Evaluation of biochemical alteration in serum electrolytes (Na\(^+\), K\(^+\)) and female sex hormones (estrogen, progesterone) levels in ovarian cancer

Abstract

Ovarian cancer is the type of cancer which initiates at ovaries. The grounds of ovarian cancer is not strong yet although doctors recognized some influences which can amplified the risk of this disease. For this purpose, total 70 individuals were included in the present study, which were grouped into two groups. Group A was normal healthy women, who were not used any hormone-related treatment. Group B was of women with ovarian cancer. Group B was also subdivided into three subgroups, on the basis of health criteria, pre-treatment phase patients, treatment phase patients, and post-treatment phase patients. The Blood sample were completely processed and analyzed for the estimation of serum electrolytes (Na\(^+\), K\(^+\)) and female sex hormones (Estrogen and progesterone) concentrations by using flame photometer and enzymatic reagent kits. The outcomes of the present study showed significant alteration in serum sodium, potassium, and estrogen level while in-significant alteration in serum progesterone level was observed indicating the profound effect of cancer manifestation on serum sodium, potassium and estrogen levels.

Introduction

Ovarian cancer is the seventh most common cancer in women. It is the fifth leading cause of cancer death in women. Ovarian cancers actually represent a group of different tumors that arise from the diverse type of tissue contained within the ovary. Ovarian cancer is a cancerous growth arising from different parts of the ovary. Most (>90%) ovarian cancer are classified as “epithelial” and were believed to arise from the surface (epithelium) of the ovary. The fallopian tube could also be the source of some ovarian cancer. Hereditary form of ovarian cancer can be caused by mutations in specific genes (most notably BRCA1 and BRCA2, but also in genes for hereditary nonpolyposis colorectal cancer). Infertile women and those with a condition called endometriosis; those who use postmenopausal estrogen replacement therapy are at increased risk. Estrogen (estradiol, estrone, estriol) are predominately female hormones and in adults, they are important for maintaining the health of the reproductive tissues, breasts, skin, and brain. Excessive estrogens can cause fluid retention weight gain, migraine and over stimulation of the breasts, ovaries, and uterus, leading to cancer, endometriosis, polycystic ovaries uterine fibroid tumors insufficient estrogen levels or fluctuations of estrogen can lead to hot flashes vaginal dryness rapid skin aging urinary problems excessive bone loss and possible acceleration of dementia.

Estrogens have long suspected as etiologic factors of ovarian cancer. Although usage of estrogen-based oral contraceptives is known to reduce ovarian cancer risk, its effect is primarily attributed to reduction in ovulation frequency. Ovarian tissue estrogen levels are at least 100-fold higher than circulating levels and those in the follicular fluid of ovulatory follicles are even higher. It is logical to speculate that genomic damage of OSE cells covering the ovulating follicles or in inclusion cysts may in part be caused by the high levels of estrogen in the follicular fluid or in the ovarian stoma in addition to including genetic damages of OSE cells Syed et al. have reported estrogen receptor (ER) mediated growth stimulatory responses of normal and malignant OSE cells to estradiol-17 beta and estrone worthy of mentioning is the observation that estradiol-17 beta and estrone are equal potency in stimulating growth OSE cells although it is well known that estrone is a much less potent estrogen when compared to estradiol-17 beta this is an important finding after menopause estrone is the major circulating estrogen produced as a result of aromatization from androstenedione in skin and adipose tissue.

The mitogenic action of estrogen appears critical to the etiology and progression of human gynecologic cancers. The principal biological activities of estrogens are to influence the growth, differentiation, and function of reproductive tissues. Estrogens interact with their receptors to mediate various signaling pathways that are likely associated with the risk of ovarian cancer. Estrogen receptors exist in two forms, estrogen receptor alpha (ER\(\alpha\)) and estrogen receptor beta (ER\(\beta\)). which is the predominant estrogen receptor in the ovary. Although the exact role of ER\(\beta\) in ovarian carcinogenesis remains to be determined, recent in vivo and in vitro studies suggest that ER\(\beta\) is involved with the control of cellular proliferation, motility and apoptosis in ovarian cancer; and loss of ER\(\beta\) expression is associated with tumor progression.

