SIGNIFICANCE OF TUMOR MARKERS IN DIAGNOSIS, RESPONSE TO TREATMENT AND FOLLOW UP OF OVARIAN CANCER

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ABBREVIATIONS

AFP	Alpha fetoprotein
BSO	Bilateral Salphingo Oorephectomy
CA-125	Cancer antigen
CEA	Carcinoembryonic Antigen
ch/th	Chemotherapy
gp	Group
LDH	Lactate Dehydrogenase
ng	Nanogram
OCA	Ovarian cancer antigen
OSE	Ovarian surface epithelium
S.D	Standard Deviation
TAH	Trans abdominal hysterectomy
TM	Tumor markers
β-hCG	Beta antigen of Human chorionic gonadotropin

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INTRODUCTION

INTRODUCTION

Cancer of the ovary remains one of the outstanding problems in the spectrum of malignant disease. It constitutes the fifth commonest cause of cancer deaths among women. Ovarian cancer is the most common cause of death from gynecological cancers in the Western world. (Alice and Nelly 2003) There are many genetic and environmental factors, which can influence a woman's risk of getting ovarian cancer. A strong family history of breast or ovarian cancer is one of the most important and best-defined epidemiological risk factors.

Ovarian surface epithelium-derived ovarian carcinoma is the most lethal gynecological malignancy in North America. 5–10% of epithelial ovarian cancer involves strong family histories. Thus, the familial component is one of the most important and best-defined risk factors for ovarian cancer. A woman's lifetime risk for ovarian cancer is 1.4% but is estimated to be 15–60% for women with a strong family history and/or those who inherited a germ line mutation in certain cancer susceptibility genes, suggesting that this increased risk has a genetic component. A strong family history refers to those having two or more first-degree relatives (parents, siblings and children) diagnosed with breast or ovarian cancer, and in some circumstances with features of a type of bowel cancer (hereditary non-polyposis colon cancer, HNPCC, also called Lynch Syndrome II), at age 45 or younger. There are at least three types of family history of ovarian cancer indicative of a putative autosomal dominantly inherited cancer susceptibility syndrome:

- Hereditary site-specific ovarian cancer,
- Lynch syndrome II
- Hereditary breast/ovarian carcinoma.

The discovery of DNA mismatch repair genes such as *MSH2* and *MLH1* for the Lynch Syndrome II (Lindblom *et al* 1993 and Peltomaki *et al* 1993), and the identification of BRCA1 and BRCA2 tumor suppressor proteins in hereditary breast/ovarian cancer syndrome (Boyd 2003 and Miki *et al* 1994) have advanced our knowledge on the etiology of familial ovarian cancer. Mutations in the BRCA1 and BRCA2 genes, in

particular, account for as much as 90% of cancers in women with familial ovarian cancer histories and the lifetime risk for ovarian cancer in women carrying a BRCA1 or BRCA2 mutation is estimated to be as high as 60–70% (Antoniou *et al* 2003). The majority of BRCA1 or BRCA2 mutations are presumed to lead to premature protein truncations as a result of frame shift deletions/insertions or nonsense mutations and alter the functions of BRCA protein. Whereas the functions of the BRCA1 and BRCA2 proteins have yet to be fully elucidated, BRCA genes are believed to be tumor suppressor genes, where they inhibit the growth of cancer cells through their roles in the maintenance of genome integrity, DNA repair, cell cycle control and apoptosis (Welcsh and King 2001).There is embryological and *in vitro* evidence that Ovarian surface epithelium is the origin of ovarian epithelial carcinomas (Alice and Nelly 2003). Ovarian surface epithelium is a simple mesothelium that overlies the surface of the ovary. It is important to note that the adult Ovarian surface epithelium and the Mullerian epithelia arise from a common embryonic origin, the celomic epithelium. In early development, Ovarian surface epithelium cells form part of the celomic epithelium and the celomic epithelium adjacent to the presumptive gonads invaginates to give rise to the Mullerian ducts, i.e. the primordia for the epithelia of the oviduct, endometrium and endocervix. The relevance of this close developmental relationship between the Ovarian surface epithelium and the Mullerian epithelia could explain the frequent acquisition of architectural and functional characteristics of the Mullerian epithelia during neoplastic progression of Ovarian surface epithelium and the similarities between Ovarian surface epithelium -derived carcinomas and Mullerian epithelial malignancies. Ovarian surface epithelium cells from ovaries of women with strong familial history of ovarian cancer frequently undergo Mullerian metaplasia in adult life (Alice and Nelly 2003). The increasing incidence of cancers throughout the world is one of the major concerns of our generation, every day new discoveries are made and research is continuously been done in this regard .The most common cancer of the females is the breast cancer and the second most widely spread are the cancers of gynecological regions, most of them involving cervix, uterus and ovary the most important part of the female reproductive system. Like the rest of the world we in Pakistan are also facing a continuously increasing incidence of cancers of the ovary and

most of the cases are detected at a time when treatment is difficult, or they are diagnosed late due to social and financial reasons.

The population of Pakistan has been reported to have the highest rate of breast cancer of any Asian population (excluding Jews in Israel) and one of the highest rates of ovarian cancer worldwide. To explore the contribution that genetic factors make to these high rates, a case-control study, 120 case subjects with ovarian cancer, and 200 female control subjects from two major cities of Pakistan (Karachi and Lahore). The prevalence of BRCA1 or BRCA2 mutations among case subjects with ovarian cancer was 15.8%. Mutations of the BRCA1 gene accounted for 84% of the mutations among case subjects with ovarian cancer (Liede *et al* 2002).The ovaries are infrequently the primary sites of any disease except, notably neoplasm. Indeed carcinomas of the ovaries account for more deaths than do cancers of the cervix and uterine corpus together. In 1966 they caused about 14,800 deaths in the USA alone. It is less their frequency than their lethality (because of their silent growth) that makes them so evil (Kumar et al 1997).

