DOI: https://doi.org/10.53350/pjmhs2115113372

**ORIGINAL ARTICLE**

# Circulating Expressions of Gonadotropin Releasing Hormones and Risk of Ovarian Cancer

GULSHAN PARVEEN[1], ALI HASSAN[2], MUHAMMAD HASSAM REHM[3], AWAIS ALTAF[1], NUREEN ZAHRA[1], ARIF MALIK[1], ANAM NAZ[4], HUMA SATTAR[1]

[1]*Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore-Pakistan*
[2]*Department of Biochemistry, Sahiwal Medical Collage, Sahiwal*
[3]*Community Medicine Department, Sahiwal Medical Collage, Sahiwal*
[4]*Centre for Research in Molecular Medicine (CRiMM), The University of Lahore-Pakistan*
*Correspondence to Gulshan Parveen, Email: gulshanparveen14@gmail.com, Cell: 0333-1461527*

## ABSTRACT

**Background:** Ovarian cancer (OC) is a worst type of malignancy in the field of gynecology. This is because ovarian tumors diagnosed at advanced stage of disease. The exact mechanism for its development is still unknown.
**Aim:** The aim of this study is to measure the levels of steroidal hormones and their function in ovarian cancer progression.
**Methods:** In the present study, fifty ovarian cancer patients and fifty control individuals were taken and serum was separated from their blood samples. The levels of steroid hormones were measured by ELISA kit methods.
**Results:** Result of the current study determined the levels of $E_2$, progesterone, testosterone, FSH, LH, 17-β-HSD-I, 17-β-HSD-II, cortisol and aromatase were extensively higher in patient group in comparison with healthy individuals.
**Conclusion:** Current study concluded the Study concluded that overexpression of steroid hormones may lead to enhance tumor survival in ovarian cancer through various signaling mechanisms.
**Keywords**: Ovarian cancer, Estradiol, FSH, LH, progesterone

## INTRODUCTION

Ovarian cancer is the 8th common malignant women diagnosed disease in worldwide. The risk of ovarian cancer in its reproductive age is 1.3% for each woman. It is 5th common cancer related cause of death for women and considered to be as deadliest gynecological malignancy. There are other reasons for late prognosis is unspecific symptoms of the disease[1]. Approximately 50% of the ovarian cancers patients are suffer from weight loss and low performance in their work. Ovarian cancer originates from multiple sites from the inside of body. In case of ovarian surface epithelium, ovarian cancer derives from fallopian tubes[2]. It is hormone dependent cancer which affected by hormonal intracellular signaling mechanisms. This outcome declares itself after the disease and has therapeutic potential target against ovarian cancer. In hypothalamus, gonadotropin releasing hormone (GnRH) is released from neurons and regulate secretion of luteinizing hormone (LH) and follicle stimulating hormones (FSH) from anterior pituitary. These hormones facilitate ovulation, synthesis of corpus luteum, formation of progesterone and estrogen as well as maturation of follicles[3]. There are two main theories have presented related to ovarian cancer expansion. Theory of incessant ovulation hypothesized that ovarian cancer is enhanced due to number of ovulatory cycles and exposure of epithelium rupture follicles with mitotic stimuli[4]. The hypothesis related to gonadotropin explains that increase secretion of gonadotropin stimulates transformation, malignant and proliferation of ovarian epithelium within the inclusion cysts during ovulation[5]. Reproductive steroid hormones including estrogen and androgens significantly involved in survival of ovarian cancer. Study of Salazar et al (1996)[5] explained that high frequencies of metaplastic and hyperplastic alterations in ovarian epithelium is strongly linked with progression of ovarian cancer. According to epidemiological data, the induction of ovarian cancer is strongly linked with the metabolism and induction of estrogen. The experimental analysis suggested that ovarian cancer has regulated mechanisms which can either hormone dependent caner including endometrial or breast cancer. These mechanisms were also studies in various other tumors[6]. The imbalance between progesterone and estrogen is potent for development of cancer. Prevalence rate of ovarian cancer is much advanced in industrial countries as compared to developing countries. Birth rate is comparatively low in industrial countries as compared to developed countries. Linkage between ovarian cancer and sex steroids can be explained through the mechanisms taking place in menstrual cycles in which ovarian surface epithelium is significantly involved in wound repair and ovulation. Proliferative activity of ovarian surface of epithelium is linked with ovulation and synthesis of corpus luteum. During menstrual phase, high exposure of ovarian surface of epithelial to FSH and LH may lead to enhance tumor survival and metastasis[7]. In the following study, we provide the role of various steroid hormones in ovarian cancer development via signaling mechanism.