Progestrone is a hormonal balance particularly of estrogens it enhances the beneficial effect of estrogens while mitigating the problems associated with estrogen excess natural progesterone also helps regulate apoptosis. Progesterone is the “pro-gestational” hormone and plays a critical role in the conception and full-term pregnancy) Progesterone (p4) or cellular responses to p4 appears to offer protection against ovarian carcinogenesis. The progesterone receptor (pr) gene locus is commonly observed in OCA specimens (~75 %, (29-31)) and this genetic alteration is associated with poor prognosis. These findings thus implicate PR as tumor suppress or gene. Epidemiological data provide additional support that P4 or response of OSE cells to the steroid affords a protective role against ovarian carcinogenesis.
cancer incidence was observed among women with progesterone deficiency. In contrast increased parity is associated with a reduction in ovarian cancer risk, the protective effect of pregnancy may be attributable to exposure of the OSE to high levels of P4 during pregnancy in concordance with twin pregnancies. 

Tumor lysis syndrome (TLS) contributes to the assemblage of electrolyte abnormalities caused by the rapid and immediate release of intracellular content into the bloodstream. The syndrome is marked by hyperkalemia. Metabolic acidosis and acute renal impairment could also arise. The release of intracellular potassium and organic and inorganic phosphate in the blood cells of apoptosis result in the evolution of hyperkalemia respectively. TLS can develop before administration of high dose chemotherapy, resulting in the rapid destruction of tumor cells. Hyponatremia is a frequent complication of electrolyte in patients with cancer could be caused by different mechanisms. True volume, which has pronounced stimulus for vasopressin (ADH) release, could result in hyponatremia. True volume depletion is typically found in hemorrhagic, diarrhea, vomiting, drainage of ascites or pleural effusion, or peritoneum ileus. Moreover, salt wasting nephropathy been associated with cisplatin or ifosfamide tubular toxicity, adrenal insufficiency attributable to tumor metastasis in both adrenal glands and cerebral salt wasting that has been set out patients with intra-cranial may be also produced hyponatremia in patients with cancer. Our hypothesis is that manifestation of ovarian cancer cause alteration in serum electrolytes level (Na+, K+) and estrogen, Progesterone levels. Therefore we estimated the level of Electrolytes (Na+, K+) in ovarian cancer and female sex hormones( Estrogen and Progesterone) in ovarian cancer.

Materials and methods

The present study was conducted to evaluate serum electrolytes (Na+, K+) and female sex hormones (estrogen, progesterone) levels in ovarian cancer. Samples were collected from INMOL (Institute of Nuclear Medicine and Oncology). All biochemical analysis was conducted at IMBB (Institute of Molecular Biology and Biotechnology) The University of Lahore.

Experimental design

Total 70 individuals were selected for the present study, which were grouped into two groups. Group A was comprised of normal healthy women, who were not using any hormone-related treatment. Group B was with women having ovarian cancer. Group B was also subdivided into three subgroups, on the basis of health criteria, pre-treatment phase patients, treatment phase patients, and post-treatment phase patients. Pre-treatment phase patients were those who were diagnosed for ovarian cancer but they were not using any therapy for their treatment. Treatment phase patients were used chemotherapy or radiotherapy for 2-3 month while post-treatment phase patients those who had completed their chemo and radiotherapy (Table 1).

Blood collection

2-3ml of blood was drawn from the patient’s antecubital vein using the aseptic technique. Blood was transferred into the red top vacutainer, immediately after collection. Vacutainer was labeled with patient’s name and sample code. Blood was allowed to clot. Then it was centrifuged at 3000 r.p.m for 10minutes and serum was separated. Serum was allocated into the eppendorf tubes and was labeled with the same sample code as that of the mother tube. From all the patients and controls bio samples were collected and processed in the same way and the tubes were stored at −20°C till the time of analysis.