In addition to their role in Cancer diagnosis, some Tumor markers levels are measured before treatment to help Doctors plan appropriate therapy. In some types of cancers, tumor marker levels reflect the extent of the disease and can be useful in predicting how well the disease will respond to treatment. Tumor Markers levels may also be measured during treatment to monitor a patients response to treatment. A decrease or return to normal level of a tumor marker may indicate that the cancer has responded well to the therapy .If the Tumor marker level increases, it may indicate that the tumor is not responding to the therapy .As a end part of our study measurements of tumor markers levels may be used after treatment as a part of follow up care to check for a recurrence or relapse. Tumor markers help to assess a cancers response to treatment and check for recurrence. Scientists continue to study these uses of tumor markers as well a s their potential role in early detection and diagnosis of cancer, and help the doctors to understand better the role of tumor markers in the detection, diagnosis and treatment for that person. (www.marystolfacancerfoundation). Currently the only tumor marker to have a well–defined and validated role in the management of Ovarian cancer is CA-125.Changes in the level of CA-125 can be used as a reliable indication of response or

progression according to various criteria, but its value as part of a screening tool and during routine follow-up remains a subject of ongoing trails. Other markers remain experimental and do not have a well-defined contribution to make at present. (Meyer and Rustin 2000).CA-125 is the most reliable marker for monitoring the course of epithelial ovarian cancer; CA-125 assay is not an adequate screening test for this malignancy but it can represent an useful adjunct to clinical examination and ultrasound in the differential diagnosis of ovarian masses in postmenopausal women; Serial measurement of CA-125 assay, the concomitant determination of other tumor markers does not add further information when compared to CA -125 alone. Conversely in Patients with preoperative negative assay the measurement of one or more of other antigens could be of clinical relevance. (Maggino 2000)

The use of serum CA-125 measurement as a means to assess the response to surgery and chemotherapy in ovarian cancer is now well documented. Good prognostic significance is attributed to rapid decline in CA-125 levels after chemotherapy in patients with advanced ovarian cancer. Preoperative serum CA-125 levels may successfully discriminate benign from malignant adnexal masses in menopausal women but women in their reproductive years the specificity is low. (de Buruijn *et al* 1997).The tumors of the Ovary are of many types most common are the epithelial tumors and the Germ cell tumors. Epithelial cancer of the ovary is a relatively uncommon gynecologic cancer in the United States, with approximately 25,580 new cases and 16,090 deaths anticipated in 2004. (Schildkraut and Thompson 1988), Most patients present with advanced disease, which is managed with surgical resection followed by platinum-based chemotherapy. During the past decade, advances in chemotherapy have resulted in improved survival and in more effective treatment of relapsed disease. In addition, a better understanding of genetic risk factors has permitted a tailored approach to preventive strategies, such as bilateral salpingo-oophorectomy in selected women

FAMILIAL SYNDROMES

A strong family history of breast cancer, ovarian cancer, or both sometimes occurring at an early age and in the same woman may be related to the presence of an inherited mutation in one of two genes, known as *BRCA1* and *BRCA2*. The *BRCA1* and *BRCA2* genes are located on chromosomes 17q and 13q, respectively, and their gene products are involved in DNA repair. (King *et al* 2003 and Scull *et al* 1996) Because a mutated allele for the *BRCA1* or *BRCA2* gene may be inherited from either parent, it is important to obtain a complete family history during risk assessment. Women with a germ-line mutation in *BRCA1* are reported to have a lifetime risk of ovarian cancer that ranges from 16 to 44 percent, and a lifetime risk of breast cancer that ranges from 56 to 87 percent. (Struewing *et al* 1997 and Ford *et al* 1996) Ovarian cancer may develop at an earlier age in women with germ-line *BRCA1* mutations than in those with the sporadic form of the disease, although it is important to recognize that ovarian cancer may occur at any age in mutation carriers. Like BRCA1, the BRCA2 protein is localized in the nucleus and is involved in DNA repair through its association with the protein RAD51 (Taytigian *et al* 1996). Women with germ-line mutations in *BRCA2* have a lifetime risk of breast cancer that is similar to that for carriers of the *BRCA1* mutation, and their lifetime risk of ovarian cancer is approximately 10 percent. (Struewing *et al* 1997 and Tonin *et al* 1996). Men with *BRCA1* or *BRCA2* mutations are at risk for male breast cancer and may also have an increased risk for developing pancreatic cancer, as compared with men who do not have the mutation (Tonin *et al* 1996 and Frank *et al* 2002) The natural history of ovarian cancer that develops in the setting of *BRCA1* or *BRCA2* germ-line mutations appears to be characterized by a more indolent course than that of sporadic disease (Ben *et al* 2002 and Rubin *et al* 1996). A second familial disorder that carries with it an increased risk of ovarian cancer is referred to as the Lynch syndrome II, it is caused by inherited germ-line mutations in DNA mismatch repair genes, such as MSH2 (mutS homologue 2) or MLH1 (mutL homologue 1) (Chung and Rustig 2003) Affected families have a predominance of hereditary nonpolyposis colon cancer, often on the right side of the colon and sometimes in association with other cancers, such as those of the endometrium.

The symptoms of ovarian cancer are nonspecific and often suggest the presence of upper abdominal disease. Patients may report abdominal fullness, dyspepsia, early satiety, or bloating as the result of increased abdominal pressure from ascites or involvement of the omentum. Patients with early-stage disease presents with pelvic pain due to ovarian torsion, although most patients with early-stage disease are asymptomatic. Physical findings are diverse and typically include a palpable ovarian mass. In this regard, ovarian cancer should be considered in any pre menopausal woman with an unexplained enlargement of the ovary or any postmenopausal woman with a palpable ovary.

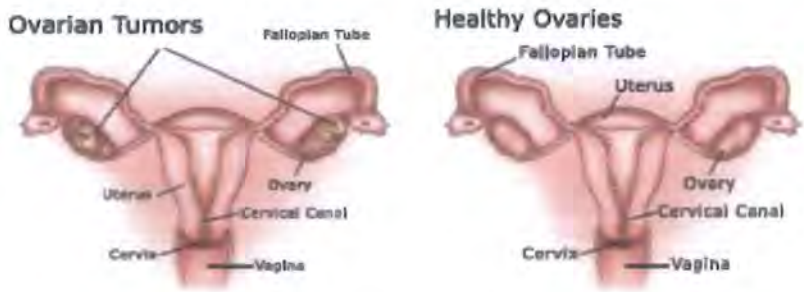
ANATOMY OF THE OVARY

Each ovary is an oval shaped structure, measuring 2 x 4 cm, and is attached to the back of the broad ligament by the mesovarium. Ovary usually lies against the lateral wall of the pelvis in a depression called ovarian fossa, bounded by the external iliac vessels above and by the internal iliac vessels behind the position of Ovary is extremely variable and it is often found hanging down in the recto uterine pouch (pouch of Douglas). A thin fibrous Capsule the Tunica Albuginea surrounds the ovaries, This capsule is covered externally by a modified area of peritoneum called germinal epithelium surrounds the ovaries.

Before puberty, ovary is smooth, but after puberty ovary becomes progressively scarred as successive corpora lutea degenerates after menopause the ovary becomes shrunken and its surface is pitted with scars The ovaries are organs responsible for the production of female germ cells, the ova and female sex hormones estrogen and progesterone in the sexually mature female (Richard 1992).