## MATERIAL AND METHODS

Current study was designed to measure the levels of biochemical variables in ovarian cancer patients. Fifty patients sample (ovarian cancer) were collected from INMOL hospital, Lahore and fifty healthy individuals were taken as control group. All the selected patients were screened at the Institute of Biology and Biotechnology (IMBB), The University of Lahore. The ovarian cancer patients with stage IV were included in this study. Blood sample (5 ml) of blood sample was taken from anticubital vein of each person. Then, serum was separated by centrifugation and stored at -75 ºC for further biochemical analysis. Ovarian cancer patients (stage IV) with the history of hormonal imbalance were included in this work and subjects with medication of mental disorders, metabolic diseases, malnutrition syndrome and chronic infection were excluded from in current study. It was confirm that the control individuals were not any type of medications. Complete blood count (CBC) of the ovarian cancer patients were measured by the automated hematology blood analyzer of Sysmex version XP-2100. The levels of estradiol, LH, FSH, cortisol, aromatase, 17-β-HSD-I, 17-β-HSD-II, progesterone and testosterone were determined by commercially available ELISA kit method.
**Statistical analysis:** In this study, statistical analysis was done by SPSS statistics 17.0. Results of all variables were measured by independent sample t-test. Data was expressed by Mean±SD and changes were considered significant, when p-value were less than 0.05.

## RESULTS

**Hematological profile of healthy individuals versus patients suffering from ovarian cancer:** Data depicted in table 1 and figure 1 shows the hematological report of ovarian cancer patients versus control individuals.

Mean values of neutrophils, lymphocytes, monocytes, WBCs and creatinine were significantly increased in ovarian cancer patients (4.75±1.47%, 3.81±0.81%, 2.01±0.60%, 9.62±1.51 k/mm$^3$ and 2.53±0.21 mg/dl) as compared to control individuals (1.23±0.98%, 2.41±0.5%, 1.21±0.21%, 3.60±0.28 k/mm$^3$ and

1.31±0.51 mg/dl) respectively. On the other hand, decreased trend of RBCs (2.52±0.20 million/mm$^3$ vs 8.58±0.709 million/mm$^3$), PLTs (196.11±30.91 $10^9$ /L vs. 315.83±9.17 $10^9$ /L), Hct (35.27±3.93% vs. 55.29±1.59%), Hb (10.34±1.33 g/dL vs. 13.61±2.30 g/dL) and BMI (21.43±4.31 Kg/m$^2$ vs. 32.11±3.62 Kg/m$^2$) were recorded in ovarian cancer patients in contrary with healthy control correspondingly.

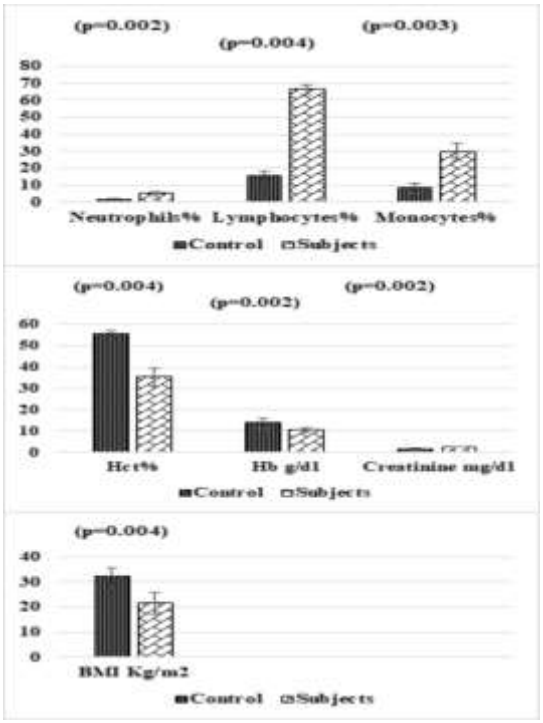


Figure 01: Hematological Profile Of Women Suffering From Ovarian Cancer Versus Contrl

