The Application of CA 125 in the Diagnosis and Follow up of Ovarian Cancer

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Authors' contributions

This research was conducted in collaboration between the two authors. The authors involved in the study design, protocol writing, results interpretation and final manuscript draft, reading and approval. Author RE M managed the field data collection. Authors RM S and A MA preformed the laboratory techniques and statistical analysis. The two authors read and approved the final manuscript.

Abstract: Ovarian cancer represents the sixth most commonly diagnosed cancer among women in the world, and causes more deaths per year than any other cancer of the female reproductive system. Despite the high incidence and mortality rates, the etiology of this disease is poorly understood. The main objective of this study is to detect the relation of CA 125 levels with the diagnosis and follow up of ovarian cancer patients, and to access its role in the diagnosis and follow up of ovarian cancer in Sudanese patients. Ovarian cancer Samples were examined for the socio-demographic characteristics, histological and cytological features and the CA 125 levels, which was measured from the serum of ovarian cancer patients using Electrochemilminescence techniques by Roche Cobs e 411 instrument. Laboratory analysis of selected 124 ovarian cancer samples showed the following: CA125 level elevated (more than 35U/ml) in 94 samples (75.8%) in the first reading of CA125 (before treatment), in the second sample (after treatment) showed increasing of CA125 level in 74 samples (59.68%), and the third sample (after treatment) showed increase of CA125 in 54 (43.55%), this result showed gradual reduction in CA125 levels from first reading, to second , and to third, in which the first samples mean was 1160_1837, second samples mean was 510_930 and the means of third samples was 94_167. In conclusion CA125 levels elevated in ovarian cancer patients (75.8%) and these levels decreased with the ongoing therapy, which is important in the follow up of patients. The study recommended the using of CA125 in diagnosis and follow-up of ovarian cancer patients. Further studies should be done using large sample size.

Keywords: Ovarian cancer diagnosis; H&E; Electrochemiluminescence; Sudan.

1. INTRODUCTION

There are 11th most common cancers among women, ovarian cancer is the fourth leading cause of cancer- related death among women, and is the deadliest of gynecologic cancers. Mortality rates are slightly higher for Caucasian women than for African-American women (WHO, 2014). During the years (2009–2010), 6771 new cancer cases were registered in Sudan. Of those, 3646 (53.8%) cases were in women and 3125 (46.2%) were in men. The most commonly diagnosed cancer among women was breast followed by leukemia, cervix, and ovary (WHO, 2014). Serum level of CA125 is used to monitor response to chemotherapy, relapse, and disease progression in ovarian cancer patients. In Sudan the diagnosis of ovarian cancer detected by using ultrasound, CA125 levels, and fine needle aspiration or biopsy (FNA) for histological diagnosis (SJPH, 2015). A tumor marker is a biomarker found in the blood, urine, or body tissues that can be elevated in cancer, among other tissue types. There are many different tumor markers, each indicative of a particular disease process, and they are used in oncology to help detect the presence of cancer. Most tumor markers are tumor antigens, but not all tumor antigens can be used as tumor markers (Brandtzaeg, 1998). CA 125 is a protein that is a so-called tumor marker or biomarker, CA 125 is present in greater concentration in ovarian cancer cells than in other cells. It was first identified in the early 1980s, and the function of the CA 125 protein is not currently understood (Suprasert and Chalapati, 2013). CA 125 is usually measured from a venous blood sample. It can also be measured in fluid from the chest or abdominal cavity. The tests currently in use are all based upon the use of an antibody that is directed against the CA 125 protein (monoclonal antibody technique). The normal values for CA 125 may vary slightly among individual laboratories. In most laboratories, the normal value is 0- 35 units/ml (Suprasert and Chalapati, 2013). The potential role of CA-125 for the early detection of ovarian cancer is controversial and has not yet been adopted for widespread screening efforts in asymptomatic women. Although CA 125 has been used clinically for over 20 years, its use in some settings is still poorly defined, and the social and emotional impact of this test on patients is immense (Pepin et al., 2011).