TUMORS OF THE OVARY

Tumors of the ovary make up an amazing diversity of pathological entities. This diversity of pathological to the three cell types that make up the normal ovary: the multipotential surface (coelomic). Covering epithelium is the totipotential germ cells, and the multipotential sex cord-stromal cells, each of these cells type give rise to a variety of tumors. It is evident that neoplasm of surface epithelium origin account for the great majority of all primary ovarian tumors, and in their malignant forms account for 90% of all ovarian Cancers, These epithelial tumors are the ones that require most attention. Germ cells and sex cord-stromal tumors are much les frequent and, although they constitute 15-20% of all ovarian tumors, are collectively responsible for less than 10% of cancers of the ovary (Richard 1992)



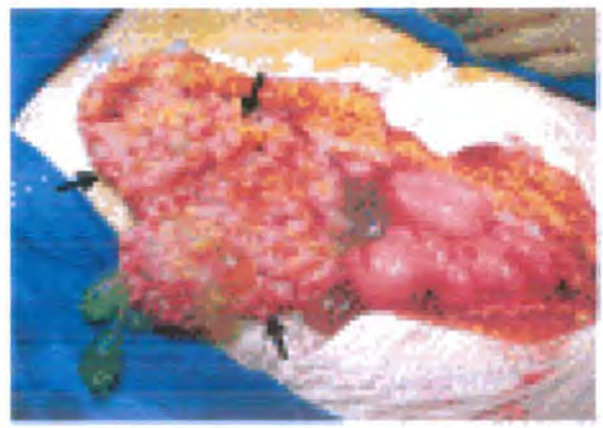


Figure-1

Typical, Intraoperative Appearance of Stage III Epithelial Ovarian Cancer.

The entire omentum (arrows) has been replaced by multiple tumor implants. (Photograph courtesy of Dr. Young B. Kim, Beth Israel Deaconess Medical Center, Boston.) NEJM

TYPES OF OVARIAN TUMORS

Surface epithelial-Stromal Tumors

Serous Tumors

Mucinous Tumors

Endometroid Tumors

Cystadenofibroma

Brenner Tumor

Germ cell tumors

Teratomas

SURFACE EPITHELIAL-STROMAL TUMOR

These neoplasms are derived from the coelomic epithelium. They can be strictly epithelial (e.g., serous, mucinous tumors) or they can have a distant stromal component like cystadaenofibroma, Brenner Tumor

SEROUS TUMOR

This type of ovarian tumors may be small in size ranging from 5-10 cm in diameter, but most are large, spherical to ovoid, cystic structures, up to 30-40 cm in diameter; serous tumors are mostly common in the age group falling between 30 – 40 years. They are commonly known as cytadenomas or cystadenocarcinom and these account for about 60% of all ovarian cancers. The prognosis for patients with invasive serous cystadenocarcinoma after surgery, followed by radiation and chemotherapy are poor and mostly depends on the time of diagnosis.

MUCINOUS TUMORS

They most resemble the serous tumors differing in only the epithelium, which consist of mucinous secreting cells. Tumor is common in the same age group i.e. 30-40 years, these tumors account for only 10 % of all ovarian tumors. The prognoses for mucinous cystadenocarcinoma is better then that for serous counterpart.

ENDOMETROID TUMORS

These tumors are solid or cystic but sometimes they develop as a mass projecting from the wall of an endometriotic cyst filled with brown colored fluid, although benign and borderline forms exists these tumors are usually malignant and bilateral in 30 % cases. if tumor is well differentiated there is a 62% 5 year survival rate with aggressive forms it drops to 23%.

CYSTADENOFIBROMA

It is a variant of serous cyst adenoma. These are small tumors usually benign and multilocular. Carcinomattous transformation is rare.

BRENNER TUMOR

Brenner is a an uncommon ovarian solid usually unilateral tumor consisting of an abundant stroma containing nests of traditional epithelium resembling that of urinary tract (Kumar *et al* 1997)

GERM CELL TUMORS

Germ cell tumors are derived from the primordial germ cells of the ovary Where as malignant germ cell tumors can arise in extragonadal sites, such as the mediastinum and the retro peritoneum, the majority of germ cell tumors arise in the gonad from the undifferentiated germ cells. The variation in the sites of these cancers is explained by the embryonic migration of the germ cells from the caudal part of the yolk sac to the dorsal mesentery, prior to their incorporation into the sex cords of the developing gonads

TERATOMAS

These germ cell tumors comprise 15-20 %of total ovarian cancers. They usually arises in the first two decades of life, younger the patient greater is the chance of malignancy. But luckily 90% of these germ cells are benign cystic mature teratomas another type of teratomas are immature malignant teratomas, they are found early in life the mean age being 18 years.

DYSGERMINOMAS

Dysgerminomas are the most common malignant germ cell tumors, accounting for about 30% to 40% of all ovarian cancers of germ cell origin.[394] The tumor represents only 1% to 3% of all ovarian cancers, but as many as 5% to 10% of ovarian cancers in patients younger than 20 years of age. Seventy-five percent of dysgerminomas occur between the ages of 10 and 30 years, 5% under the age of 10 years, and rarely over the age of 50 years. Because these malignancies occur in young women, 20% to 30% of ovarian malignancies associated with pregnancy are dysgerminomas. Much has been, and continues to be learned about the management of dysgerminoma by analogy with its male counterpart, testicular seminoma. The dominant route of spread in both is nodal, via the gonadal lymphatics to the renal hilar and para-aortic nodes. Both are exquisitely radiosensitive and curable with modest doses of radiotherapy, 2,500 to 3,500 cGy, even when bulk disease up to 5 cm is being treated. For this reason, excellent long-term survival rates are obtained when surgical removal, using oophorectomy or hysterectomy/bilateral salpingo-oophorectomy, is followed by radiotherapy to para-aortic and pelvic nodes. About 75% of dysgerminomas are stage I at diagnosis, it is, confined to one or both ovaries. About 85% to 90% of stage I tumors are confined to one ovary; 10% to 15% are bilateral. In fact, dysgerminoma is the only germ cell malignancy that has this significant rate of bilaterality, other germ cell tumors rarely being bilateral.

