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Diagnostic Value of Serum Osteopotin in Ovarian Cancer Patients

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Abstract:

Amongst all gynecological cancers, ovarian cancer is the most lethal malignancy worldwide, aggressive local invasion and the lack of sensitive early screening methods, poses the most difficult in early diagnosis, furthermore, its high mortality rate has made it one of the most investigated fields in gynecological oncology during this year in USA. The aim of the study is to assess the level of serum biomarker osteopontin (OPN), among ovarian cancer women at Khartoum State - Sudan. and compare the findings of osteopontin (OPN) serum concentration with the control group, and correlate with study variables. Then estimate the predictive values of this marker. Osteopontin testing were performed to all serum samples to determine the concentrations by enzyme linked immunosorbent assay for quantitative determination of osteopontin. By the end of this study, concludes that epithelial ovarian cancer is the most common followed by germ cell tumors. Serum level of osteopontin biomarker within the reference range in the control group. In contrast, increasing serum level in the ovarian cancer patients, a general agreement that a

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combination of osteopontin may increase diagnostic sensitivity and specificity when combined with the golden markers cancer antigen 125.

Key words: ovarian cancer, biomarker, osteopontin, epithelial OV

1. OVARIAN CANCER

Ovarian cancer has been called the "silent killer" because symptoms often become apparent only when the cancer has spread and is harder to treat. It's the fifth leading cause of cancer-related death in women in the United States and is the leading cause of gynecologic cancer deaths. Despite being one-tenth as common as breast cancer, it is three times more lethal, and carries a 1:70 lifetime risk. This year, approximately 20,180 women will be diagnosed with ovarian cancer, and 15,310 will die in USA from the disease [1]. The high mortality rate of ovarian cancer is due to the lack of a screening strategy to detect early-stage disease. Ovarian cancer presents with very few, if any, specific symptoms. Twenty percent of patients are diagnosed at stage I and II when the disease is still confined to the ovary. In patients diagnosed with advanced disease, the 5-year survival rate ranges from 20% to 25%, depending on the stage and grade of tumor differentiation [2]. Of these patients, 80% to 90% will initially respond to chemotherapy, but less than 10% to 15% will remain in permanent remission [2].

Over the past quarter of a century, several scientific developments have challenged traditional concepts in ovarian cancer. First, it was recognized that ovarian cancer is not a homogeneous disease, but rather a group of diseases-each with different morphology and biological behavior. Approximately 90% of ovarian cancers are carcinomas and, based on histopathology, immunohistochemistry, and molecular genetic analysis, at least five main types are currently distinguished: high-grade serous carcinoma (HGSC,70%); endometrioid

carcinoma (EC,10%); clear-cell carcinoma (CCC,10%); mucinous carcinoma (MC, 3%); and low-grade serous carcinoma (LGSC, <5%) [3,4]. These tumor types (which account for 98% of ovarian carcinomas) can be reproducibly diagnosed by light microscopy and are inherently different diseases, as indicated by differences in epidemiologic and genetic risk factors; precursor lesions; patterns of spread; and molecular events during oncogenesis, response to chemotherapy, and prognosis [3,4]. Much less common are malignant germ cell tumors and potentially malignant sex cord-stromal tumors. The biomarker expression profile within a given histotype is consistent across stages. Ovarian cancers differ primarily based on histologic type [5,6].

The International Federation of Gynecology and Obstetrics in US (FIGO) stages ovarian tumors on a scale of I to IV according to how well- or poorly-organized the tumors are and whether the cancer is metastasized. Stage I is cancer that is localized and contained in the ovary or ovaries. Stage II is cancer that has spread to other pelvic organs such as the uterus, bladder, or rectum, but is confined to the pelvis [6]. Stage III is cancer that has spread to the lymph nodes and/or abdominal lining and organs, with possible superficial liver metastases. Stage IV is cancer that has spread to distant organs, such as the brain, bone, lungs, or liver parenchyma [6,7].

