MEASURING CANCER SPECIFIC ANTIGEN CA-125 IN PATIENTS OF A TERTIARY HEALTH CARE CENTRE

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Abstract : The discovery and detection of cancer markers may provide earlier diagnosis of cancer and improved therapeutic intervention. Tumor markers can be produced directly by the tumor or by non-tumor cells as a response to the presence of a tumor. Serum CA 125 is widely used as a tumor marker for epithelial ovarian cancer. CA 125 test along with ultrasound, may be used to look for early signs of ovarian cancer in people with a very high risk of the disease. Serum level of cancer antigen 125 is one of the most often used methods for screening, monitoring the response to treatment, the eventual relapse and disease progression in cancer patients.

Index Terms **- Ovarian cancer, tumor markers, CA 125.**

I. INTRODUCTION

Cancer is a cumulative disease involving abnormal cell growth with the potential to invade or spread to other parts of the body and destroy the surrounding tissues. These are malignant in nature (Chatterjee, 2011). They contrast with benign tumors which do not spread to other parts of the body. However, benign tumors can grow very large. Malignant tumors may shed cells that spreads to other parts of the body. This is called metastasis (Chatterjee and Mukherjee, 2011, 2012).

A cancer or tumor marker is a biomarker, usually proteins, found in blood, urine, or body tissues that can be elevated by the presence of one or more types of cancer. The discovery and detection of cancer markers may provide earlier diagnosis of cancer and improved therapeutic intervention (Chatterjee and Sarkar, 2016). There are many types of cancer markers, each indicative of a particular disease process. An elevated level of a cancer marker can indicate cancer; however, there can also be other causes of the elevation (false positive values).

Tumor markers can be produced directly by the tumor or by non-tumor cells as a response to the presence of a tumor (Chatterjee, 2012). Cancer Antigen 125 (CA-125) is found on the surface of many ovarian cancer cells. It can also be found in other cancers and in small amounts in normal tissues.

CA 125 is a high molecular glycoprotein of 200 kDa which was first described by Bast *et al.* (1981). It contains 25% carbohydrates, and circulates in serum connected to another glycoprotein with molecular mass of 1000 kDa. Initially, CA125 was detected through a monoclonal antibody which recognizes a single transmembranar domain mucinous protein, MUC 16 (Michurina *et al.*, 2014; Jankovic and Tapuskovic, 2005).

Serum CA 125 is widely used as a tumor marker for epithelial ovarian cancer (Chakraborty *et al.*, 2015). Cancer Antigen 125 (CA-125) is a protein found on the surface of many ovarian cancer cells. It can also be found in other cancers and in small amounts in normal tissues. A CA 125 test may be used to monitor certain cancers during and after treatment. In some cases, a CA 125 test along with ultrasound, may be used to look for early signs of ovarian cancer in people with a very high risk of the disease. If a cancer is found in another site, (metastatic cancer), a higher-than-normal CA-125 test result may mean that ovarian cancer is the main site.

II. MATERIALS AND METHODS

In the present study, the CA 125 level was measured in the patients suffering from ovarian cancer. The patients were diagnosed by the physicians of Barasat Cancer Research & Welfare Centre. For the study, permission from ethical committee was taken. Official concent letter was also taken from patients to do the research work.

A. Reagents and solutions:

i. Cancer Panel VAST® Callibrators - 1ml/vial.
ii. CA-125 Enzyme Reagent - 13ml/vial. - One vial containing enzyme labeled antibody and biotynilated monoclonal mouse IgG specific for CA-125 in buffer, dye and preservative.
iii. Wash solution concentrate - 20ml/vial. - One vial containing surfactant in buffer saline.
iv. Substrate Solution 'A' - 2 x 7ml/vial. - Two vials containing tetramethylbenzidine (TMB) in buffer.
v. Substrate Solution 'B' - 2 x 7ml/vial. - Two vials containing hydrogen peroxide in buffer.
vi. Stop solution - 2 x 8ml/vial. - 2 vials containing a strong acid (1N HCL).
vii. Distilled or deionised water.

B. Instruments:

i. Streptavidin coated microwells - 96 well microplates coated with streptavidin.
ii. Pipettes capable of delivering 0.025, 0.05, and 0.1 ml volumes.
iii. Microplate washer or squeeze bottle.
iv. Microplate reader with 450nm wavelength absorbance capability.
v. Absorbent paper for blotting the microplate wells.
vi. Microplate cover for incubation period.