Dysgerminomas affect younger women (85% are under 29 years of age) and are present in two thirds of cases as stage IA. Dysgenetic gonads tend to develop dysgerminoma Therapeutic concepts have changed dramatically over the past few years, largely because of the recognition of dysgerminomas' chemosensitivity, allowing cure without ovarian ablation by surgery or radiotherapy in many cases; the advent of improved techniques of imaging the retro peritoneum, with lymphography, and CT and MRI scanning that allow detection of disease under 3 to 5 cm in diameter when it is eminently curable; tumor markers, AFP and the beta subunit of human chorionic gonadotrophin (Beta-hCG), which distinguish pure dysgerminoma from the mixed germ-cell tumors; and the emerging emphasis in treatment on preservation of childbearing capacity, presumably without compromising cure. Many aspects of management are controversial, such as the need for

complete surgical staging; the extent of the primary operative procedure in unilateral, bilateral, and metastatic disease; the role of postoperative observation in stage IA; the choice between radiotherapy and chemotherapy; the decision as to which chemotherapy drugs should be used; and the need for a second-look operation. The subject was recently reviewed in greater depth than is possible here. In the 25% of patients who present with metastatic disease, the tumor most commonly spreads via the lymphatics. It also can spread hematogenously, or by direct extension through the capsule of the ovary, with exfoliation and dissemination of cells over the peritoneal surfaces. Metastases to the contralateral ovary occur and may be present when there is no other evidence of spread. An uncommon site of metastatic disease is bone, and when it does occur here, the lesions are seen principally in the lower vertebrae. Metastases to the lungs, liver, and brain are seen most often in patients whose disease is longstanding or recurrent. Metastatic disease to the mediastinum and supraclavicular lymph nodes is usually a late manifestation of disease.

UNCOMMON OVARIAN TUMORS

There are several types of rare malignant ovarian tumors that together compose about 0.1% of ovarian malignancies. These lesions include small cell carcinomas, lipoid cell tumors and primary ovarian sarcomas.

SARCOMAS

Malignant mixed mesodermal sarcomas of the ovary are rare, and only about 100 cases have been reported. Most are heterologous, and 80% occur in postmenopausal women. The presentation is similar to that of most ovarian malignancies, although these tumors are biologically aggressive, and the majority of patients have metastases to organ parenchyma, such as the liver and lung, and to the retroperitoneal lymph nodes. Although 20% to 25% of all benign and malignant ovarian neoplasms are of germ cell origin, only about 3% of these tumors are malignant. Germ cell malignancies account for less than 5% of all ovarian cancers in Western countries but they represent up to 15% of ovarian cancers in Asian and black societies, where epithelial ovarian cancers are much less common. In the first 2 decades of life, almost 70% of ovarian tumors are of germ cell

origin, and one third of these are malignant Germ cell tumors account for two thirds of the total ovarian malignancies. Germ cell cancers are seen in the third decade, but thereafter become quite rare.

DIAGNOSIS

When an adnexal mass is 2 cm or larger in a premenarchal female or 8 cm or larger in other premenopausal females, surgical exploration is frequently required. In young patients, blood tests should include serum β -hCG and AFP levels, a complete blood count, and liver function tests. A chest radiograph should be performed because germ cell tumors can metastasize to the lung or mediastinum. A karyotype should be obtained preoperatively on all premenarchal females, because of the propensity for these tumors to arise in dysgenetic gonads. A preoperative CT scan may document the presence and extent of retroperitoneal lymphadenopathy or liver metastases, but because these patients require surgical exploration, a more extensive and time-consuming preoperative evaluation is unnecessary. If postmenarchal patients have predominantly cystic lesions up to 8 cm in diameter, they may undergo a trial of hormonal suppression for two menstrual cycles.

TUMOR MARKERS

Tumor markers are substances that can often be detected in higher than normal amounts in the blood, urine, or body tissues of some patients with certain types of cancer. Tumor markers are produced either by the tumor itself or by the body in response to the presence of cancer or certain benign. Ideally a tumor marker should be able to detect sub clinical disease helpful in monitoring the response to treatment and identify the signs and symptoms of recurrence so that the treatment could be managed likewise. The use of tumor marker to monitor response to treatment is particularly helpful in ovarian cancer where there is often a lack of clinically or radiologically measurable disease.

TUMOR MARKER FOR EPITHELIAL OVARIAN CANCER

Cancer antigen CA-125 or ovarian cancer antigen OCA

CA-125 is the most reliable marker for monitoring the course of epithelial ovarian cancer (Maggino and Gudduci 2000) this has also been reported at many places in the literature. Serum 125 assay is very useful for both the differential diagnosis of ovarian masses, particularly in the menopause, in monitoring to response of chemotherapy and follow up of patients with histological proven ovarian tumor, although its role in the screening of malignancy is controversial (Gadduci *et al* 2004). Serum CA-125 is a reliable biological marker for staging and restaging of patients with Lymphoma. Serial measurements are useful in conjunction with other markers for monitoring response to treatment. Higher CA-125 levels are associated with advanced disease, aggressive histology, mediastinal and or abdominal involvement, bulky tumor, high tumor burden, effusions, contiguous extra nodal extension, high LDH activity or elevated beta 2-Microgloulin levels (Lazzarino *et al* 1998). A reduction in the serum CA-125 level correlates well with clinical response. It is also useful in monitoring the patients for recurrence of ovarian cancer In many studies where emphasis has been put on finding CA-125 as a source of tumor diagnosis its level has been found to be higher in the malignant tissues, there was a significant association between the tissue marker and the histological type (Higher CA-125 was associated with serous and endometroid tumor) and between the marker and survival, no relation with Stage was found, There was a correlation between the CA-125 level in the cytosol and serum both variables being dependent with a correlation co-efficient of 0.44.This good correlation speaks in favor of usefulness of CA-125 determination in serum in the follow up of ovarian cancer, tumors having a high expression of CA-125 were found to have a double relative risk of death, independent of Tumor stage (de la Cuesta *et al* 1999).

Bast *et al* (1981) defined CA-125 as a 200kd glycoprotein recognized by murine monoclonal antibody OC 125 as a marker for epithelial malignancies. The CA-125 antigen has shown to be derived from coelomic epithelial cells and was initially defined by a monoclonal antibody, OC 125, isolated and characterized by Bast and colleagues.

The OC 125 monoclonal antibody was obtained through the immunization of BALB/c mice with a cell line, OVCA 433, derived from cells in the ascities fluid of a patient with serous papillary Adencarcinoma of the ovary.