Table 1: Hematological profile of ovarian cancer patients vs. control

| Parameters | Control (N=50) Mean±SD | Patients (N=50) Mean±SD | p≤0.05 |
|---|---|---|---|
| Neutrophils % | 1.23±0.98 | 4.75±1.47 | 0.007 |
| Lymphocytes % | 2.41±0.51 | 3.81±0.81 | 0.004 |
| Monocytes % | 1.21±0.21 | 2.01±0.60 | 0.003 |
| RBCs (million/mm$^3$) | 8.58±0.70 | 2.52±0.20 | 0.001 |
| WBCs (k/mm$^3$) | 3.60±0.28 | 9.62±1.51 | 0.001 |
| PLTs ($10^9$ /L) | 315.83±9.17 | 196.11±30.91 | 0.005 |
| Hct% | 55.29±1.59 | 35.27±3.93 | 0.004 |
| Hb (g/dL) | 13.61±2.30 | 10.34±1.33 | 0.002 |
| Creatinine (mg/dl) | 1.31±0.51 | 2.53±0.21 | 0.002 |
| BMI (Kg/m$^2$) | 32.11±3.62 | 21.43±4.31 | 0.004 |

Profile of steroidal hormone of patients suffering from ovarian cancer

Data expressed in table 02 and figure 02 explains the significance of various steroid hormones ovarian cancer progression in contrary with control Mean value of estradiol was significantly increased in ovarian cancer patients (40.22±4.77 pg/ml) as compared to control individuals (13.32±2.22 pg/ml) with statistically significant p=0.001.

Increase trend of LH (46.21± 5.21 mU/ml vs. 15.54± 2.22 mU/ml, p=0.007) and FSH (20.41±4.88 mU/ml vs. 9.55±2.88 mU/ml, p=0.001) were recorded in patients suffering from disease as compared to control individuals respectively. Moreover, mean values of cortisol and aromatase were significantly raised in these patients (22.01±3.88 µg/dL and 9.52±1.66 ng/ml) in comparison with control individuals (4.12±1.54 µg/dL and 2.54±1.41 ng/ml) with statistical significant p value=0.003 and 0.006 correspondingly. Highest trend of 17-β-HSD-I (8.18±2.44 pg/mL vs. 4.72±1.22 pg/mL) and 17-β-HSD-II (66.14±2.99 pg/mL vs. 15.32±2.67 pg/mL) were observed in women suffering from ovarian cancer in comparison with control individuals respectively. The mean value of testosterone was also increased in ovarian cancer patients (29.44±4.99 pg/mL) versus control individuals (8.32±2.78 pg/mL). On the other hand, the levels of progesterone (6.21±2.34 ng/ml vs. 20.34±4.32 ng/ml) were significantly reduced in ovarian cancer patients in comparison with control individuals respectively.