C. Reagent preparation:

1. Wash buffer - dilute the contents of wash concentrate to 1000ml with distilled or deionised water in a suitable storage container.
2. Working substrate solution - pour the contents of the maber vial labelled Solution 'A' into the clear vial labelled Solution 'B'. I shake it rigourously.

D. Test procedure:

1. Select the number of coated wells needed for formatting the microplates for each calibrator, control and patient sample to be tested.
2. Pipette out 0.025ml (25µl) of the appropriate serum reference callibrator, control or specimen into the assigned well.
3. Add 0.1 ml (100µl) of the appropriate enzyme reagent to each well.
4. Swirl the microplates gently for 20-30 seconds to mix the reagents and then cover the microplates.
5. Leave it for incubation for a period of 60 minutes in room temperature.
6. After incubation I decant the contents of the microplate and tap and blot the plate dry using an absorbent paper.
7. Then add 0.35 ml (350µl) of wash buffer and then decant. I repeat this step two more times.
8. Then add 0.1 ml (100µl) of working substrate solutions to all the wells.
9. This is kept for incubation for 15 minutes.
10. Next add 0.05 ml (50µl) of stop solution to each well and mix by rotation so that a uniform yellow colour is obtained.
11. Finally read the absorbance in each well at 450nm in a microplate reader.

III. RESULTS

A dose certain graph is used to ascertain the concentration of each corresponding marker in unknown specimens. The observance is recorded as obtained from the printout of microplate reader. The observance for each duplicate serum reference versus the corresponding marker concentration in appropriate units have been plotted on linear graphs. The best fit curve is drawn through the plotted points.

To determine the concentration of the corresponding cancer marker for an unknown, the absorbance of the duplicates for each unknown on the vertical axis of the graph have been located, the intersecting point on the curve have been identified, and the concentration (in ng/ml) from the horizontal axis of the graph have been observed.

The normal value of the following cancer marker in a healthy adult is found to be:
Cancer Marker - CA 125
Value (in U/ml) ≤35

Most women with ovarian cancer lump that can be felt in the pelvic area have CA-125 values greater than 65μ/ml.

Results with high values can be inferred as -
(a) Cancerous conditions with high value include: ovarian cancer in the fallopian tubes or the endometrium.
(b) Non-cancerous conditions with high value include: Pelvic inflammatory disease, endometriosis, uterine fibroids, liver disease, pancreatitis, first trimester of pregnancy, the menstrual cycle, and lupus.

IV. DISCUSSION

Traditional methods for the diagnosis of cancer are endoscopy, biopsy and cytology specimen tests, as well as imaging/radiology tests, such as X-ray, positron emission computed tomography-computer tomography (PET-CT) and magnetic resonance imaging (MRI). Unfortunately, these diagnostic methods are not very powerful for the diagnosis of cancer at early stages, and some of these methods are quite time-consuming, expensive; thus not available for to a large number of people. Therefore, the development of simple and rapid strategies that are specific and reliable for the diagnosis of cancer at early stages is of utter importance.

Colorimetric immunoassays for tumor marker detection have attracted considerable attention, due to their simplicity and high efficiency. The traditionally used colorimetric immunoassays for the detection of tumor markers are based on enzyme-linked immunosorbent assays, and the great achievement of nanotechnology has further opened opportunities for the development of such kind of immunoassays.

Large quantities of colorimetric assays for detecting tumor markers are proposed based on enzyme-linked immunosorbent assay (ELISA). This method in colorimetric immunoassays for tumor marker detection is the two antibody “sandwich” ELISA, which measures the amount of antigen between two layers of antibodies. In this assay, a capture antibody is firstly immobilized onto a solid support. The tumor marker, which is present in a biological sample or standard mixture, is then bounded and concentrated onto the support surface during incubation. After that, the solid support is incubated with a solution containing the detection antibody, thus the detection antibody will also bind with the tumor marker. Since the detection antibody is also tagged with certain enzymes, the color change caused by the enzyme-catalytic reaction can be thus used to quantify the amount of target tumor marker.

Ovarian cancer represents one of the most important health problems all over the world, being responsible for a high number of deaths annually. Serum level of cancer antigen 125 is one of the most often used methods for screening, monitoring the response to treatment, the eventual relapse and disease progression in these patients (Gireada *et al.,* 2016).

In spite of higher levels of CA 125 in ovarian carcinoma cases compared to other diagnosis in data, results confirm the high false positive rate and non-specificity associated with CA 125 as a biomarker as it was seen to increase in numerous other diseases (Parambath *et al.*, 2016).

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