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ORIGINAL PAPER

Elevated estrogen receptor beta and oxidative stress in postmenopausal women with ovarian cancer

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ABSTRACT

Introduction and aim. Ovarian cancer in postmenopausal women is associated with hormonal dysregulation and oxidative stress. This study investigates the relationship between estrogen receptor beta (ERβ), selected reproductive hormones, and oxidative stress markers in women with ovarian cancer compared to healthy controls. To our knowledge, this is one of the first studies to evaluate these parameters in an integrated way, offering new insights into the pathophysiological mechanisms underlying postmenopausal ovarian cancer.

Material and methods. Blood samples were collected from 45 postmenopausal women with ovarian cancer immediately after diagnosis to be compared with 45 healthy women. ERβ and some hormones were evaluated using an enzyme-linked immunosorbent assay. Chemiluminescence imunoassays and miniVIDAS, while spectrophotometric methods were used to evaluate variables associated with oxidative stress.

Results. The results show a significant increase in beta estrogen receptor values for women with ovarian cancer 12.69±1.79 ng/mL, p<0.001 compared to healthy women 0.47±0.06 ng/mL. Furthermore, a significant increase was observed in the values of each estrogen (E2) 18.4±2.19 pg/mL vs. 16.20±3.45 pg/mL, p=0.001, anti-Müllerian hormone (AMH) 15.56±2.88 pmol/L vs. 1.22±0.29 pmol/L, p<0.001, and total oxidant status 2.93±0.63 µmol/L vs. 0.65±0.09 µmol/L, p<0.001. On the contrary, a significant decrease in total antioxidant capacity 3.22±0.72 mmol/L vs. 10.04±1.50 mmol/L, p<0.001. The results also show a positive correlation between the values of total oxidants and the hormones studied, compared to the negative correlation with total antioxidants.

Conclusion. The significant increase in the values of ERβ as well as the estrogen hormone that may be derived from adipose tissue in women with ovarian cancer in the postmenopausal stage, has multiple effects, for example, by altering some hormones such as progesterone, dehydroepiandrosterone sulfate, testosterone, and AMH. These hormonal disturbances resulting from granulosa cell tumors play a role in increasing the metabolic rate and therefore increasing the oxidative stress of cells.

Keywords. β-estrogen receptors, hormonal disorders, ovarian cancer, oxidative stress, postmenopausal

Introduction

Ovarian cancer affects postmenopausal women and is the second most deadly gynecologic cancer worldwide. About 90% of all ovarian cancers are epithelial ovarian carcinomas, according to histological subgroups.[1] High-grade serous ovarian cancer (HGSOC), the most prevalent and deadly variety, is responsible for 70 to 80% of ovarian cancer fatalities.[2] This is because the pathology is asymptomatic, which causes the diagnosis to be made after the disease has already advanced and has a poor

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prognosis. Surgery is typically the first step in treatment and then platinum-based chemotherapy. However, resistance to treatment frequently arises despite early favorable responses, leading to a poor 5-year survival rate of 20% in stage IV and 40% in stage III.[3]

Estrogen receptor β and ovarian cancer

When estrogen attaches to the ER, receptor1dimerization occurs. In the cytoplasm, "ligand-bound ER dimer molecules translocate" to the cell's nucleus. Estrogen receptor elements (ERE) are specific DNA sequences present in the coding regions of target genes to which ER dimers attach in the nucleus.[4,5] ER-mediated transcriptional-1regulation causes changes in the expression of specific genes involved in several cellular activities, such as division, growth, survival, and metabolism (Fig. 1). G protein-coupled estrogen receptor 1 (GPER1) is a binding to the membrane protein receptor that binds to estrogen and activates a number of subsequent signaling cascades, including the pathway involving the PI3K, AKT and m and the mitogen-activated protein kinase (MAPK) circuit. This further enhances classical genomic signaling. Rapid cell proliferation, motility, and survival alterations can be caused by this so-called non-genomic signal.[6,7]