As an interesting historical note, the designation of "125" refers not to the apparent mass of the antigen but rather to the 125th attempt by the Bast group to develop a monoclonal antibody to ovarian cancer. Subsequent biochemical and histo-chemical studies using the OC 125 antibody have demonstrated CA-125 antigen to be associated with coelomic epithelium, including pleura, pericardium, peritoneum and Mullerian epithelia. Normal ovarian epithelial cells do not express CA-125; mucinous papillary ovarian tumors and, to a greater extent, serious papillary tumors are positive. CA-125 is a glycosylated protein, and secretion of the antigen into the circulation appears to also require phosphorylation of the O-glycosylated form. The OC 125 monoclonal antibody recognizes and ~40 kDa proteolytic fragment of the native 200-kDa antigen, the molecular cloning of the CA-125 antigen is been recently reported by Lloyd and colleagues. The cloned sequence codes for a new mucin, designated MUC16, and is characterized by a high serine, threonine, and proline content in an *N*-terminal region composed of nine partially conserved, tandemly repeated sequences of 156 amino acids each and a C-terminal region containing a possible Trans membrane domain and a potential tyrosine phosphorylation site.

CA-125 is produced by a variety of cells, but particularly by ovarian cancer cells. Studies have shown that many women with ovarian cancer have elevated CA-125 levels. In women with ovarian cancer being treated with chemotherapy, a falling CA-125 level generally indicates that the cancer is responding to treatment. Increasing CA-125 levels during or after treatment, on the other hand, may suggest that the cancer is not responding to therapy or that some cancer cells remain in the body. Abnormal CA-125 levels may be found in fluids of different origin ascities, pleura, pericardium, amniotic fluids, CA-125 is a very good tumor marker in Ovarian and Lung cancer, The sensitivity of CA-125 in ovarian cancer is related to stage histological types (low levels in mucinous Adenocarcinoma and the marker is useful in the early detection of recurrence (sensitivity 80%) and in therapy, prognosis and disease monitoring (Richard 1992)

For now, serum immunoassay for the level of CA-125 is the best-established screening and diagnostic marker for ovarian cancer.

SIGNIFICANCE OF TUMOR MARKERS

CA-125 is a tumor marker that may be elevated in non-mucinous epithelial tumors but is neither sensitive nor specific enough to be used as a diagnostic tool. However, CA-125 serum concentration variations can be used for monitoring chemotherapy efficiency (Carcenac, 2004). CA-125 is positive in 62.5 % in cases of common epithelial carcinoma, 100 % positive in serous carcinoma, but it is negative in patients with germ cells and sex chord stromal tumors. CEA is positive in 40.6% cases of epithelial carcinoma, most frequently elevated in patients with mucinous carcinoma, pseudomyxoma peritonei, and krukenberg tumor. AFP is positive only in endodermal sinus tumors; LDH is elevated in 41% cases of epithelial carcinoma (Konishi et *al* 1986).

Although much is to be studied it remains a well-established fact that CA-125 is a reliable and accurate means of monitoring response to treatment and confirming relapse in ovarian cancer patients. However, its role in follow up after initial treatment is less certain and the subject of clinical trial. Serial changes in Ca 125 can be used as a reliable indicator of disease response or progression so that patients can be classified as responding or progressing to either standard or CA-125 criteria (Guppy and Rustin 2002)

CA-125 or OC is one of the most commonly used tumor marker for epithelial cancer of the ovary and it is very useful in differentiation of histological types and clinical stage of ovarian tumors in combination with other tumor markers. Different serum tumor markers are used for different histological types of ovarian cancer, which are described later. All serum tumor markers in patients with complete response to chemotherapy significantly decreased after 3 courses of chemotherapy (Konishi et *al* 1986).In patients with a history of ovarian cancer, three progressively rising serum 125 values in the normal range at 1-3 month interval are associated with a high likelihood of tumor recurrence. Patients with such a pattern should undergo immediate investigation to rule out and/or identify recurrent cancer (Wilder *et al* 2003).

TUMOR MARKERS FOR NON-EPITHELIAL OVARIAN CANCER

The tumor marker for non epithelial ovarian cancer, The germ cell tumors are Human beta chorionic gonadotrophin and alpha feto protein are probably the best known tumor marker in clinical practice and are invaluable in the diagnosis, treatment, and follow up of ovarian germ cell tumors. Serum placental alkaline phosphate and LDH are also sometimes helpful as markers for dysgerminoma (Kumar *et al* 1997)

BETA HUMAN CHORIONIC GONADOTROPHIN

Human chorionic gonadotropin (β-hCG) is normally produced by the placenta during pregnancy. In fact, HCG is sometimes used as a pregnancy test because it increases early within the first trimester .It is also used to screen for choriocarcinoma (a rare cancer of the uterus) in women who are at high risk for the disease, and to monitor the treatment of trophoblastic disease (a rare cancer that develops from an abnormally fertilized egg).

Elevated β-hCG levels may also indicate the presence of cancers of the Testis, ovary, liver, stomach, pancreas, and lungs. Pregnancy and marijuana use can also cause elevated β-hCG levels

ALPHA FETO-PROTEIN (AFP)

Alpha-Feto protein (AFP) is normally produced by a developing fetus .AFP levels begin to decrease soon after birth and are usually undetectable in the blood of healthy adults (except during pregnancy). An elevated level of AFP strongly suggests the presence of either primary liver cancer or germ cell cancer (cancer that begins in the cells that give rise to eggs or sperm) of the ovary or testicles. Only rarely do patients with other types of cancer (such as stomach cancer) have elevated levels of AFP. Non-cancerous conditions that can cause elevated AFP levels include benign liver conditions, such as cirrhosis or hepatitis: ataxia telangiectasia: Wiscott-Aldrich syndrome: and pregnancy.

LACTASE DEHYDROGENASE (LDH)

Lactate dehydrogenase (LDH) is a glycolytic enzyme that may be elevated in the serum of patients with gonadal and extragonadal dysgerminomas. A case of unilateral ovarian pure dysgerminomas showed remarkably elevated levels of serum LDH, after complete excision of tumor serum LDH returned to normal.

Lactate dehydrogenase is a protein found throughout the body. Nearly every type of cancer as well as many other diseases can cause LDH levels to rise. Therefore, this marker cannot be used to diagnose a particular type of cancer. But many examples cited in the literature reported elevated levels of ovarian dysgerminomas suggest that serum LDH and its isoenzymes pattern are useful tumor markers for diagnosis and post therapy surveillance in patients with ovarian dysgerminomas (Yoshimura *et al* 1988).

LDH levels can be used to monitor treatment of some other cancers, including testicular cancer, Ewing's sarcoma, non-Hodgkin's lymphoma, and some types of leukemia. Elevated LDH levels can be caused by a number of non-cancerous conditions, including heart failure, hypothyroidism, anemia, and lung or liver disease (Meyer and Rustin 2000).