2. DEFINITION OF STUDY AND STUDY AREA

Retrospective and hospital case study to assess the role of CA125 levels in the diagnosis and follow up of ovarian cancer, this study was done in the National cancer institute (NCI), Wad Medani, Gezira state - Sudan. The NCI is references hospital in central Sudan, established in 1999 as the second cancer center in Sudan. It is the only cancer center outside the capital of Sudan, Khartoum. All discovered patients who attended NCI clinic during the study period from Jan 2013 to June 2015, and was diagnosed histologically with ovarian cancer and agreed to participate.

3. THE ORDINARY HAEMATOXYLIN AND EOSIN (H&E) STAIN

The formalin fixed specimens of prostate samples were dewaxed, hydrated in descending grades of alcohol concentration, at 100%, 95% through 70% to distilled water for 2 minutes in each stage. For staining of the nucleus, the sections treated with Mayer's Haematoxylin for 8 minutes and differentiated by rinsing in acid alcohol for seconds, bluing in running tap water for 8minutes, counterstaining in Eosin for 1 minute, and rinsed in water.

The sections dehydrated in 70% alcohol through 95% and 100% alcohol, and then blotted in a filter paper, cleared in xylene and mounted in DPX, after that the smears were ready for microscopic examination.

Interpretation of the Results

Nucleus; deep blue color. Cytoplasm and background. tissue; pink colour. RBCs; orange colour (John et al., 1996; Grogan, 1992).

4. THE CA 125 TEST

Sandwich principle, total duration of assay: 18 minutes.

1st incubation

20μL of sample, a biotinylated monoclonal CA 125 specific antibody, and a monoclonal CA 125 specific antibody labeled with a ruthenium complex) form a sandwich complex.

2nd incubation

After addition of streptavidin-coated micro particles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. Results are determined via a calibration curve which is instrument specifically generated by two point's calibration and a master curve provided via the reagent barcode. < 35 unit/ml is normal level and > 35 unit/ml is abnormal or elevated level (Nadji and Morales, 1983).

5. RESULTS

First CA 125 levels and histological staging (diagnosis)
There were elevated levels of CA 125 in 94 (75.80%) in the first diagnosis of the study population and the means of the first level were 1160-1837. Increasing of CA 125 level was direct proportion with the stage of tumor (table 1).

First CA 125 levels and first ultrasound findings(diagnosis)
There was close relation between the first CA125 levels and ultrasound findings, all patients that had any degree of ascites had elevated CA125level. According to ascites classification, the highest group is those with massive ascites (52 cases); in which 43cases had elevated CA125 level (>35 ml) and 9 cases had normal CA125 level (<35). The lowest group those who had no ascites (9 cases); in which 3 cases had normal CA125 level and 6 cases had elevated CA125 level. Concerning pelvic mass; 91 cases had pelvic mass, in which

72 cases had elevated CA125 levels and 19 cases had normal CA125 level. Cyst observation; 85 cases had cyst observation, in which 70 cases had elevated CA125 levels and 15 cases had normal CA125 level (table 2).

Second CA 125 levels and second histological staging(follow up)

In the second diagnosis of CA125 the overall population reduced to 118. The elevated levels of CA125 was shown in 74 (59.68%), and the means of the second level was 510_930. Also in the second reading increasing of CA 125 level was direct proportion with the stage of tumor (table3).

Second CA125 levels and second ultrasound findings(follow up)

According to ascites classification, the highest group are those with massive ascites and high CA125 level in 25 (20.16%), the lowest group contains those who had no ascites and normal levels of CA125. Concerning pelvic mass the results showed that who had pelvic mass were 5 (4.03%), 4 (3.22%) of them had elevated CA125 levels. For cyst observation the results showed that only 2 (0.81%) were having cyst while both of them having elevated CA125 levels. In the follow up there was a relation between CA125 level and Ultrasound findings (table 4).

Third CA125 levels and histological staging(follow up)

For the third diagnosis of CA125 the overall population also reduced again to 78. The elevated levels of CA125 was shown in 54 (43.55%), and the means of the third level was 94_167, compared to 94 (75.80%) in the first diagnosis and 74 (59.68%) in the second diagnosis. There was increasing of CA 125 level with the stage of tumor (table 5).