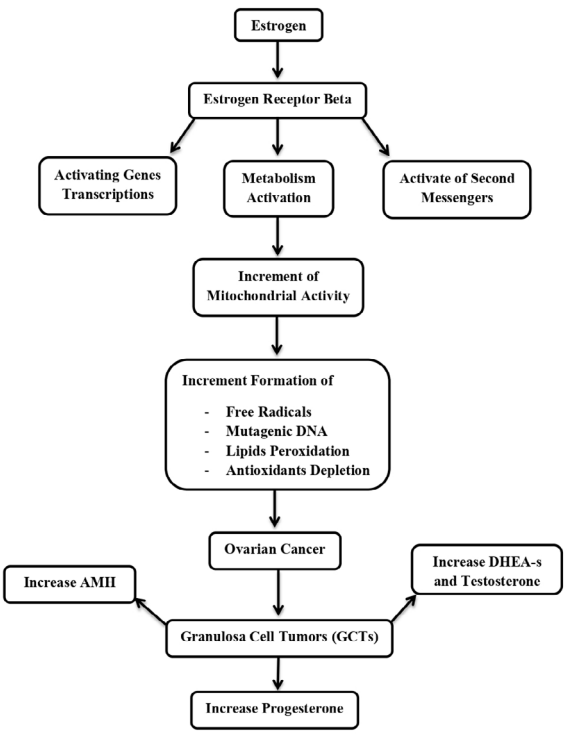

Fig. 1. Impact of estrogen and estrogen receptor on ovarian cancer

Anti-Mullerian hormone and ovarian cancer

The production by the tumor directly causes endocrine symptoms in the majority of cases of granulosa cell tumors (GCTs) cases (two-thirds). Although there are occasional reports of androgenic effects, most of the effects are estrogenic. Menstrual irregularities are observed in premenopausal women, whereas prepubertal women can experience early isosexual development as a result of these effects. Postmenopausal bleeding is the most common symptom among older women. This hormone release has led to a well-documented correlation between GCT and endometrial hyperplasia, as well as adenocarcinoma.[8] AMH is a highly helpful indicator of cancers originating from granulosa cells of ovarian follicles because it is released exclusively by these cells in women. According to reports, 76-93% of women with "granulosa cell tumors (folliculomas)" have elevated AMH levels (Fig. 2). AMH levels could be noticeably higher. AMH levels may rise to 16 months before a tumor becomes clinically noticeable. Compared to estradiol and inhibin, AMH appears to be a more specific biomarker for these tumors and its levels are correlated with tumor size.[9,10] Additionally, AMH is a highly sensitive and specific marker that helps patients with folliculoma who have had the ovaries removed detect a recurrence early. Even 10 to twenty years after the main tumor has been removed, there is a significant chance of recurrence.[11]

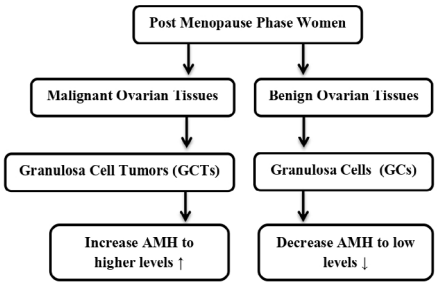

Fig. 2. AMH levels in benign and malignant ovarian cancer in women in the postmenopause phase