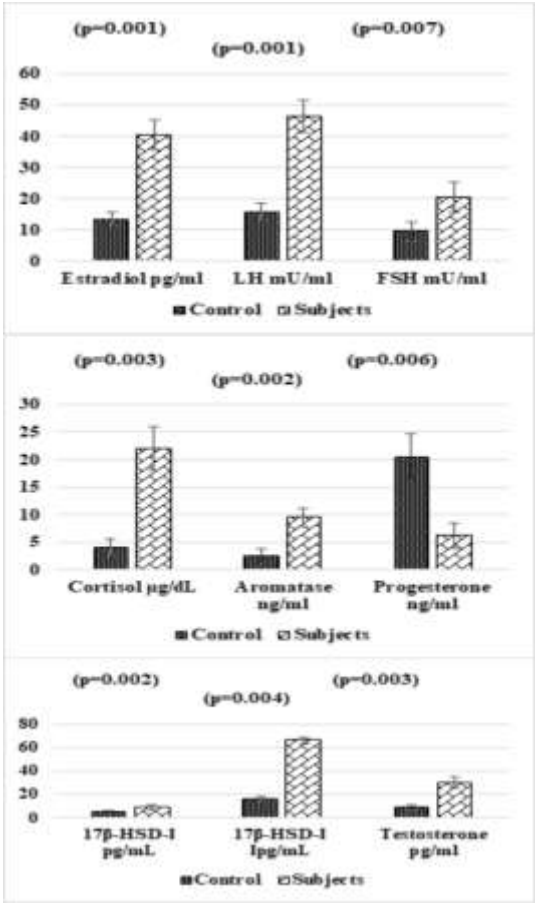


Figure 02: Hormonal Profile Of Ovarian Cancer Patients Versus Control

Table 2: Profile of steroidal hormones of patients suffering from ovarian cancer

| Parameters | Control (N=50) Mean±sd | Subjects (N=50) Mean±sd | (p<0.05) |
|---|---|---|---|
| Estradiol (pg/ml) | 13.32±2.22 | 40.22±4.77 | 0.001 |
| Luteinizing hormone (LH) (mU/ml) | 15.54± 2.22 | 46.21± 5.21 | 0.007 |
| Cortisol (µg/dL) | 4.12±1.54 | 22.01±3.88 | 0.003 |
| Aromatase (ng/ml) | 2.54±1.41 | 9.52±1.66 | 0.006 |
| 17-β-Hydroxydehydrogenase-I (17β-HSD-I) (pg/mL) | 4.72±1.22 | 8.18±2.44 | 0.002 |
| 17-β-Hydroxydehydrogenase-II (17β-HSD-II) (pg/mL) | 15.32±2.67 | 66.14±2.99 | 0.003 |
| Progesterone (ng/ml) | 20.34±4.32 | 6.21±2.34 | 0.002 |
| Follicle stimulating hormone (FSH) (mU/ml) | 9.55±2.88 | 20.41±4.88 | 0.001 |
| Testosterone pg/mL | 8.32±2.78 | 29.44±4.99 | 0.004 |

## DISCUSSION

In ovarian cancer, the importance of gonadotropins for granulosa and theca cells steroidogenesis is well understood e.g. FSH and LH enhance the levels and activity of P450 in granulosa cells and luteal cells which resultantly enhance the formation of estradiol[8]. During ovarian carcinoma, the mechanism of action of LH and FSH is mediated by seven transmembrane receptors. Ligand binding triggers conformational changes on receptors consequently secrete Gsα subunit, stimulate adenylate cyclase and protein kinase A (PKA), and cause elevated synthesis of cAMP. PKA can affect the activity and expression of steroidogenic machinery such as the transcriptional activity of StAR promoter is enhanced by activation of adenylate cyclase through FSH and LH ligand binding. High expression of StAR protein is linked with cAMP dependent steroidogenic factor-1. Moreover, this ligand binding interaction may leads to the activation of MAPK/ERK signaling pathways, which induces proliferation, metastasis, survival and angiogenesis of ovarian cancer.

Throughout the reproductive years, the progesterone, estrogen, estrone (E1), 17β-estradiol (E2) and estriol (E3) are produced and released from the ovaries. E2 is considered to be a more active form of natural estrogens, which collectively with progesterone to play a potent role in normal uterine function, maintenance and establishment of pregnancy as well as development of the mammary gland. Ovarian theca and granulosa cells are responsible for the synthesis of ovarian steroid hormones, which can be possible by the stimulation of gonadotropins FSH and LH. The signaling of LH may response to theca cells by enhancing the concentration of steroid synthesizing enzymes. These enzymes are used for the transformation of cholesterol to 5-androstenedione and testosterone. FSH signaling may response to granulosa cells by stimulating the concentration of estrogen synthesizing enzymes[9]. In premenopausal women, estradiol abstains from the synthesis in the ovary. In menopause women, estradiol is synthesized from locally in several tissues, including adipose tissue, brain, and liver. Estrogen is also produced by estrogen precursors and androgen. In the blood, these estrogen precursors combined with sex steroid binding globulins. After that, they are transported into ovary and taken up ovarian epithelial cells by transporters such as organic anion transporting peptides (OATPs). In postmenopausal women, the significance of visceral adipose tissue for E2 formation is revealed by increased levels of 5-androstendione, dehydroepiandrosterone sulfate (DHEA-S) and estrone sulfate (E1-S) in ovarian carcinoma cells[10].