Third CA125 levels and ultrasound findings (follow up)

According to ascites classification, the highest group is those with massive ascites and elevated CA125 level 26 (20.97%), the lowest group contains those who had no ascites and elevated levels of CA125 in 4 samples (3.23%). Concerning pelvic mass the results showed that who had pelvic mass were 4 (3.23%), and all of them had elevated CA125 levels. Cyst observation showed that 5 (4.03%) were having cyst while 2 (1.61%) of them where having elevated CA125 levels. There was a relation between CA125 level and Ultrasound findings (table 6).

Table 1: First CA 125 levels and histological staging(Diagnosis)

Stage	<35	>35	Total
I	5	1	**6**
II	7	20	**27**
III	8	41	**49**
IV	10	32	**42**
	30	**94**	**124**

Table 2: First CA125 levels and first ultrasound findings(Diagnosis)

Ultrasound findings		<35	>35	Total
Ascites	No ascites	3	6	9
	Mild	7	13	20
	Moderate	11	32	43
	Massive	9	43	52
Pelvic mass	Yes	19	72	91
	No	11	22	33
Cyst	Yes	15	70	85
	No	15	24	39
Total		**30**	**94**	**124**

Table 3: Second CA 125 levels and histological staging(Follow up)

Stage	<35	>35	Total
I	6	0	**6**
II	8	17	**25**
III	18	29	**47**
IV	12	28	**40**
	44	**74**	**118**

Table 4: Second CA125 levels and second ultrasound findings (Follow up)

Ultrasound findings		<35	>35	Total
Ascites	No ascites	10	10	20
	Mild	16	19	35
	Moderate	10	20	30
	Massive	8	25	33
Pelvic mass	Yes	1	4	5
	No	43	70	113
Cyst	Yes	0	2	2
	No	44	72	116
Total		**44**	**74**	**118**

Table 5: Third CA125 levels and histological staging (Follow up)

Stage	<35	>35	Total
I	0	0	**0**
II	6	12	**18**
III	11	22	**33**
IV	7	20	**27**
	24	**54**	**78**

Table 6: Third CA125 levels and ultrasound findings

Ultrasound findings		<35	>35	Total
Ascites	No ascites	9	4	13
	Mild	5	6	11
	Moderate	5	17	22
	Massive	6	26	32
Pelvic mass	Yes	0	4	4
	No	24	50	74
Cyst	Yes	3	2	5
	No	21	52	73
Total		**24**	**54**	**78**

6. DISSICUSION

Laboratory analysis of selected 124 showed that there were elevated levels of CA 125 in 94 (75.80%) in the first diagnosis of the study population, and the means of the first level were 1160_1837. Increasing of CA 125 level was direct proportion with the stage of tumor, this results nearly agree with J. M. Riedinger et al., (2006), who found that the evaluated levels of CA 125 among their

study population were in (89.6%) (Riedinger, 2006). Also agree with Target Ovarian Cancer (2014) they found that in most healthy women the level of CA125 is usually less than 35. However, the level of CA125 in the blood can rise for many reasons, which include endometriosis, menstruation, ovarian cysts, and sometimes ovarian cancer.

The small number of patients in stag I with elevated levels may be due to the justification of the Foundation for Women's Cancer (2011), they found that about 50% of stage I ovarian cancer patients have normal values and the majority of patients with a certain tumor cell type

called mucinous ovarian cancers have normal values. Also there was close relation between the first CA125.

Also there was close relation between the first CA125 levels and ultrasound findings, all patients who had any degree of ascites had elevated CA125 level. The highest group is those with massive ascites (52 cases); in which 43cases had elevated CA125 level (>35 ml) and 9 cases had normal CA125 level (<35). Majority of patients who have pelvic mass and cyst have high CA125 levels.

These findings agree with Cancer Research UK (2016) they said that, to decide if an abnormality is more likely to be cancer or not, doctors can use a tool called the risk of malignancy index (RMI). This index combines the results of the ultrasound, CA125 blood levels and menopausal status (whether or not you are past the menopause). This gives doctors a final score (Cancer Research UK, 2016).

National Ovarian Cancer Coalition (2016),said that there is no consistently-reliable screening test to detect ovarian cancer, there are tests available and should be offered to women, especially those women at high risk for the disease; one of them is CA-125 Test, this blood test determines if the level of CA-125, a protein produced by ovarian cancer cells, has increased in the blood of a woman at high risk for ovarian cancer, or a woman with an abnormal pelvic examination (National Ovarian Cancer Coalition, 2016).