Oxidative stress and ovarian cancer

An imbalance between the body's antioxidant defense mechanisms and the generation of reactive metabolites, or free radicals, including oxidants such as reactive nitrogen species (RNS) and reactive oxygen species (ROS), is what leads to oxidative stress (OS). This imbalance may have a cumulative effect and damage biological molecules and cells. Increased oxidant production can lead to an OS state, which can irreversibly damage oxidative biomolecules such as proteins, lipids, and nucleic acids. This OS state is caused by the stimulation of the antioxidant defense mechanism. The development and progression of cancer are closely associated with these lesions. Prior research has indicated that OS has a major impact on the development of cancer.[12] The RNS or ROS can encourage molecular genetic alterations that can accelerate the development and spread of cancers and increase their resistance to treatment (Fig. 1). Although previous studies have explored hormonal disturbances or oxidative stress in ovarian cancer, few

have comprehensively investigated the concurrent alterations in estrogen receptor beta (ERβ), reproductive hormones, and markers of oxidative stress in postmenopausal women. To our knowledge, this is one of the first case-control studies to examine the integrated relationship between ERβ expression, hormonal profiles, and systemic redox status in this patient population.[12]

Aim

The study aims to explain the effect of the presence of estrogen receptors and estrogen hormone in the postmenopausal stage and its effects on disturbances in other hormone levels such as progesterone, testosterone, DHEA, and AMH, as well as high levels of total oxidant status and low levels of antioxidant capacity in women with ovarian cancers compared to healthy women of similar age.

Material and methods

Sample collection and processing

This retrospective study included postmenopausal women diagnosed with ovarian cancer, confirmed by histopathological examination and ultrasound. The patients were age-matched with a control group of healthy women. Blood samples were collected after ethical approval from both the patients and the Iraqi Ministry of Health. Under the supervision of specialist physicians, samples were obtained immediately after diagnosis at the Maternity and Children's Teaching Hospital in Babylon Governorate, Iraq, between February 1 and November 30, 2024. For comparison, blood samples were also taken from healthy controls. Serum was separated from all samples for analysis of hormonal, oxidative stress, and antioxidant parameters.

Study design

The experimental design of this research is a case-control study. Samples were taken from (45) women diagnosed with ovarian cancer after clinical, laboratory, and ultrasound diagnosis, and before starting chemotherapy or radiation therapy to compare with (45) women apparently healthy as control. Patients with chronic diseases or hormonal or endocrine problems were also excluded. The sample size was calculated according to the following equation:

$$n = Z2P\ (1 - P) / d2$$

Ninety samples were gathered; the 'Z score (1.96), the population (0.14)", the number 1 of samples (n), and the absolute marginal error (d), which is equal to 5%, are all indicated.

Inclusion criteria: All women who are suspected of ovarian cancer. Women in the postmenopausal phase.

Exclusion criteria: All women suffering from autoimmune diseases, diabetes mellitus, chronic kidney diseases, hypertension, viral infections such as HPV and any chronic disease. All women with estrogen- or estrogen-like effect therapy.

Procedures

ERβ levels were measured using a competitive quantitative ELISA technique (Catalog No: ab285292). The optical density (OD) was measured at 450 nm. The standard curve included the following concentrations: 20, 10, 5, 2.5, 1.25, 0.625, 0.313, and 0 ng/mL.

Serum estradiol concentrations were determined using a competitive ELISA method (Catalog No. E-FS-E117). The OD was measured at 450 nm. The assay had a detection limit of 5 ppb.

Progesterone levels were quantified using a competitive ELISA (Catalog No: EIAP4C21). The OD was read at 450 nm. The assay demonstrated a sensitivity of 47.9 pg/mL, calculated by adding two standard deviations to the mean OD obtained after 20 replicates of the zero standard.

Dehydroepiandrosterone sulfate (DHEA-S) levels were assessed using a competitive immunoluminometric assay on a fully automated chemiluminescence immunoassay (CLIA) analyzer (MAGLUMI system). The assay used an anti-DHEA-S monoclonal antibody labeled with FITC and a purified DHEA-S antigen labeled with ABEI. Each reagent cartridge was equipped with an RFID tag to ensure proper identification and performance. The procedure strictly followed the manufacturer's operating instructions.

Testosterone and AMH: These hormones were measured using an enzyme-linked fluorescent assay based on competitive immunoassay principles. The solid-phase receptacle functioned both as the solid phase and as a pipetting system. All reagents were predispensed in sealed strips and ready to use. The assay was fully automated on the MiniVIDAS® system, with all steps performed by the instrument. The reaction mixture was repeatedly cycled through the SPR to obtain optimal binding and detection.