Table I The study plan was given in the table in which total 70 samples were shown, 10 are from control and 60 for ovarian cancer

<table>
<thead>
<tr>
<th>Sr.#</th>
<th>Groups</th>
<th>Health status</th>
<th>No. of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A Controls</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>B Women</td>
<td>pre-treatment phase</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>with ovarian cancer</td>
<td>treatment phase</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>post-treatment phase</td>
<td>20</td>
</tr>
</tbody>
</table>

Results

Blood analysis

All the serum tubes were brought to the room temperature before analysis. Serum estrogen and progesterone were determined through enzyme Immunoassay test kit.

Sample analysis

The samples were processed and analyzed for the estimation of estrogen, progesterone and serum electrolytes (Na+, K+) levels.

i. Estimation of sodium and potassium

The flame photometer (FP) FP 10 and FP 20 (Supplied with dilution) flame photometer series was designed to measure sodium potassium and lithium concentrations in sera. These instruments use lithium as an internal reference in order to obtain a greater accuracy and enable a precise compensation of changes in the flame itself.

ii. Preparation of blank and the standard solutions

The containers were cleaned thoroughly for the preparation of the samples, the standard, and the blank solutions. Double distilled water was used which had a conductivity of < 5 μS/cm.

iii. Blank solution for the Serum

The 15mEq/L Lithium solution was supplied with the instrument (P/N 71010300) was used to dilute the samples and the standards under test.

iv. Standard solution for the Serum program

A 1/200 ratio the following potassium and sodium solutions were diluted: P/N 71010030 (K 5 – Na 140)mEq/L for Serum reckonings was used P/N 71010020 (K 100- Na 100)mEq/L for urine reckonings was used 15mEq/L Lithium solution was diluted.

Principle

Metal ions in solution aspirated in low-temperature flame (i-e aerosol form). Electrons of ion excited to high energy states. Excited electrons return to ground state, and lose excited energy. The discrete wavelength of visible light is emitted. Optical filter isolate light wavelength from others light wavelength. Amount of light emitted can be detected electron suitable photo detector. Amount of light emitted is directly proportioned to the number of ions in flame. Electrical signal from the photo detector is amplified and displayed on the digital readout.

Citation: Zafar N, Khan MS, Kiran Q. Evaluation of biochemical alteration in serum electrolytes (Na+, K+) and female sex hormones (estrogen, progesterone) levels in ovarian cancer. MOJ Cell Sci Rep. 2019;6(1):1-5. DOI: 10.15406/mojcsr.2019.06.00122
**Procedure**

The coiled compressed air hose was connected to the output connector on the compressor. The other end of the hose was attached to the intake connector on the rear panel of the instrument. The gas tube was fitted on the nipple and carefully tightened the ring clamp around the tube so as to avoid gas leakage. Check that the knob is locked turning it all the way. The compressor’s power supply cable was connected from the compressor’s socket. The glass tube vertically was inserted through the opening in the top part of the instrument’s housing. The power supply cable was connected to the socket. The PVC tube was inserted into the diluting solution tank this tube is connected to the filter by means of a small stainless-steel pipe. The Supplied intake probe tip was fitted onto the cone point of the tube anchored to the front panel. The instrument was turned on by means of a switch and push key. The gas tank’s tap was opened. Switch was pressed on the rear of the instrument. Ignition key was pushed. The air compressor was delivered the pressure required in order to supply the spray chamber and opened the gas valve. Air pressure was measured by means of a manometer which was placed on the rear of the instrument and should bar (Atm). The sparking device is activated at this time in order to ignite the gas within the burner. The flame was checked through the special window on the side of the instrument. The flame was approximately 3cm (1-3/16") high and colored blue (Oxidizing flame). The knob was adjusted to obtain the correct air pressure. The sample under test was taken after a 1/200 dilution with the 15mEq/L Lithium solution was effected. The values of the k⁺ and Na⁺ concentration in the sample were displayed as soon as the integrating process was completed. The sample was removed and the progressive ID number was automatically increased (Table 2).