Moya O'Doherty (2016), compared between Ca125 level and Human epididymis protein 4 (HE4), and found that the best available marker for epithelial ovarian cancer is still considered to be CA-125 due to a combination of reliability and general availability. HE4 is more sensitive than specific than CA-125, however the former is not in routine use. NICE still therefore recommends CA-125 rather than HE4. CA-125 is used for the diagnosis of epithelial ovarian cancer (Moya O'Doherty; 2016). In the second diagnosis of CA125 the overall population reduced to 118, due to death and seeking cure in other hospitals in Khartoum or even abroad. The elevated levels of CA125 was shown in 74 (59.68%), and the means of the second level was 510_930. this reduction can be justified to treatment. These agreed with J. M. Riedinger et al, (2006), he found that Change in CA 125 levels after the first cycle of induction chemotherapy (CT) is an independent predictor of epithelial ovarian tumor outcome, the results indicate that CA 125 variations before the second CT course enable to differentiate responder and non-responder patients (Riedinger, 2006). Also there was a relation between second reading of CA125 level and Ultrasound findings, the majority of patients who have ascites, pelvic mass and cyst have high CA125 levels. American cancer society (15) published that, screening tests and exams are used to detect a disease like cancer in people who don't have any symptoms. Perhaps the best example of this is the mammogram. The two tests used most often to screen for ovarian cancer are transvaginal ultrasound (TVUS) and the CA-125 blood test, in many women with ovarian cancer, levels of CA-125 are high. This test can be useful as a tumor marker to help guide treatment in women known to have ovarian cancer, because a high level often goes down if treatment is working (American Cancer Society, 2016).

For follow up of patients in the third diagnosis of CA125, the overall population also reduced again to 78, also can be due to death or seeking cure in elsewhere. The elevated levels of CA125 was shown in 54 (43.55%), and the means of the third level was 94_167, compared to 94 (75.80%) in the first diagnosis and 74 (59.68%) in the second diagnosis, this reduction in CA125 levels was caused by having therapy. This is agree with Digant Gupta et al., (2006), they found that reduction in CA125 after three months of therapy was associated with better overall survival in ovarian cancer Digant Gupta et al., 2006).

Also there was a relation between the third reading of CA125 level and Ultrasound findings, the majority of patients who have ascites, pelvic mass and cyst have high CA125 levels. This is agree with Maurie Markman, M.D (1996), published that, over more than a decade of clinical use, CA-125 has proven itself to be one of the most useful tumor markers in cancer medicine. The major clinical utility of this serum marker is in following the clinical course of women with known ovarian cancer. Other potential uses of CA-125 include the evaluation of the effectiveness of new anti neoplastic agents in this malignancy, and in the modification of treatment strategies in individuals whose CA-125 levels fail to decline at an acceptable rate following the institution of therapy. At the present time, the use of CA-125 as a method to screen for ovarian cancer should be considered investigational (Markman, 1996).

Digant Gupta and Christopher (2009), said that CA125 is the gold standard tumor marker in ovarian cancer. Serum level of CA125 is used to monitor response to chemotherapy, relapse, and disease progression in ovarian cancer patients. Thus, it is reasonable to investigate whether CA125 may have utility as a prognostic indicator as well in ovarian cancer (Digant Gupta and Christopher, 2009).

7. CONCLUSION

There was increasing of CA 125 level with the stage of tumor in the first diagnosis and in follow up of patients after treatment. Also there was a relation between CA125 level and Ultrasound finding in the first diagnosis and in follow up of patients after treatment.

Therefore CA125 can be used in supporting the diagnosis and in follow-up of treatment of ovarian cancer patients. Screening programs can be used to help earlydetection in elder women having one symptom of ovarian cancer. Further studies should be done using large sample size.

ETHICAL APPROVAL

Ethical clearance was obtained from ethical committee of the ministry of Health, Gezira Estate. The samples were collected from the bank of the Laboratory and all cases were reviewed for histological type, histological grade. Grading was done according to Nottingham Grading System.

The samples information's from the records, numbered samples, and no patients' name. Ethical approval for this study was obtained from the research ethical committee from the Gezira State Ministry of Health.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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