1.1 Risk Factors for Ovarian Cancer

According to American Joint Committee on Cancer 2012 and American Cancer Society2016, there are several specific factors that change a woman's likelihood of developing ovarian cancer include:

Age, the risk of developing ovarian cancer gets higher with age and, rare in women younger than 40. Most ovarian cancers develop after menopause. Half of all ovarian cancers are found in women 63 years of age or older [8]. *Obesity*,

Melinda M and coauthors [10] have looked at the relationship of obesity and ovarian cancer. Overall [8,9], it seems that obese women have a higher risk of developing ovarian cancer and obesity is associated with a weak adverse effect on the survival of women with ovarian cancer [10].

Reproductive history, several studies have suggested that the ovarian cancer risk reductions associated with parity and oral contraceptive use are weaker in postmenopausal than premenopausal women; yet little is known about the persistence of these reductions as women age. This question gains importance with the increasing numbers of older ovarian cancer women. parity women have a lower risk of ovarian cancer than nulliparity. The risk goes down with each full-term pregnancy and, women who have their first full-term pregnancy after age 35 or nulliparity have a higher risk of ovarian cancer [11].

Breastfeeding, the evidence that breastfeeding protects against ovarian cancer is well established epidemiologically, recent evidence finds a 37% reduction for ovarian cancer for women who have breastfed for a year or more [12]. Reduced risk of ovarian cancers related to prolong periods of time during which women do not ovulate or have their menstrual cycles. Later onset of puberty and first menstrual cycles, and an earlier menopause, both of which mean fewer lifetime ovulatory cycles, are associated with decreased risk of ovarian cancer. *Contraceptive*, women who have used oral and an injectable contraceptive have a lower risk of ovarian cancer. and the risk is lower the longer the contraceptives are used [13]. *Gynecologic surgery*, tubal ligation may reduce the chance of developing ovarian cancer by up to two-thirds and, hysterectomy also seems to reduce the risk of getting ovarian cancer by about one-third [11]. *Fertility drugs*, researchers have found that using the fertility drug for longer than one year may increase the risk for developing ovarian tumors, the risk seemed to be increase the risk of low malignant potential ovarian tumor [10].

Estrogen therapy and hormone therapy, recent studies done by Muhammad Zahid eta l[14], suggest women using estrogens after menopause have an increased risk of developing ovarian cancer for at least 5 years, the increased risk is less certain for women taking both estrogen and progesterone [14].

About 5 to 10% of ovarian cancers are a part of family cancer syndromes resulting from inherited mutations in certain genes like what happened in *hereditary breast and ovarian cancer syndrome*, this syndrome is caused by inherited mutations in the genes BRCA1 and BRCA2, these genes are tumor suppressor genes involved in the regulation of cellular proliferation, chromosomal stability, and DNA repair which linked to a high risk of breast cancer as well as ovarian, fallopian tube, *primary peritoneal cancers*, pancreatic cancer and prostate cancer, are also increased [15]. According to American collage of Obstetrician and Gynecologist in 2017 [16] the lifetime ovarian cancer risk for women with a BRCA1 mutation is estimated to be between 35% and 70%. For women with BRCA2 mutations the risk has been estimated to be between 10% and 30% by age 70. These mutations also increase the risks for primary peritoneal carcinoma and fallopian tube carcinoma. In comparison, the ovarian cancer lifetime risk for the women in the general population is less than 2% in USA [16].

PTEN tumor hamartoma syndrome (Cowden disease) people are primarily affected with thyroid problems, thyroid cancer, and breast cancer. Women also have an increased risk of ovarian cancer. It is caused by inherited mutations in the PTEN gene. Women with *Hereditary nonpolyposis colon cancer* (Lynch syndrome) have a very high risk of colon cancer and also have an increased risk of developing of ovarian and endometrial cancer and many different genes include MLH1, MLH3, MSH2, MSH6, TGFBR2, PMS1, and PMS2 which reduces ability to repair damage to its DNA. The lifetime risk of ovarian cancer in women with *hereditary nonpolyposis colon cancer* (HNPCC) is

about 10%. Up to 1% of all ovarian epithelial cancers occur in women with this syndrome [16].