**Reagents**

**Procedure**

The desired number of the coated well was secured in the holder. 25ul of standards, specimen, and controls were dispensed into appropriate wells. 100ul of Estradiol-HRP Conjugate Reagent was dispensed into each well. 50ul of rabbit anti-Estradiol (E2) reagent was dispensed to each well which was thoroughly mixed for 30seconds. The solution was Incubated at room temperature (18-25°C) for 90minutes. During the incubation, a fixed amount of HRP-labeled E2 competes with the endogenous E2 in the standard, sample, or quality control serum for a fixed number of binding sites of the specific E2 antibody. Thus, the amount of E2 peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of E2 in the specimen increases. Unbound E2 peroxidase conjugate is then removed and the wells washed. Next, a solution of TMB Reagent is added and incubated at room temperature for 20Minutes, resulting in the development of blue color. The color development is stopped with the addition of 1N HCl, and the absorbance is measured spectrophotometrically at 450nm. The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled E2 in the sample. The value of E2 for each sample and control were analyzed by automatically analyzer.

**Reference values**

Potassium 3.4-5.2 mEq/L

Sodium 135-147 mEq/L

**Table 2 Estimation of electrolyte levels in ovarian cancer patients**

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Category</th>
<th>Electrolyte level(Na⁺) mEq/L</th>
<th>Electrolyte level(K⁺) mEq/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>137.30±1.154</td>
<td>3.93±0.351</td>
</tr>
<tr>
<td>2</td>
<td>Pre - treatment</td>
<td>151.40±7.469</td>
<td>3.93±0.351</td>
</tr>
<tr>
<td>3</td>
<td>Treatment phase</td>
<td>157.80±3.271</td>
<td>4.36±1.003</td>
</tr>
<tr>
<td>4</td>
<td>Post-treatment phase</td>
<td>154.80±8.526</td>
<td>3.60±0.393</td>
</tr>
</tbody>
</table>

**Table 2 Estimation of electrolyte levels in ovarian cancer patients**

The recorded mean value of sodium (Na⁺) was 137.30±1.154 in control individuals, 151.40±7.469 for Pre-treatment phase patients, 157.80±3.271 for treatment phase patients, and 154.80±8.526 for Post-treatment phase patients. The mean value of potassium (K⁺) was 3.93±0.351 in control individuals was 5.38±1.198 for Pre-treatment phase patients, 4.36±1.003 for treatment phase patients and 3.60±0.393 Post-treatment phase women, respectively.

**Estimation of estradiol (E₂)**

Serum estradiol (E₂) was measured by using Estradiol (E2) enzyme Immunoassay test kit (catalog no: BC-1111)

**Estimation of progesterone**

Serum progesterone was measured by using Enzyme Immunoassay for the Quantitative Determination of Progesterone Concentration (Cat. No. BC-1113)

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progesterone-HRP conjugate for a constant amount of rabbit anti-
progesterone. In the incubation, goat anti-rabbit IgG-coated wells are
incubated with 25ul progesterone standards, controls, patient samples,
100ul progesterone-HRP Conjugate Reagent and 50ul rabbit anti-
progesterone reagent at room temperature (18-25C) for 90minutes.
During the incubation, a fixed amount of HRP-labeled progesterone
competes with the endogenous progesterone in the standard, sample,
or quality control serum for a fixed number of binding sites of the
specific progesterone antibody. Thus, the amount of progesterone
peroxidase conjugate immunologically bound of the well progressively
decreases as the concentration of progesterone in the
specimen increases. Unbound progesterone peroxidase conjugate is
then removed and the wells washed. Next, a solution of TMB Reagent
is then added and incubated at room temperature for 20minutes,
resulting in the development of blue color. The color development
is stopped with the addition of stop Solution, and the absorbance is
measured spectrophotometrically at 450nm. The intensity of the
color formed is proportional to the amount of enzyme present and
is inversely related to the amount of unlabeled progesterone in the
sample. The concentration of progesterone for each sample was
determined by automatically analyzer.