PROGNOSIS OF OVARIAN TUMOR

The overall 5-year survival rate of patients diagnosed with ovarian cancer is ~40%, a figure that has not changed during the last two decades despite clinical advances in aggressive cytoreductive surgery and chemotherapeutic regimens. Detection of ovarian cancer at early stages is associated with much higher 5-year survival, ~90% for stage I and ~70% for stage II. Early stage disease, however, comprises only ~25% of cases. Pre malignant precursors of epithelial ovarian cancer, which comprise ~90% of cases, have not been identified. Clinical research has focused on modalities for identifying women at high risk, and improved ovarian cancer screening and early detection. Although at least 90% of all ovarian cancers appear to be sporadic, the remaining 10% are associated with an inherited susceptibility, identified initially by family studies and, most recently, by the identification of inheritance of germ line mutations in BRCA 1 or 2 genes, which appear to account for at least 90% of inherited ovarian cancers.

The traditional standard clinical screening method for ovarian cancer has been palpation of a suspect adnexal mass as part of annual pelvic examination. This screening procedure is clearly inadequate, as ~70% of women with ovarian cancer has advanced disease at the time of initial diagnosis. More recently, diagnostic pelvic ultrasound has been implemented, using first tran abdominal and later trans vaginal techniques, which can directly assess the ovaries and determine their morphology. Color Doppler flow studies provide added evaluation of ovaries, particularly in cases with abnormal morphology. These modalities have been recommended as useful screening approaches, but although these screening methods can detect ovarian cancer in clinically asymptomatic women, there currently is no established evidence that these screening methods improve outcome for women in any risk group.

In the initial clinical report, 83% of ovarian cancer patients had elevated serum CA-125 levels; these patients all had stage III or IV epithelial ovarian cancers. However, early detection of ovarian cancer requires that the screening tumor marker be consistently elevated in patients with early stage disease. At best, serum CA-125 levels are elevated in only 50% to 60% of women with stage I disease. The largest prospective studies addressing the utility of CA-125 screening as a technique for the early detection of ovarian cancer to date have been those of Jacobs and colleagues. These studies demonstrated that asymptomatic postmenopausal women with CA-125 levels above 30 U/ml had a significantly greater likelihood of developing ovarian cancer.

Subsequent to the initial derivation and characterization of the original OC 125 monoclonal antibody, a number of new monoclonal CA-125 antibodies have been reported As reviewed by the International Society of Oncodevelopmental Biology and Medicine (ISOBM) TD-1 Workshop, these antibodies, 26 in number, were classified into two groups based on the two distinct major CA-125 epitope regions, OC 125-like and M11-like.They recognize The availability of these monoclonal antibodies has resulted in the development and use of an improved second generation of immunoassays, employing pairs of "catcher" and I and non-isotope-labeled "detector" antibodies with improved performance, as defined by superior receiver operator characteristic (ROC) curves over the original homologous OC 125-based radio immunoassay ROC curves graphically describe the relationship between the true-positive rate (sensitivity) and false-positive rate

(1-specificity) of the assay, as the cutoff criterion of the assay is varied. Quantitative analysis of these ROC curves has demonstrated that these second-generation assays, first available in 1995 and presently the standard assay used in the United States, specifically provide clinically important improved discrimination between benign gynecologic tumors and early stage (Stage I and II) ovarian cancer, although even the new and improved assays still show relatively low sensitivity and specificity for early stage ovarian malignancies.

With a cut off value of 30- 35 U/ml, CA-125 exhibits a sensitivity of 50% to 60% for early disease, with specificity approaching 99%, in populations of apparently healthy postmenopausal women. Lack of CA-125 specificity is due largely to a spectrum of normal physiological changes and benign clinical conditions that can result in moderate elevations of CA-125 levels above the nominal 35 U/ml cutoff value. CA-125 specificity is lower in premenopausal women and in women with benign gynecologic conditions, including ovarian cysts, pelvic endometriosis, and uterine fibroids. CA-125 levels are also elevated in women with inflammatory processes, such as pericarditis, and pregnancy. To further improve CA-125 screening performance, Skates and colleagues defined an algorithm incorporating age and absolute levels and rate of change of CA-125 that increases sensitivity compared to a single cutoff value This approach was used as part of a multimodal pilot randomized control trial of ovarian screening employing ultrasonography and CA-125. The positive predictive value of 21% obtained with this multimodal screening strategy for ovarian cancer is substantially higher that that achieved in postmenopausal women by any other strategy and suggests that sequential use of CA-125 and ultrasonography is the most cost-effective approach to screening for ovarian cancer. Future improvements in circulating tumor markers and diagnostic technology may improve the ability to screen for and detect early ovarian cancer. Promising new circulating markers that appear to add complementary independent information to CA-125 include lysophosphatidic acid (LPA), mesothelin, and kallikrein 10, the last two of which are elevated in 76% and 56% of ovarian cancer patients, respectively. Last, O'Brien and colleagues have described additional candidate markers over expressed in ovarian cancer, including cyclins D_1 and E; the matrix metalloprotease pump-1 MMP-7, matrilysin; and hepsin, a multidomain cell surface serine protease, and have proposed that

these antigens may have utility as novel targets for dendritic cell-based immunotherapy against ovarian cancer.

OBJECTIVES OF THE STUDY

CA-125 is an ideal marker for the early detection of ovarian cancer; its role in clinical follow-up during and after therapy is well documented. The objectives of this study were to determine the absolute value and rate of decline of CA-125 after the initial courses of chemotherapy, predict final clinical response after complete courses of chemotherapy, as predictor of prognosis. To see the effect of AFP, LDH, CEA and β-hCG in addition to CA-125 for the diagnosis , response and follow up of ovarian cancer.

This topic is selected to analyze the patients with diagnosed cases of ovarian Cancer for the serum levels of tumor Markers most commonly used in Ovarian Cancers of both epithelial and Germ cell type.

METHOD
AND
MATERIAL

METHOD AND MATERIAL

This study has been done at Nuclear Medicine Oncology and Radiotherapy Institute (NORI), Islamabad. Started in mid 2004, it is basically a cancer treatment and radiation therapy center, Blood of patients having different types of ovarian malignancies was taken as samples because all the patients from different hospitals of the city and from distant and remote areas which do not have any other nearby facility comes to NORI hospital for treatment. During the research 130 samples of ovarian cancer patients were collected, with the average age of 42.8 years.

SAMPLE COLLECTION

1.3ml of blood is collected in dry tubes. Kept for 20 minutes at room temperature.

2. Serum is separated from cells by centrifugation at 3000 rpm for 20 min.

3. Serum samples are kept frozen at <-18 ^{0}C in capped polystyrene tubes till the assay is run. Samples were aliquoted to avoid repeated freezing and thawing. The data sheet given below was used to collect information at the time of sample collection.