Peutz-Jeghers syndrome is a rare genetic syndrome caused by STK11 gene mutations this syndrome develops polyps in the stomach and intestine in teenagers. Women with this syndrome have an increased risk of both epithelial ovarian cancer and sex cord tumor with annular tubules (SCTAT). *Personal history of breast cancer* has an increased risk of developing ovarian cancer, because one subtype of breast cancer shares many genetic features with high-grade serous ovarian cancer, a cancer that is very difficult to treat, according to researchers supported by the National Institutes of Health [17]. The findings suggest that the two cancers are of similar molecular origin, which may facilitate the comparison of therapeutic data for subtypes of breast and ovarian cancers [17].

There are many lowering ovarian cancer risk factors including, history of pregnancy has a 50% lower risk of ovarian cancer than women who were never pregnant (nulliparous), and a protective effect is shown in women with multiple pregnancies, oral contraceptive, tubal ligation and hysterectomy also have been associated with a reduced risk of ovarian cancer [18].

1.2 Causes Ovarian Cancer:

There are many theories about the causes of ovarian cancer, can be classified to *exogenous* and *endogenous* factors. The *exogenous* factors including, Estrogen therapy and hormone therapy, smoking and alcohol induced, exposure to carcinoids materials and radiation, diet with heavy fatty and proceeding meat [10]. *Endogenous* factors, the hormonal imbalance is important causes of ovarian cancer because it's hormonal dependent cancer, also researchers find a relationship between ovulation and the risk of developing ovarian cancer [15,16]. Genetic mutations either inherited mutations in the BRCA1 and BRCA2 genes, as well as the genes related to other family

cancer syndromes linked to an increased risk of ovarian cancer, such as PTEN tumor hamartoma syndrome, Peutz-Jeghers syndrome, MUTYH-associated polyposis, and the many genes that can cause hereditary nonpolyposis colon cancer (MLH1, MLH3, MSH2, MSH6, TGFBR2, PMS1, and PMS2). or acquired like the TP53 tumor suppressor gene or the HER2 oncogene mutation,

1.3 Incidence and Prevalence:

According to American cancer society an estimated 22,400 new cases of ovarian cancer in 2017 and about 14,080 deaths will occur in 2017, accounts for 5% of all cancers in women [20], and A total of 7,378 new cases were reported in the UK in 2014 and it has the highest mortality of all gynecological cancers, accounting for 6% of all cancer deaths in women [19] Although ovarian cancer occurs most commonly after menopause (average age is 63), it may develop at any age. A woman's risk of developing ovarian cancer in her lifetime is 1 in 71, and her risk of dying from the disease 1 in 95. The 5 years survival rate for ovarian cancer is relatively low (46%) because most patients are diagnosed with distance stage disease, for which survival is 29%. Survival also varies subsequently by age, with women younger than 45 much more likely to survive 5 years than women 75 and older (77% versus 20%) [20].

1.4 Diagnosis of ovarian cancer:

History, is nonspecific in that symptoms in early-stage disease are either absent or vague and may resemble menopausal symptoms and intestinal illnesses. Individuals in later stages may report indigestion, gas, nausea, vomiting, loss of appetite, a feeling of fullness after small meals, pelvic or abdominal pain, swelling, increased frequency or urgency of urination, unexplained change in bowel habits, unexplained weight gain or loss, pain during intercourse, ongoing fatigue, lower back pain, shortness of breath, and, in rare cases, postmenopausal

vaginal bleeding. These symptoms usually do not become apparent until the later stages of the disease when the cancer mass is large enough to interfere with pelvic organs such as the bladder or rectum, or after the cancer has metastasized to the abdominal cavity. Obtaining a personal obstetric and gynecologic history and a family history of gynecologic disease may be important in diagnosis [21]. A number of case–control studies investigating symptoms in women with ovarian cancer and comparing them to symptoms in women without ovarian cancer demonstrate that patients with ovarian cancer are symptomatic for a variable period before diagnosis and challenge the perception of ovarian cancer as the "silent killer" [22].

