 (SD 5.9) years. Normal healthy controls had mean age of 50.7 (SD 3.8) years. Serum TSA, glycoprotein, ferritin, and CA-125, can provides useful information for patients in a diagnosis of ovarian cancer particularly in early-stage disease. Ferritin discriminates between healthy controls and patients with epithelial ovary cancer's, particularly in early-stage disease. In order to test for EOC, serum ferritin and CA125 together offer highest level of diagnostic efficiency.

Conclusion: Early identification and diagnosis of metastasis in women with ovarian cancer is beneficial for therapy and can be utilized to determine why cancer cells develop chemoresistance.

Keywords: Total sialic acid, CA125, Malondialdehyde, Glutathione, Caspase-3, Comparative study, AL-Hadi University College.

INTRODUCTION

Total sialic acid (TSA) is involved in tumor development and metastasis **(1).**

Due to the lack of early diagnostic indicators, epithelial ovarian cancer is frequently discovered at a late stage. The serum sialic acid ratio may aid in the detection of ovarian cancer because sialic acid linking of N-glycans in modulations occurs even in early stages of the disease **(2).** The amount of free or attached sialic acid was related with both the growth and severity of the tumor. Sialic acid is utilized to assess the efficacy of various malignancy therapies and diagnostic developments **(3).**

Chemical markers called glycoprotein oligosaccharide chains are utilized to identify proteins that will be employed outside of the cell. Glycoproteins include a range of polypeptide hormones.

Glycolipid and glycoprotein residues that are membrane-bound can be found on the cell surface **(4).** Patients with ovarian cancer may have high serum glycoprotein levels because of aberrant protein glycosylation, which indicates that the cells have undergone malignant transformation **(5).** In order to diagnose ovarian cancer, patients can benefit from the information provided by CA125 **(6)**.

A glycoprotein called CA 125, which is typically generated by cancerous cells with epithelial development, acts as a tumor marker. The histological grade of primary ovarian neoplasms and serum levels of CA 125 were related, showing that ovarian neoplasms are associated with high levels of this glycoprotein **(7).** Due to iron overload increasing the levels of oxidative stress and mutagenesis, ovarian epithelial cells may be subjected to a sublethal quantity of oxidative stress and may therefore become of causing cancer **(8).**

Patients' serum iron levels were downregulated, and ferritin levels were overexpressed, which may be a potential target for the detection and therapy ovary cancer's **(9)**.

A common marker for ovarian cancer diagnosis is CA125. In the EOC groups, a considerable rise in CA125 was unmistakably seen. Ferritin was examined and revealed a comparable rise in the EOC groups **(10).** The ferritin is necessary for preserving the cellular redox state. It has been shown that ferritin regulates the proliferation of cancer cells in ovarian cancer **(11).**

The intrinsic apoptotic pathway was activated as a result of the induced formation of intracellular reactive oxygen species (ROS). Apoptosis is generated along with changes in ROS signaling and induction of apoptosis **(12).**

In healthy cells, ROS operate as antioxidants, but in cancer cells, they are powerful pro-oxidants that activate the apoptotic pathways **(13).** Caspases are specific cysteinyl aspartate-degrading proteases utilized in programmed cell death, while apoptotic caspases include executioner caspase-3 **(14).**

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Through the process of apoptosis, caspases maintain homeostasis. The activity of caspase-3 is necessary to control the proliferation of many different types of cells. One of the primary caspases that are activated and killing the cell is caspase-3 [15].

In nearly all cancers ROS are involved in cancer etiology and progression. Elevated rate of ROS has been detected, where they promote many aspects of tumor development and progression [16].

Depletion of glutathione (GSH) is a common sign of apoptotic cell death induced by a variety of events such as the activation of death receptors [17]. Lipid peroxidation is a metabolic process that results in the oxidative destruction of lipids by ROS. This procedure may result in lipid breakdown in the cell membrane, which could damage the cells [18]. Cancer and other human diseases have been linked to oxidative stress-induced lipid peroxidation [19].