Total antioxidant capacity (TAC) was measured using the CUPRAC method (Cupric Reducing Antioxidant Capacity), which is based on the redox reaction involving copper (II) ions. The absorbance was recorded at 450 nm using a spectrophotometer. A 1 mM uric acid solution was used as the standard.[14]

$$\text{Antioxidant} + Cu^{2+} \longrightarrow Cu^{+}$$

$$Cu^{+} + \text{2.9-dimethyl-1,10-phenanthroline} \longrightarrow \text{complex (at 450 nm)}$$

The total oxidant status (TOS) was determined spectrophotometrically using a method developed by Erel. This technique quantifies total oxidant molecules in serum on their ability to oxidize ferrous ion–o-dianisidine complexes to ferric ions. Glycerol, present in the reaction medium, enhances the oxidation process. In an

acidic environment, the resulting ferric ions form a colored complex with xylenol orange. The intensity of the color, measured at 530 nm using a spectrophotometer, is directly proportional to the oxidant concentration in the sample. The assay is calibrated with hydrogen peroxide, and results are expressed in micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 Eq/L).

Statistical analysis

Statistical analysis was done using SPSS version 20 (IBM, Armonk, NY, USA), independent T test and the p-value less than 0.05, and the correlation analysis is performed in Person and (r) is the correlation coefficient. Normal distribution assessed by the Shapiro-Wilk test.

Ethical considerations

The study was approved by the Ethics Committee of the Women's and Maternity Teaching Hospital and Babylon University (IRB No. 2-17, dated February 12, 2024). Informed consent was obtained from all participants.

Results

Table 1 presents the results of female patients with ovarian cancer in terms of age and weight, as well as the results of some of the hormones studied, in addition to the values of total oxidation and total antioxidants.

Table 1. Age, BMI, TOS, TAC, and ERβ results in combination with some associated hormones

Parameters	Groups	Means±SD	95% confidence interval		p
			Lower	Upper	
Age (years)	Patients	56.66±3.11	55.77	57.74	0.440
	Control	57.17±3.12	56.27	58.22	
BMI (kg/m^2)	Patients	25.92±3.71	24.90	27.12	0.001
	Control	22.43±2.56	21.74	23.24	
ERβ (ng/mL)	Patients	12.69±1.79	12.15	13.42	<0.001
	Control	0.47±0.06	0.45	0.49	
Estrogen (pg/mL)	Patients	18.4±2.19	17.58	19.01	0.001
	Control	16.20±3.45	15.12	17.03	
Progesterone (ng/mL)	Patients	0.82±0.15	0.76	0.87	<0.001
	Control	0.32±0.1	0.28	0.35	
Testosterone (ng/dL)	Patients	7.31±1.21	6.97	7.69	<0.001
	Control	4.07±0.7	3.82	4.30	
DHEA-S (ng/mL)	Patients	1.13±0.22	1.07	1.20	<0.001
	Control	0.66±0.1	0.63	0.70	
AMH (pmol/L)	Patients	15.56±2.88	14.62	16.57	<0.001
	Control	1.22±0.29	1.15	1.29	
TOS (μmol/L)	Patients	2.93±0.63	2.78	3.14	<0.001
	Control	0.65±0.09	0.61	0.68	
TAC (mmol/L)	Patients	3.22±0.72	2.99	3.51	<0.001
	Control	10.04±1.50	10.20	11.80	

The correlation between oxidation antioxidants with the studied hormones, which include estrogen receptors, estrogen, progesterone, testosterone, DHEAs, and AMH, as well as with BMI as shown in (Table 2). There are positive correlations between TOS and BMI, also with the hormones studied. On the contrary, negative correlation for TAC with these parameters.

There is a positive correlation between estrogen and the beta estrogen receptor with progesterone, DHEA-s, testosterone, and AMH and the results appear in (Table 3).