Pelvic examination, many conditions that can affect women's health are often evaluated through pelvic examination. These conditions include malignant diseases, such as ovarian, uterine, vaginal, and cervical cancer; infectious diseases, such as bacterial vaginosis, candidiasis, genital warts, genital herpes, trichomoniasis, and pelvic inflammatory disease; and other benign conditions, such as cervical polyps, endometriosis, ovarian cysts, dysfunction of the pelvic wall and floor, and uterine fibroids. Pelvic examination is a common part of the physical examination; 44.2 million pelvic examinations were performed in the United States in 2012. Although it is a common part of the physical examination, it is unclear whether performing screening pelvic examinations in asymptomatic women has a significant effect on disease morbidity and mortality [23,24].

Routine imaging tests, are noninvasive diagnostic imaging such as ultrasound performed with a transvaginal probe, computed tomography (CT), and magnetic resonance imaging (MRI), may help distinguish between benign and cancerous tumors. X-ray procedures are used if involvement of the colon or urinary tract is suspected. In women who have gastrointestinal symptoms, examination of the GI tract with

upper and lower endoscopy is indicated to help rule out GI conditions and evaluate for bowel obstruction, and Positron Emission Tomography (PET)by radioactoring sugars to detect small group of cancer cells [21].

Laboratory investigations, included complete blood count (CBC), chemistry profile with a liver function tests(LFT) combined with alpha-fetoprotein (AFP), total serum proteins, and cancer antigen 125.Histopathological examination of ovarian tumors one of the most important method to differentiate between ovarian cancer types, used in staging and also in predicting the prognosis [25].Tumor markers and Malignancy Indices, prospectively acquired evidence from the United Kingdom Collaborative Trial of Ovarian Cancer Screening Cancer (UKCTOCS) - with 46,237 women triaged using MMS in whom serial CA-125 measurements were interpreted via the risk of ovarian cancer algorithm (ROCA) - has shown that screening by using ROCA doubles the number of screen-detected EOC compared with a fixed cut off of 35 IU/ml [19]. A Risk of Malignancy Index (RMI) was developed to estimate the probability of malignancy and the need to refer the patient to a tertiary hospital for optimal treatment. RMI is calculated by multiplying the menopausal status by the CA125 value and by certain sonographic features. Risk of Malignancy Index (RMI) = M x CA125 x U. RMI > 200 = Suspicious for malignancy.

1.5 Osteopontin (OPN):

Osteopontin (OPN) is a bone associated, extracellular matrix glycosylated phosphoprotein which is produced by several cell types, including osteoblasts, osteoclasts, immune cells, endothelial cells, epithelial cells and extra-osseous cells. Due to differences in post-translational modification (PTM) from different cellular sources, OPN has a molecular weight ranging from 41 to 75 kDa, which may have a cell type-specific structure and function. OPN plays a major role in various normal

physiological processes, including bone remodeling, immune-regulation, inflammation and vascularization. In addition, OPN has also been shown to be involved in carcinogenesis with multi-functional activities. OPN and certain integrins are able to promote angiogenesis through enhanced endothelial cell migration, proliferation and the subsequent formation of capillaries, which are all essential requirements for the process of angiogenesis. It has been shown to correlate with tumorigenesis, as well as with the progression and metastasis of different malignancies in both experimental and clinical studies [26].

The upregulation in clinical investigations, elevated OPN levels have been shown to correlate with increased tumor burden, a poor prognosis and reduced survival, although discrepancies remain. For example, the increased expression of OPN in plasma and tumor tissues has been identifed in breast cancer patients and has been shown to be associated with metastatic disease and decreased survival [26].

Similarly, in advanced gastric cancer, patients with OPN-positive cancer have a decrease in their 5-year survival, when compared with those with OPN-negative cancer. In colorectal cancer patients, increased OPN mRNA levels have been shown to significantly correlate with stage, lymph node metastasis and lymphatic or venous invasion, as well as with lower disease-free and overall survival rates [27]. A study on patients with pancreatic ductal adenocarcinoma demonstrated that serum levels of OPN are elevated with advanced tumor grades [27]. Elevated OPN levels have also been strongly associated with increased stage, grade and tumor size in melanoma patients. In early stage, non-small cell lung cancer patients high OPN plasma levels were observed which were reduced after surgical tumor resection. However, in those patients which showed recurrence after surgery OPN plasma levels re-elevated, indicating that OPN is not only a potential diagnostic marker, but also has potential as a tool for detecting

tumor recurrence after treatment. Thus, OPN may be a useful biomarker to monitor cancer progression and a significant predictor of poor prognosis and survival rates [28] [29].