PATIENTS AND METHODS

The participants of this study comprised 36 women with epithelial ovarian cancer patients, and 36 women with benign ovarian mass (pathological controls). Other 36 women were considered normal healthy controls, who attended for treatment in Al-Amal National Cancer Hospital from January, 2022 till June, 2022.

The pathological examination of each instance revealed that they were either ovarian benign tumors or epithelial ovarian carcinoma.

Before starting any cancer treatment, patients were evaluated at the time of diagnosis. Determine the clinicopathological stage for ovarian cancer based on FIGO (International Federation of Gynecology and Obstetrics) guidelines from 2015.

The blood was centrifuged for 10 minutes at 3000 rpm after it had been allowed to coagulate at room temperature. Resultant sera were isolated; an assay was performed by hand. Serum TSA, glycoprotein and GSH were examined using methods of kits. Serum ferritin, CA125 and caspase-3 were examined using methods of ELISA kits. The level of serum MDA was determined by a modified procedure of Guided and Shah [20].

Ethical Consideration:

This study was ethically approved by the Institutional Review Board of AL-Hadi University College (Baghdad, Iraq). Written informed consent was obtained from all participants. This study was executed according to the code of ethics of the World Medical Association (Declaration of Helsinki) for studies on humans.

Statistical Analysis

The collected data were introduced and statistically analyzed by utilizing the Statistical Package for Social Sciences (SPSS) version 20 for windows. Qualitative data were defined as numbers and percentages. Quantitative data were tested for normality by Kolmogorov-Smirnov test. Normal distribution of variables was described as means and standard deviation (SD), ANOVA test/Kruskal-Wallis test was used for comparison between groups. P value ≤ 0.05 was considered to be statistically significant.

RESULTS

Epithelial ovarian cancer patients had a mean age of 52.8 (SD 9.7) years, and benign ovarian mass (pathological controls) had a mean age of 49.6 (SD 5.9) years. Normal healthy controls had mean age of 50.7 (SD 3.8) years.

The levels of TSA and glycoprotein increased significantly, as seen in **Table 1** in sera of ovarian cancer when comparing to healthy controls, there was a significant increase in ovarian cancer when comparing to pathological controls with ($P<0.001$), while there was no significant differences in serum TSA and glycoprotein levels when compared pathological controls to healthy controls. Both ferritin and CA125 significantly increased in sera of ovarian cancer when comparing to healthy controls, a significant increase in ovarian cancer when compared to pathological controls with ($P<0.001$), while there was no significant differences in serum ferritin and CA125 levels when compared pathological controls to healthy controls.

Also, **Table 1** indicated that the amount of caspase-3 had significantly increased in sera of ovarian cancer and pathological in comparison to healthy controls and there was a significant increase in ovarian cancer in comparing to pathological controls with ($P<0.001$).

There was increased significantly in the level of MDA in ovarian cancer women in comparison to healthy controls, there was increased significantly in ovarian cancer in comparison to pathological controls with ($P<0.001$). The level of GSH in ovarian cancer women was significantly decreased when compared to healthy controls, there was significantly decreased in ovarian cancer women when compared to pathological controls with ($P<0.001$), while there was no significant differences in serum MDA and GSH levels when compared pathological controls to healthy controls.

Table (1): Biostatistical calculations for some biochemical markers and the comparison of levels of healthy controls, pathological controls and ovarian cancer.

Serum biochemical markers	Ovarian cancer (n=36)	Pathological controls (n=36)	Healthy controls (n=36)	*P* Value
Total sialic acid (mg/dL)	80.32 ± 3.44	55.11 ± 8.23	51.84 ± 6.36	P<0.001 NS
Glycoprotein (mg/dL)	49.8 ± 6.45	27.4 ± 5.23	29.5 ± 3.66	P<0.001 NS
Ferritin (ng /mL)	98.33 ± 17.6	34.80 ± 7.1	35.22 ± 5.5	P<0.001 NS
CA125 (U/mL)	255.33 ± 14.3	17.85 ± 3.7	15.54 ± 2.2	P<0.001 NS
(CASP3) (ng/mL)	2.75 ± 0.09	2.42 ± 0.08	2.21 ± 0.15	P<0.001
(GSH) (mg/dL)	24.5 ± 6.1	33.2 ± 4.8	35.3 ± 7.4	P<0.001 NS
(MDA) (nmol /mL)	13.21 ± 1.8	10.11 ± 1.5	9.49 ± 1.3	P<0.001 NS

* Significant at 0.001 level of significance using ANOVA test/Kruskal-Wallis test between independent means.