Patient Data Sheet

Registration no. /Serial no:_____
Name: _____
Father's/Husband'sName:_____
Sex: _____ Age_____
Telephone/Contactno_____
Family History:
Diabetes:_____
B.P:_____
Smoker:_____
Mother: _____ Sister:_____
Aunt: _____

Tests Performed:

1- Blood CP:
Urea:_____, Creatinin:_____, Electrolytes (Na, K, Cl):_____
2- Biopsy: _____ 3- CT scan: _____
Histopathology:_____

Tumor Markers:

CA-125: _____BHCG: _____α-fetoprotein: _____LDH: _____

Patient History:

Treatment Protocol
Tumor Markers
CA-125: _____BHCG: _____α-fetoprotein: _____LDH: _____
Before1st ChemotherapyCycle:_____
After each Chemotherapy Cycle: _____

Epithelial Carcinoma:
CAP: _____ CP: _____Pacilitaxel/Cis-platin /Carboplatin _____

Germ cell Carcinoma
BEP: _____ PVB: _____ VIP: _____

GROUPING OF PATIENTS

1. Out of 130 patients 63 cases were dropped out of the study because their complete follow up was not available they came to the hospital after taking treatment somewhere else and only visited for tumor marker analysis. They received no treatment no chemotherapy and they did not turned up at their scheduled visit next time.

2. Those patients who came to treatment after some sort of surgery either laparatomy, Tran abdominal hysterectomy (TAH) bilateral salphingo oorephectomy (BSO). And had chemotherapy and falling tumor marker levels, and their complete follow up is available in the following pattern. This group is taken here for further investigation.

1. Baseline tumor marker level before the start of chemotherapy
2. Post chemotherapy levels at the end of last cycle.
3. Follow up first after last cycle up to 6 months after chemotherapy
4. Follow up last from 6 months up to 1 year for the first year

Out of the total 130 patients investigated in one year time, during this research,77 were taken for further tests two groups were made

Group 1. 44 patients were found to have complete picture fulfilling our requirement for the tumor marker CA-125, out of these 44,10 patients were again separated because they had complete surgery for removal of tumor and they fall in the stage 1 tumor grade .their pre chemotherapy levels were low.33 were grouped separately because they had high pre chemotherapy levels. Theses patients were sampled for only one tumor marker CA-125.

Group 2. 23 patients were sampled and 5 tumor marker tests were done for them. An elevated level of AFP and LDH strongly suggests the presence of either primary liver cancer or germ cell cancer. (Understanding Tumor Markers-Grades/Prognosis) These two Markers are a part of tests applied in the germ cell tumors.

PRINCIPLE OF THE ASSAY FOR CA-125

The CA-125 antigen assay is a one step "sandwich " type assay in which two mouse monoclonal antibodies, directed against two different epitopes of the molecule, are employed. Samples or standards are incubated in tubes, coated with the first monoclonal antibody, in presence of the second 125-labeled monoclonal antibody. Following

incubation the contents of the tubes are aspirated and washing eliminates unbound labeled antibody. The amount of bound reactivity measured in Gamma counter is proportional to the CA–125-concentration .The unknown values are determinated by interpolation from a standard curve.

REAGENTS PROVIDED IN THE KIT

All reagents in the kit are stable until the expiry date indicated on the kit labels, They were stored at 2-8^{0}C.

REAGENTS

No	Regents	Dispensed as	Condition
1	Anti-CA 125 antibody Coated tubes	100 tubes	Ready to use
2	^{125}I labeled monoclonal Antibody	01 vial of 33 ml	Ready to use
3	4 standards	01 ml vials	Ready to use
4	Control serum	01 ml vial	Ready to use
5	Wash solution (20x)	01 vial o50 ml	Diluted upto1000ml

EXPERIMENTAL DESIGN

PROTOCOL

Tube Number	No of tubes	Content of tube
1 and 2	2	0.00
3 and 4	2	14
5 and 6	2	40
7 and 8	2	160
9 and 10	2	400
11 and 12	2	Control, 46.75
13 and 14and onwards	2	Patient 1

ASSAY PROCEDURE FOR CA-125

Immunological step	Washing step	Counting
To antibody coated tubes- 100 ul of sample/ standard or control was added 300 ul of tracer was added and vortex gently. Incubated for 4 hours at 18-25 °C , Shaking at 400 rpm	Aspirated carefully the content of each tube except total cpm then washed three times with 2 ml of wash solution, then aspirated the contents of the tubes	Bound cpm (B) and total cpm (T) was counted for 1 minute

PRINCIPLE OF THE ASSAY FOR BETA HCG

The immunoradiometric assay of human chorionic gonadotrophin (β-hCG) is a sandwich type assay Mouse monoclonal antibodies directed against two different epitopes of beta subunit of hCG molecule and hence not competing are used. Total β-hCG i.e. hCG intact and hCG b-subunit is thus determined. The samples or calibrators are incubated in tubes coated with the first monoclonal antibody in the presence of second monoclonal antibody labeled with iodine 125.After incubation, the content of tubes is aspirated and the tubes are rinsed so as to remove unbound 125-labelled antibody. The bound radioactivity is determined in a gamma counter. The total β-hCG concentrations in the samples are obtained by interpolation from the standard curve. The concentration of total ß-hCG in the samples is directly proportional to the radioactivity.

REAGENTS

No	Reagents	Dispensed as	Condition
1	Anti hCG monoclonal antibody-coated tubes	100 tubes	Ready to use
2	^{125}I-labelled monoclonal anti-hCG antibody	01 vial of 22 ml	Ready to use
3	Dilution buffer (hCG buffer)	01 vial of 60 ml	Ready to use
4	Calibrators	06 vials of 0.5 ml	Ready to use
5	Control Sera	02 vials	Lyophilized
6	Wash solution (20x)	01 vial of 50 ml	Diluted up to 1000 ml with distilled water

EXPERIMENTAL DESIGN

Tube Number	No of tubes	Content of tube
1 and 2	2	0.00
3 and 4	2	8.20
5 and 6	2	27.4
7 and 8	2	82
9 and 10	2	274
11 and 12	2	820
13 and 14	2	Control 1,31-48.4
15 and 16	2	Control 2,118-178
16 and 17 And onwards	2	Patients onwards

ASSAY PROCEDURE FOR BETA HCG

Step 1 Additions	Step 2 Incubation	Step 3 Counting
To coated tubes, 50 ul of calibrators/ control/ or sample was added. 200ul of Tracer	Incubated for 1 hour at 18-25°C,shaking at >280 rpm	Contents of the tubes are aspirated carefully except for 2 total counts tubes, Wash twice with 2 ml wash solution, Bound cpm (B) and total cpm (T) was counted for 1 min

PRINCIPLE OF ASSAY FOR AFP

The immunoradiometric assay of AFP is a sandwich type assay in which two mouse monoclonal antibodies directed against two different epitopes of the molecule, are employed. The samples or calibrators are incubated in tubes coated with the first monoclonal antibody The content of tubes are aspirated and the presence of AFP in the sample is revealed by incubation with the second 125-labelled antibody. The contents of the tubes are aspirated and the bound radioactivity is determined in a gamma counter. The AFP concentrations in the samples are obtained by interpolation from the standard curve. The concentration of AFP in the samples is directly proportional to the radioactivity.