In ovarian cancer, OPN is usually overexpressed. in diagnosis of ovarian cancer, OPN has been intensively studied; for example, two recent published meta analyses [30][31]. showed that serum OPN levels were generally elevated in ovarian neoplasm patients, indicating that OPN is a potential diagnostic marker for ovarian cancer. these higher levels of OPN are associated with poorer prognosis. OPN as a sole biomarker has a sensitivity of 81.3%. When combined with (CA125) there is a 33.7% specificity and 93.8% sensitivity. A potential use of OPN is in early noninvasive detection of ovarian cancer, as levels may be measured in urine [31].

2. MATERIALS AND METHODS

2.1 Materials

A total of 90 Sudanese ladies age range (16-80) years old attending Gynological Oncology clinics in Omdurman Military hospitals - Khartoum state from May 2015 to December 2016 was included in the study. The study was analytical comparative cross-sectional study. The sample population was divided into two main groups; study group including 53 (58.8%) Ovarian cancer patient with an age of 16 to 80 years, and control group including 37 (31.2%) aged match apparently healthy individuals according to the study Inclusion criteria including of Sudanese women diagnosed with primary ovarian cancer and excluded any women diagnosed with other cancer types rather than ovarian cancer.

History and background data were collected from participants using verbal interviews and pre-designed questionnaire. Clinical presentation includes an enlarged ovary on a pelvic exam, ascites, and histopathological examinations to regulate the tumor type, ovarian cancer type, and staging of the

disease, then followed by metastatic status of cancer. Five ml blood samples were collected from each participant; sera were separated, and then stored at -20oC for subsequent testing. Biomarker testing were performed to all serum samples to determine the concentrations of serum Osteopontin (OPN). Informed and written consents were obtained from all participants prior to involvement in the study.

2.2Methods

Enzyme llink Immunosorbent assay kits for Osteopontin (OPN) determination, the CUSABIO Human Osteopontin (OPN) ELISA Kit for the quantitative determination of human osteopontin (OPN) concentrations in serum, plasma, urine, tissue homogenates.

Principle, this assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for OPN has been pre-coated onto a microplate. Standards and samples were pipetted into the wells and any OPN present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for OPN was added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) was added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution was added to the wells and color develops in proportion to the amount of OPN bound in the initial step. The color development was stopped and the intensity of the color was measured.

Preparation of Reagents and sample, allowed all reagents and required number of strips to reach room temperature (18-25°C) prior to use, distilled water was used to make reagents preparation. Wash Buffer(1x) after warmed up to room temperature mixed gently until the crystals completely dissolved. 30 ml of Wash Buffer Concentrate (20 x) was diluted into distilled water and 600 ml prepared of Wash Buffer (1 x), thawed samples inverted several times prior to testing.

Assayed Procedure, secured the desired number of Microtiter wells in the frame holder, dispensed 25 µL of each Standard, Control and samples with new disposable tips into appropriate wells, 75µl of HRP-conjugate was added to each well. Mixed well and then incubated for 1hour at 37°C. Aspirated each well and washed four times for a total of five washes, then filling each well with Wash Buffer (300µl) by multi-channel pipette, and let it stand for 20 seconds, complete removal of liquid at each step was performed any remaining was removed. Added 50µl of Substrate A and 50µl of Substrate B to each well, mixed well, incubated for 15 minutes at 37°C. the plate Kept in the dark. Added 50µl of Stop Solution to each well, the plate taped gently to ensure thorough mixing. Finally, the optical density of each well was determined within 10 minutes, using a microplate reader set to 450 nm.

Calculations of Results, by using automated method, in this method the results have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. The concentrations of the samples were read directly from this standard curve. Detection range 0.78ng/ml – 50ng/ml

2.3 Quality control

The controls were running with calibration curve for the OPN, a statistically significant number of controls were assayed to establish mean values and acceptable ranges to assure proper performance. Using control sera at both normal and pathological levels. The checking of the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods were done.