There is also a positive association between AMH and the hormones studied in women with ovarian cancer (Table 4).

Obesity is considered a risk factor for ovarian cancer due to the adipose tissues from estrogen sources. Therefore, a positive correlation between BMI and estrogen, progesterone, DHEA, testosterone, and AMH for women with ovarian cancer in the postmenopausal period (Table 5).

Table 2. TOS and TAC correlations with ERβ and some related hormones[a]

Hormones		Eβ	Estrogen	Progesterone	DHEA-s	Testosterone	AMH	BMI
TOS	r	0.930*	0.904**	0.398**	0.781**	0.836**	0.873**	0.486**
	p	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001
TAC	r	-0.946**	-0.930**	-0.333**	-0.785**	-0.781**	-0.902**	-0.438**
	p	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001

[a] * – the correlation significant at p less than 0.05 (2-tailed), ** – the correlation significant at p less than 0.01 (2-tailed)

Table 3. Correlations of estrogen and ERβ with some related hormones[a]

Hormones		Progesterone	DHEA-s	Testosterone	AMH
Estrogen	r	0.390**	0.267*	0.289**	0.342**
	p	<0.001	0.011	0.001	0.001
ERβ	r	0.877**	0.828**	0.841**	0.944**
	p	<0.001	<0.001	<0.001	<0.001

[a] * – the correlation significant at p less than 0.05 (2-tailed), ** – the correlation significant at p less than 0.01 (2-tailed)

Table 4. Correlations of AMH and related hormones[a]

Hormones		ERβ	Estrogen	Progesterone	DHEA-s	Testosterone
AMH	r	0.944**	0.342**	0.816**	0.754**	0.703**
	p	<0.001	0.001	<0.001	<0.001	<0.001

[a] * – the correlation significant at p less than 0.05 (2-tailed), ** – the correlation significant at p less than 0.01 (2-tailed)

Table 5. Correlations of BMI and related hormones[a]

Hormones		ERβ	Estrogen	Progesterone	DHEA-s	Testosterone	AMH
BMI	r	0.113	0.463**	0.415**	0.340**	0.386**	0.440**
	p	0.289	<0.001	<0.001	0.001	<0.001	<0.001

[a] * – the correlation significant at p less than 0.05 (2-tailed), ** – the correlation significant at p less than 0.01 (2-tailed)

Discussion

The notion that postmenopausal obesity is associated with increased mortality from ovarian cancer is supported by the findings of this large prospective investigation. However, among women who were slim and of normal weight, the death rate from ovarian cancer

did not increase steadily. Only women who were overweight or obese had a higher risk. As predicted, there was no indication of increased risk with rising BMI between overweight and obese women who had previously taken postmenopausal estrogen.[15]

The possible roles of estrogen and androgenic hormones in the development of ovarian cancer provide biological reasons for the relationship between BMI and the death rate from ovarian cancer. After menopause, the main origin of endogenous estrogen is adipose tissue, and postmenopausal women have higher circulating estrogen levels. In the culture of cells from malignant and normal sources, estradiol and estrone promote cell proliferation.[16] Therefore, estrogens of extra glandular origins may encourage ovarian epithelial cells to proliferate and undergo malignant transformation. The documented elevated risk associated with long-term postmenopausal estrogen use lends credence to an estrogen-related role in the development of ovarian cancer. Elevated blood testosterone concentrations have been linked to high BMI in postmenopausal women. Additionally, in vitro proliferation of both malignant and normal cells is stimulated by testosterone and 5-dihydrotestosterone. In a prospective analysis of blood hormone levels and the appearance of ovarian cancer, prediagnostic levels of androstenedione and dehydroepiandrosterone were linked to an increased risk of ovarian cancer.[17]