DISCUSSION

Complementary markers are required to increase sensitivity and specificity, particularly in cases of early-stage disease. Each tumor marker is effective for screening and identifies biological alterations that signify the presence of patients with malignant or benign ovarian tumors. Results revealed there was a significant increase in levels of TSA and glycoprotein in sera of ovarian cancer when comparing to healthy controls, there was a significant increase in ovarian cancer when comparing to pathological controls with (P<0.001), while no significant differences in TSA and glycoprotein levels when compared pathological controls to healthy controls. These values can be utilized to distinguish benign from malignant ovarian tumors. Glycoprotein and TSA levels, particularly in cases where the disease is in its early stages, can be used to diagnose ovarian cancer. Glycoprotein and TSA are altered in many cancer types, but they are more elevated in ovarian cancer. Results matched those of **Zhou *et al.*** [(1)] on the cell surface, sialic acids are frequently found as glycolipids or glycoproteins. Sialic acids are crucial for the development and spread of tumors.

As malignant diseases advance, **Tummalacharla *et al.*** [(21)] discovered a considerable increase in TSA, indicating that these markers may be helpful for following disease progression and therapeutic interventions. Patients with ovarian cancer may have high serum glycoprotein levels as a result of the cells' malignant transformation [(5)]. Changes in TSA or glycoprotein expression may help distinguish between benign and malignant epithelial ovarian tumors and can also help us better understand how cancers behave. Results were consistent with that reported by **Li *et al.*** [(22)].

Using specific changes in glycoprotein expression or glycosylation occupancy, it might be possible to distinguish between benign and malignant epithelial ovarian tumors using our growing understanding of the biology of ovarian cancer. Elevated glycoprotein release simply indicates the existence of tissue damage and the explanation of tissue proliferation as the etiological reason for glycoprotein behavior in cancer. Results were consistent with that reported by **Stavrovskaya *et al.*** [(23)].

Increased p-glycoprotein levels are linked to the development of treatment resistance to many drugs in cancer cells; they can prevent apoptosis and reduce caspase-3 activation.

Results revealed that, as compared to healthy controls, ovary cancer's serum had significantly higher levels of ferritin and CA125, a significant increase in ovary cancer's in comparison to pathological controls with (P<0.001), while there was no significant variations in serum ferritin and CA125 levels when compared pathological controls to healthy controls. These values may be utilized for distinction between benign from malignant ovarian tumours. Ferritin and CA125 provides useful information for patients in a diagnosis of ovarian cancer especially in earlystage disease. The findings agreed with those from **Zhao *et al.*** [(24)]. Ferritin distinguishes between healthy individuals and people with (EOC), particularly in the early stages of the disease; the most accurate diagnosis is obtained when serum ferritin and CA125 are combined. In addition to the usual CA125 test, serum ferritin could be used as an EOC biomarker [(24)].

Our findings demonstrated a relationship between the rise in serum ferritin and the ovary cancer's prognosis. Furthermore, ovary cancer's patients had increased ferritin expression comparing to those with benign ovarian tumours. The prognosis of EOC was associated with patients' higher ferritin levels and decreased blood iron levels and may represent a potential target for epithelial ovarian cancer detection and treatment [(9)]. In the EOC groups, there was a noticeable increase in CA125. At the same time, serum ferritin was examined and revealed a similar increase in the early stages of disease.

Results indicated that there was a significantly increased in level of caspase-3 in sera of ovarian cancer and pathological in comparison to healthy controls and there was a significant increase in ovarian cancer in comparing to pathological controls with (P<0.001). Tumor development is the net

consequence of cell proliferation and cell loss through apoptosis. Caspase-3 expression was deemed to be directly related to apoptosis. **Thomas *et al*. [15]**, found caspases preserve homeostasis by triggering apoptosis. Caspase-3 activity is required to regulate the proliferation of a wide range of cell types. Caspase-3 is one of the main caspases that is activated and destroying the cell.