Discussion

Ovarian cancer is the most lethal malignancy of the female
reproductive tract.19 The effect of major epidemiologic risk factors for
ovarian cancer has been reviewed in the light of hormonal hypotheses,
progesterone, and estrogens. The role of inclusion cyst formation and
Mullerian epithelium differentiation in the pathology of the disease
are also briefly outlined. Although based on limited data, the observed
tendency in current evidence suggests possible etiologic roles for
raised estrogens and decreased progesterone in the pathogenesis of
ovarian cancer.20 The present study was based on the evidence that
the serum sodium and potassium level gradients altered significantly
in women with ovarian cancer. The present experimental work was
aimed to investigate the effect of electrolytes (Na+, K+) and female sex
hormone (estrogen and progesterone) in ovarian cancer. It has been
reported that the mechanism encountered for these abnormalities are
multifactorial in origin. An understanding of the mechanism involved
in their pathogenesis is of paramount importance for their prevention
and treatment in cancer patients.21

Significant elevation in serum sodium level (P = 0.004) was observed
in women with ovarian cancer(pre-treatment phase, treatment phase,
post-treatment phase) as compared to the control which is deviation
with the work of Hasegawa et al.22 They conducted hatacinaromatous
meningitis is associated with ovarian cancer complicated by SIADH.
Laboratory studies showed hypotension, low serum osmolality,
 elevated urinary sodium level, and urine osmolality. The possible
mechanism underlying the serum sodium concentration is regulated
by the balance of water intake, renal filtration, and reabsorption of
sodium, and ADH-mediated water conservation by the collecting
duct. Water balance is normally mediated by thirst, the secretion of
ADH, the feedback mechanisms of the renin-angiotensin-aldosterone
system, and renal handling of filtered sodium and water.23

Significant elevation in serum potassium level (P = 0.035) was observed
in women with ovarian cancer as compared to the control group.
The pre-treatment phase patients show a high level of potassiumas compared to other groups which were a variant with the
work of John et al.24 The elevated serum level of potassium could
be due to the administration as well as chemotherapy that cause cell
lysis which results in the liberation of intracellular potassium into the
blood and extracellular fluid. Significant elevation in serum estrogen
level (P = 0.000) was observed in women with ovarian cancer as
compared to control group in all three phases which are settlement
with the work of Syed et al. Estrogens interact with their receptors
to mediate various signaling pathways that are likely associated with
the risk of ovarian cancer.25

In-Significant difference in serum progesterone level (P = 0.955)
was observed in women with ovarian cancer (pre-treatment phase,
treatment phase and post-treatment phase) as compared to control in
contrary with the work of Heinonen et al. where Peripheral serum
concentrations of progesterone were measured in 27 postmenopausal
women with malignant, borderline, or benign ovarian tumors and
in 10 women without ovarian neoplasm. Mucinous ovarian tumors
were associated with the highest progesterone levels, compared with
other histologic types of ovarian tumor. The results indicated that
mucinous ovarian tumors are able to secrete progesterone and that
the stage of malignancy has no effect on this hormonal activity. The
outcomes of present study showed significant relationship between
serum sodium level (P=0.004), significant serum potassium level with
ovarian cancer (P = 0.035), significant relationship between serum
estrogen level with ovarian cancer (P = 0.000) was observed while
and in-significant relationship between serum progesterone level with
ovarian cancer (P = 0.955) was recorded in group B (ovarian cancer
patients) as compared to group A (controls).

Conclusion

It was inferred from the outcomes of the present study that ovarian
cancer manifestation effects serum electrolyte concentrations by
altering their levels in the blood. However, the alteration was higher
in serum estrogen level as compared to progesterone level. Ensued
into each well and were incubated at room temperature (18-25C)
for 20minutes. The reaction was stopped by adding 100ul of stop
solution to each well. The contents were gently mixed 30seconds.
The observance was read at 450nm by using titer well reader within
15minutes (Table 3).

Acknowledgments

None.

Conflicts of interest

Authors declare that there is no conflicts of interest.

References


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