2.4 Statistical analysis

Raw data were entered into a spread sheet of SPSS statistical package program, data were rearranged as appropriate.

Descriptive analysis was performed to all study variables. Data was analyzed using SPSS version 21. The results expressed as mean, standard deviation, median, frequency and percentage. Descriptive statistic was done to obtained the frequencies and percentages of the study variables and clinical data. Independent–sample T-test was demonstrated to compare the mean concentration of OPN biomarker parameter OPN in OVC cancer versus healthy individual (control groups). One-way ANOVA was used to mean concentration of OVC biomarker parameters OPN across the OVC stages. Graphs were done using Microsoft excel and Graph Pad Prism version 6. P-value ≤0.05 was considering as significantly difference. All statistics tests were done in confidence interval 95%.

3. RESULTS

Clinical Results Ninety (100%) Sudanese ladies were enrolled in this study. They were distributed into two groups; Study group including 53 (58.8%) newly diagnosed ovarian cancer patients age ranged (16-80) years old, and Control group including 37 (31.2%) age match apparently healthy individuals. Study group include 32% in the reproductive age and about 68% elderly female. The frequency and percentage of signs and symptoms shown that 79.0 % from the study group suffering from abdominal bloating, 62% loss of appetite, 68% urinate more frequent, 57% irregular bowel movement, 70% presented with increased abdominal size, about 85% with abdominal pain, all study group deny history of ovarian cancer in their families, only 13% of ovarian cancer patient using pills as contraceptive as well as 4%hormonal therapy consumption, 8%Caesarean as gynecological surgery. As well as about 51% of the study group were para and multi-parity compared with 49% were nulliparous, and 45% of this study group suffering from asities when clinical examination done and confirmed by ultrasonography also signify percentage of the left (Lt), right

(Rt), and Bilateral ovarian mass as 19%, 34%, and 47% respectively.

Histopathological results, the present study showed 97% of the ovarian cancer were epithelial cell origin and only 3 % were germ cell origin. Staging of ovarian cancer among study group grading from stage 1,2,3and,4 were 11%, 13%,19% and 57% respectively.

Serum biomarker results, the present study showed that OPN mean concentration was 225.96 U/ml in the study group, and 13.52 U/ml in the control group along with mean concentration of Para/ multi parity and Nulliparous sub groups of ovarian cancer patients were 215.21 U/ml, 237.13 U/ml respectively shown insignificant difference with (P-value = 0.290) table (3.3). Mean concentrations of this marker among cancer stages 1,2,3, and 4 shown 1.6, 3.4, 2.5 and, 2.6 respectively, which shown insignificant difference with p-values (0.943) demonstrate in table (3.4) and Figure (3.13). The sensitivity 63%, Specificity 60%, Positive predictive value 59%, and Negative predictive value 55% shown on table (3.5) figure (3.15).

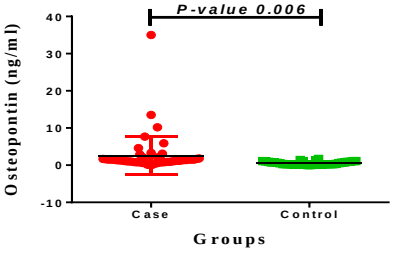

Figure (3.1) Mean concentration of OPN among ovarian cancer patient

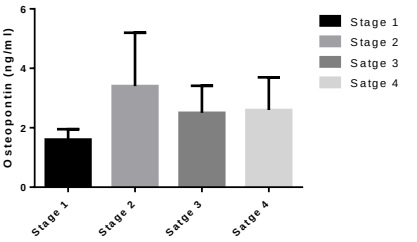

Figure (3.2) Mean concentration of OPN among ovarian cancer stages

4. DISCUSSION

Amongst all gynecological cancers, ovarian cancer is the most lethal malignancy worldwide, aggressive local invasion and the lack of sensitive early screening methods, poses the most difficult in early diagnosis, furthermore, its high mortality rate has made it one of the most investigated fields in gynecological oncology during this year in USA ovarian cancer ranks fifth in cancer deaths among women [1], a woman's risk of getting ovarian cancer during her lifetime is about 1 in 75, her lifetime chance of dying from ovarian cancer is about 1 in 100 according to Ovarian Cancer Treatment Statistics and Results of Cancer Treatment Centers of America 2012 [33].