Results revealed that the level of MDA had significantly increased in ovarian cancer women in comparison to healthy controls, there was increased significantly in ovarian cancer in comparison to pathological controls with (P<0.001). The level of GSH in ovarian cancer women was significantly decreased in comparison to healthy controls, there was significantly decreased in ovarian cancer women when compared to pathological controls with (P<0. 001), while there was no significant differences in serum MDA and GSH levels when compared pathological controls to healthy controls. Oxidative stress interferes with many cellular processes, including apoptotic mechanisms.

Tavsan *et al*. [12], found the intrinsic apoptotic pathway was activated as a result of the enhanced intracellular ROS production. Additionally, the ROS signaling changes, also the activation of apoptosis, when treating ovarian cancer.

Kopustinskiene *et al*. [13], found in healthy cells, ROS operate as antioxidants, but in cancer cells, they are powerful pro-oxidants that activate the apoptotic pathways. In nearly all cancers ROS are involved in cancer etiology and progression. Elevated of ROS has been detected, where they promote many aspects of tumor development and progression [16], because the tumor often relapses shortly after treatment. **Kim *et al*. [25]** found through the production of many aldehydes as a result of lipid peroxidation brought on by oxidative stress, the oxidative stress interferes with many cellular processes, including apoptotic mechanisms, which might reduce the ability of anticancer medicines to destroy cancer cells. Lipid peroxidation is a metabolic operation that causes oxidative of lipids by ROS. This process can lead to lipid degradation within the cell membrane resulting in cell damage **[18].**

Aldehydes produced by lipid peroxidation and brought on by oxidative stress can also interfere with apoptosis directly by attaching to the active site of caspase-3 and preventing it from doing its job. The hypothetical mechanical mechanism takes place when electrophilic aldehydes, like MDA, attach to the thiol side chain in cysteine at the active site of caspases and inhibit their activity.

These results were compatible with that reported by **Wilson *et al*. [26].** The tetrapeptide aldehyde used to characterize caspase-1 is an example of an electrophilic aldehyde that binds covalently to the group of sulfhydryls present in the cysteine at the active caspase site and inhibits caspase action. Important apoptosis regulators include depletion of glutathione (GSH) and protein post-translational modifications **[17].**

It has been discovered that glutathione depletion has a direct impact on how the permeability transition pore develops, stimulate the caspase initiator, and activate caspase-3 **[27].** Electrophilic aldehydes like MDA may bind covalently to the nucleophilic group like (thiol) of the cysteine at the active caspase site resulting in caspase inhibition. Additionally, due to the effectiveness of oxidative stress, glutathione depletion directly stimulates the initiator of caspases and activates caspase-3. Aldehyde generation may reduce the caspase activity.

CONCLUSION

Numerous clinical and biological variables, including as serum TSA, glycoprotein, ferritin, and CA-125, which have been evaluated for their prognostic significance, have been used to identify epithelial ovarian cancer. These biochemical markers can provide useful information for patients in a diagnosis of ovarian cancer particularly in early-stage disease. Serum TSA, ferritin and CA-125 were a significant increase in ovarian cancer in comparison to pathological controls and the value can be used in differentiation between benign tumors from malignant ovarian tumors. Ferritin discriminates between healthy controls and epithelial ovary cancer's patients, particularly in early stage disease. In order to test for EOC, serum ferritin and CA125 together offer the highest diagnostic accuracy. Caspase-3 is the key mediators in apoptosis. Oxidative stress and excessive production of ROS, thus GSH in ovary cancer's women was decreased, while increased oxidation products are formed by lipid peroxidation including aldehydes such as MDA. Oxidative stress interferes with many cellular processes, including apoptotic mechanisms, through many aldehydes such as MDA. Inhibition of caspase-3 activity leads to increased cell proliferation and tumor development.

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