Granulosa cells in the ovaries are among the human cells that exhibit the highest levels of ERβ mRNA and protein expression.[18] This expression is sustained or elevated in granulosa cell tumors of the sex-cord stromal tumor type. However, considering the uncommon granulosa cell malignancies and few cell lines that adequately express this subtype or preserving ERβ expression.[19] Therefore, nothing is known about ERβ's functional role of ER in granulosa cell malignancies. Only two studies conducted on this particular subtype in the last five years. The mitochondria of Granulosa tumor cells include a cytoplasmic ERβ2 that binds to the proteins of the 'Bcl2 family' to prevent apoptosis.[20] According to a different study, when ERα is present, ERβ promotes tumor growth. Both results suggest that ERβ plays a tumor-promoting function in these malignancies, albeit through different methods. These results highlight the need for a more thorough comprehension of ERβ and its isoforms in granulosa cells.[21]

Like certain tumor suppressor transcripts, ERβ's mRNA may be quickly destroyed if it functions as a tumor suppressor.[22] However, to ascertain the expression of a protein, validation using a non-antibody-dependent technique is necessary. According to several findings, ERβ in particular, normal tissues, may prevent the occurrence of cancer, as it is linked to enhanced apoptosis, decreased cell proliferation, and anti-inflammatory qualities when present and activated.[22]

An analysis of ERβ and its function in malignant neoplasms requires navigating a difficult and demanding field characterized by disagreements, variant-specific roles, and structural complexity. A vital component of researching ERβ is the use of antibodies in organs with minimal to non-existent protein expression. Since each isoform may have a different impact on its activity, the complex interaction between ERβ subtypes and their cellular location adds even another dimension to their functional variety. It is worth mentioning that antibodies directed against neglected tropical diseases (NTD) would identify every variation of ERβ rather than discriminate between distinct isoforms.[23] High levels of estrogen and progesterone receptor activation are frequently associated with LGSOC. Furthermore, they typically exhibit a wild-type expression pattern, in contrast to individuals with HGSOC, and active mitogen-activated protein kinase (MAPK) pathways with KRAS and BRAF mutations.[24]

Rare ovarian cancers known as Sertoli-Leydig cell tumors (SLCT) are triggered by stromal cells and rudimentary sex cords in the ovary. These tumors usually appear during the second and fourth decades of life and account for fewer than 0.5%1of ovarian malignancies. The self-secretion of testosterone or its precursor androstenedione, by several of these tumors induces a significant androgen excess in females. The remaining tumors either do not function at all or produce estrogen. Because SLCT is so rare, knowledge of its prognostic variables is rather poor.[25] Numerous investigations have noted that the most important prognostic factors are tumor stage at assessment and level of differentiation.[26] Serum testosterone often indicates the amount of androgen produced by the ovaries in women. Adrenal androgens, such as androstenedione, are converted to a minor amount of testosterone. Adrenocortical carcinoma is known to cause markedly high serum testosterone levels, which are correlated with circulating precursor substances of adrenal androgen precursor substances.[27]

About 10% of all ovarian tumors are stromal neoplasms of the sex cord, which are formed from mesenchymal stem cells found in the ovarian cortex. These consist of Sertoli–Leydig cell tumors, granulosa–theca cell tumors, and granulosa cell tumors (GCT). Three percent of ovarian tumors are GCTs. Based on histological characteristics and clinical presentation, two different forms of GCT have been identified, the pediatric and adult form have been identified.[28]

The elevation of serum AMH for patients with GCT because of gene expression of neoplasm specimens. AMH expression was found in each of the GC tumors in a limited number of ovarian tumors, but was absent in the seven epithelial carcinomas. Although AMH positivity was consistently detectable in the GC tumors, it did not appear as prominent or diffuse in alpha-inhibin employing paraffin-fixed tissues and an antibody di-

rected against AMH. It has recently been confirmed that AMH expression can be found in young and adult-type GCT regardless of the ovarian or metastatic location.[29–31]