Even though OVC mainly develops in older women there is younger age range were reported in review study done by Wisal etal 2017 [34], among Sudanese ovarian cancer patient which agree with our study because there were thirty tow percent within reproductive age.

The results of the present work affirm that, around fifty seven percent of all ovarian cancers included in this study were diagnosed at an advanced stage and only eleven percent in early stage. Then the five-year survival rate for patients with clinically advanced ovarian cancer is only fifteen to twenty percent, in striking contrast to a five-year survival rate of over ninety percent for patients with stage I disease [35].

In this study, we found the common symptoms among OVC patients involved in the clinical presentation are abdominal bloating, pelvic pain, abdominal pain, increase

abdominal size vaginal discharge with the highest frequent, and vaginal bleeding with low frequency, these findings similar to cancer facts and figures published in 2017 by American cancer society [20]. Ultrasonography as noninvasive diagnostic test in women with pelvic, bilateral, and ascites are helpful in predicting the likelihood that mass is malignant [24]. Ovarian tumors were unilateral in 53% of cases and bilateral in 47% with right side predominance This also chimes with the findings of Jyothi Kancherla etal [25].

Histopathological distribution in our study group is similar to many published works [36] [37], ovarian epithelial cell being the most common and followed by Germ cell, which present in different age ranges included in this study, Germ cell neoplasm present among younger age in the study group, present study findings are broadly similar to Kancherla etal [25] who reported that surface epithelial tumors were most common (80%) followed by germ cell tumors (16%).

Osteopontin upregulation in clinical investigations, elevated OPN levels have been shown to correlate with increased tumor burden, a poor prognosis and reduced survival, although discrepancies remain. For example, the increased expression of OPN in plasma and tumor tissues has been identified in ovarian cancer patients and has been shown to be associated with metastatic disease and decreased survival [26]. Many key studies by Kim JH in Laboratory of Gynecologic Oncology USA[38], and also Weber GFetal,2010 [39] have demonstrated the usefulness of OPN marker with disease monitoring following oophorectomy and in detecting recurrent ovarian cancer. It has a potential to be used as a noninvasive screening test and as an early diagnosis it was shown to be significantly elevated during advanced stages of the disease. When combined with CA125 in a biomarker screening panel, high sensitivity was achieved [26]. OPN may have a lower potential than CA125 to accurately detect the presence of ovarian cancer. But have elevated NPV than CA125. However,

in this study significantly higher levels of OPN were noted among ovarian cancer patients when comparing with normal women which indicated the usefulness of using OPN as ovarian cancer biomarker.

Unfortunately, most studies of tumor markers are highly variable, not only in their methods of marker detection, but also in design and patient selection. Interpatient heterogeneity and intratumor heterogeneity are important confounding factors. In addition, the danger of bias and the problems of overfitting the data, as well as issues relating to the handling and storing of clinical specimens, are vital factors that need consideration before a study is conducted. New tumor marker tests—single or multiparametric—must, therefore, undergo rigorous validation in order that their clinical value can be assessed. Reaching to scientific evidence in this study, the serum biomarkers Osteopontin results that fall within the normal range in the control group. In contrast, Osteopontin (OPN) level significantly increased in all ovarian cancer patient included in this study,

5. CONCLUSION

By the end of this study, concludes that epithelial ovarian cancer is the most common followed by germ cell tumors. Serum level of five biomarkers within the reference range in the control group. In contrast, increasing serum level of Osteopontin (OPN) were obtained in the ovarian cancer patients, A general agreement that a combination of multiple biomarkers may increase diagnostic sensitivity and specificity over use of individual markers and, OPN appears to have good clinical value in evaluating disease.

Recommendations

Establishing OPN as ovarian biomarker with a golden standard test, and pointing to a rationale for further research assessing

potential clinical usefulness. OPN variants needed more to learn with regards to the mechanisms of action, it is clear that they have clinical prospect(s) for this protein in ovarian cancer.

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