Biological functions of estradiol can be regulated by receptor activity as well as activity of steroid metabolizing enzymes at target tissues. From these steroid metabolizing enzymes,17β-hydroxysteroid dehydrogenase (17β-HSD), steroid sulfatase (STS) and aromatase (CYP19A1) are highly significant enzymes. In ovarian cancer, the biological active E2 can be produced from steroid precursor that is mediated by sulfatase pathway and aromatase pathway. The aromatase pathway is mediated by steroid precursors, which derived from dehydroepiandrosterone (DHEA) that converted into stosterone and consequently synthesized E2 by aromatase enzyme. In case of sulfatase pathway, sulfate component is discharged from inactive E1-S to form active estrogen E1 with the help of steroid sulfates (STS). After that, E1 is converted into E2 via reducing the 17β-HSD isoenzymes. E2 is oxidized into E1 with the help of 17β-HSD enzymes. E1 has estrogenic activity through binding to estrogen receptors and can be inhibited by estrogen sulfotransferase (SULT1E1). Inactive E1-S can be activated by eliminating sulfate group[11]. It is strongly considered that precursor of all steroid hormones is cholesterol which synthesized mainly from nutrition or in the liver. After a few steps, the cholesterol is converted into pregnenolone and then into progesterone. The precursors of steroid hormone such as DHEA with its sulfate metabolite 5α-androstenediol sulfate (5-Diol-S), E1-S and DHEA-S are produced in large amount in ovarian cancer. The levels of other steroid hormones, including 5-Diol-S and DHEA are similar in pre- and postmenopausal women. There are approximately 10-30% of serum estrogen are bound to sex steroid binding globulins to presentas a reservoir for the peripheral E2 synthesis[12]. Circulating inactive plasma estrogen precursor E1-S may serve as a major source of active estrogen (E2) in women with ovarian cancer.. After the uptake of E1-S by transporters of OATPs family, it is desulfonated into E1 with the help of steroid sulfatase (STS) and then E1 is transformed into E2 by 17β-HSD as shown in figure 01. The intracellular formation of active estrogen (E2) may regulate the estrogen dependent tumor cell proliferation.

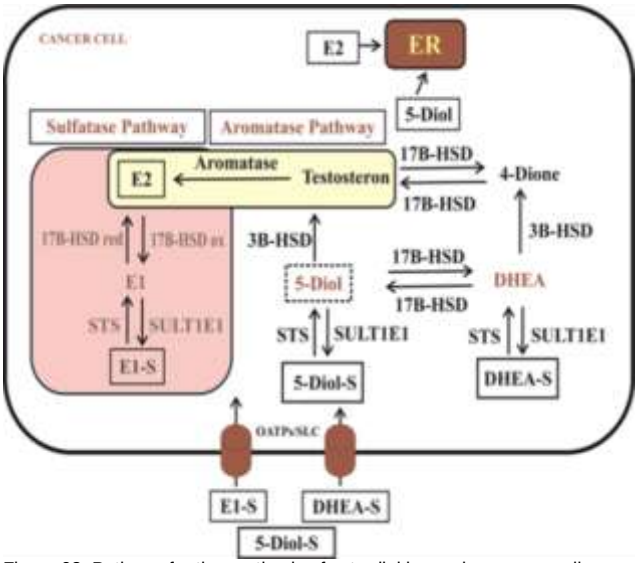


Figure 03: Pathway for the synthesis of estradiol in ovarian cancer cell.

Estrone-sulfate (E1-S) is precursor of most active form of estrogen. 17β-estradiol, dehydroepiandrosterone sulfacte (DHEA-S) and androstenedoil (5-Diol-S) are taken from blood stream into cancerous cells through transporter which is called as solute carriers (SLCs) or organic anion transporting polypeptides (OATPs). In case of sulfatase pathway, E1-S is transformed into estrone (E1) by steroid sulfatase (STS), and E1 is converted into E2 through reductive 17β-HSD). E2 is most active forms of estrogen that combines and stimulates estrogen respector (ERs). In reverse pathway, the oxidative 17β-hydroxysteroid dehydrogenase enzyme converts E2 into E1. In the mechansim of aromatase, estradiol is synthesized with the help of aromatase and testesterone is produced from 5-androstenediol (5-Diol) through STS.

## CONCLUSION

Current study concluded that tumor in ovaries stimulate the stroma to synthesize steroid hormones and provide possible information through signaling mechanism that how aberrant expression of steroid hormones stimulate and support tumor survival, growth, proliferation and metastasis in ovarian cancer.

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