Recently, it has been discovered that human ovarian epithelial cancer expresses AMH type II receptors (AMH-RII). AMH-RII mRNA was detected by RT-PCR in ovarian cancer cells and peritoneal cells in those suffering from ovarian papillary squamous cystoadenocancer. Ten out of the 15 patients had cell lines with AMH-RII transcripts. The existence of AMH-RII mRNA predicted binding to AMH in 89% of the cases. AMH-RII immunostaining was also shown in the solid tumors of four individuals.[32–34]

According to studies, the Fenton reaction is how ferrous and its metabolites increase the creation of ROS. Old blood from the ovary, which has an exceptionally high iron concentration during the development of the chocolate cyst, might increase ROS creation of ROS and cause DNA damage, hence raising the likelihood of the endometriosis turning malignantly into mast cell ovarian cancer.[35–38] The transferrin receptor 1 (TfR1) axis is boosted by the hydroxyl radicals created via H_2O_2 in the "Fenton reaction". This can result in many double-strand breaks in the DNA of the oviduct's epithelial cells, which will encourage OV. Furthermore, OS affects different immune cells, including regulatory T cells, myeloid-derived suppressor cells, neutrophils, and tumor-associated macrophages. Our findings expand current knowledge by demonstrating strong correlations between ERβ and multiple hormonal and oxidative stress parameters, which may reflect a mechanistic link between estrogen signaling and redox imbalance in the tumor microenvironment. Unlike previous studies that focused on isolated markers, this investigation combines endocrine and oxidative markers, offering a more integrative perspective on tumor biology in postmenopausal ovarian cancer.[39,40]

Study limitations

Despite the study's thoroughness, numerous aspects remain unexplored and could be investigated in future research. These include taking into account the duration of the illness and reviewing patient follow-ups both before and after the prescription of antioxidants and vitamins supplements. Furthermore, the patient's lifestyle was neglected because it is integrally related to behavior, decisions about life, and food, all of which have a strong connection with elevated oxidative stress levels.

Conclusion

This study provides novel insights into the interplay between ERβ, hormonal dysregulation, and oxidative stress in postmenopausal women with ovarian cancer, highlighting the potential of ERβ and AMH as candidate biomarkers for future diagnostic and prognostic applications. Hormonal disturbances in postmenopausal women can contribute to the occurrence of ovarian cancer in women. A large percentage of these women who have ovarian cancer have positive estrogen receptors. Estrogen at this age plays a role in increasing the growth rate of granulosa cell tumors. These tumor cells play a role in increasing the levels of estrogen, DHEA, testosterone, and AMH. These hormonal disturbances in granulosa cell tumors increase metabolic processes and thus increase oxidation levels due to mitochondrial activity and the electron transport chain, which in turn leads to a decrease in the total concentrations of antioxidants in patients. Weight gain in postmenopausal women can also be considered a risk factor for ovarian cancer. Because adipose tissue is a source of estrogen production, which is a risk factor after binding to the estrogen receptors alpha and beta, which in turn disrupts the body's hormones and balance of oxidant-antioxidants.

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Declarations

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Author contributions

Conceptualization, A.M.A.A-K.; Methodology, N.J.T.A-M.; Software, N.J.T.A-M.; Validation, A.M.A.A-K. and N.J.T.A-M.; Formal Analysis, N.J.T.A-M.; Investigation, A.M.A.A-K.; Resources, A.M.A.A-K.; Data Curation, N.J.T.A-M.; Writing – Original Draft Preparation, A.M.A.A-K.; Writing – Review & Editing, A.M.A.A-K.; Visualization, A.M.A.A-K.; Supervision, A.M.A.A-K.; Project Administration, A.M.A.A-K.; Funding Acquisition, N.J.T.A-M..

Conflicts of interest

There are no conflicts of interest.

Data availability

The corresponding author can be contacted for the raw data upon request.

Ethics approval

The study was approved by the Ethics Committee of the Women's and Maternity Teaching Hospital and Babylon University (IRB No. 2-17, dated February 12, 2024).

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