REAGENTS

No	Reagents	Dispensed as	Condition
1	Anti –AFP monoclonal antibody-coated	100 tubes	Ready to use
2	Monoclonal ^{125}I-labelled anti-AFP tracer antibody	01 vial of 22 ml	Ready to use
3	AFP Calibrators	06 vials of 0.5 ml	Ready to use
4	Control samples	02 vials	Lyophilised
5	Phosphate buffer	01 vial of 30 ml	Ready to use
6	Wash solution (20x)	01 vial of 50 ml	Diluted before use

EXPERIMENTAL DESIGN

Tube Number	No of tubes	Content of tube
1 and 2	2	0.00
3 and 4	2	3.00
5 and 6	2	10.00
7 and 8	2	40.00
9 and 10	2	150.00
11 and 12	2	400.00
13 and 14	2	Control 1, 7.3-12.3
15 and 16	2	Control 2, 87-131
17 and 18,onwards	2 each	patients

ASSAY PROCEDURE FOR AFP

Step 1 1st incubation	Step 2 Washing	Step 3 2nd incubation	Step 4 Washing and counting
To coated tubes 50 ul of calibrator /sample and 150 ul of phosphate buffer was added, mixed and incubated for 15 min at 18-25^{0}C with shaking at >280 rpm	The contents of the tubes were aspirated carefully, washed with 2 ml of wash solution and aspirated again	200 ul of tracer was added to all the tubes, mixed and incubated for 30 min at 18-25^{0}C with shaking at >280 rpm	The contents of the tubes were aspirated carefully, washed with 2 ml of wash solution and aspirated again. Bound cpm (B) and Total count (T) were counted for 1 min.

PRINCIPLE OF THE ASSAY FOR CEA

The immunoradiometric assay of Carcinoembryonic antigen (CEA) is a sandwich type assay in which two mouse monoclonal antibodies directed against two different epitopes of CEA molecule, and hence not competing are used. The samples or calibrators are incubated in tubes coated with the first monoclonal antibody in the presence of second monoclonal antibody labeled with iodine 125. After incubation, the contents of the tubes are aspirated and the tubes are rinsed so as to remove the unbound ^{125}I-labeled antibody. The bound radioactivity is determined in a gamma counter. The CEA concentrations in the samples are obtained by interpolation from the standard curve and are directly proportional to the radioactivity measured.

REAGENTS

No	Reagents	Dispensed as	condition
1	Anti CEA monoclonal antibody coated tubes	100 tubes	Ready to use
2	^{125}I- Labeled anti CEA antibody	01 vial of22 ml	Ready to use
3	Calibrators	05 vials of 0.5 ml vial, and 01 vial of 6 ml of "zero" calibrator.	Ready to use
4	Control Sera	02 vials	Lyophilized
5	Wash solution	01 vial of 50 ml	Diluted before use

EXPERIMENTAL DESIGN

Tube Number	No of tubes	Content of tube
1 and 2	2	0.00
3 and 4	2	1.00
5 and 6	2	5.00
7 and 8	2	20
9 and 10	2	100
11 and 12	2	400
13 and 14	2	Control 1 ,3.46-5.76
15 and 16	2	Control 2,17.8-27.8
17 and 18,onwards	2 each	patients

ASSAY PROCEDURE FOR CEA

Step 1 Additions	Step 2 Incubation	Step 3 Washing
To coated tubes -50 ul of calibrator/ sample/ Control was added. 200 ul of tracer Mixed	Incubated for 2 hours at 18-25^{0}C with shaking at >280 rpm aspirated again	The contents of the tubes were aspirated carefully, washed with 2 ml of wash solution. Bound cpm (B) and total cpm (T) were counted for 1 min

EXPECTED VALUES OF ASSAYS

No	CA-125	AFP	B-hCG	CEA	LDH
units	U/ml 95% population	Conc. Range (IU/ml)	(IU/ml)	(ng/ml)	U/L
female	30	0.82-9.12	<5	<5	120-320

STATISTICAL ANALYSIS

The data was subjected to Paired Student T-Test. All values are expressed as mean ± S.D, Limits of significance was set as $P<0.05$, the mean values were compared .Graphical representation has also been given with the results.

RESULTS

RESULTS

During this study 130 samples had been collected. A variety of different histological types of tumors were found, out of these 130 samples, 93 were of epithelial type. In the epithelial type there were 10 different sub-types of tumors. The first group, 1A comprises of these samples. 9 were found to be of germ cell tumor, which further had 5 different sub-types. The histological type of 28 samples was not reported in the history of the patient.

TYPES OF TUMORS

No	Epithelial tumors	No	Germ cell	No
1.	Serous carcinoma	12	Yolk sac tumor	03
2.	Adeno carcinoma	19	Immature Teratoma	02
3.	Mucinous cystadeno carcinoma	16	Dysgerminoma	02
4.	Papillary serous adenocarcinoma	12	Teratoma	01
5.	Cyst adenocarcinoma	06	Ana plastic dysgerminoma	01
6.	Papillary serous cystadenocarcinoma	10		
7.	Papillary adenocarcinoma	05		
8.	Papillary mucinous cystadeno carcinoma	02		
9.	Carcino Sarcoma	03		
10.	Endometroid carcinom	08		

In this study Patients have been divided into two main groups, one group consists of epithelial tumors and other both epithelial and germ cell tumors. All the histological types of epithelial are grouped together for convenience and all the histological types of germ cells are under the heading of germ cell tumors.

GROUP 1

43 patients were included in this group they had epithelial ovarian tumor and in these patients CA-125 was used as a diagnostic marker, CA-125 Tumor markers levels are taken at four different times pre chemotherapy, post chemotherapy, first follow up 0-6 months after chemotherapy, Last follow up 6-12 months after the end of treatment these 43 patients are further divided into 2 groups.

Group 1A. 10 patients are those who had surgery for removal of tumor were diagnosed in the first or second stage, chemotherapy after surgery. The pre chemotherapy levels of this group are within cut off level of 30 U/ml.

Group 1B.33 patients are those who did not have surgery and had elevated CA-125 pre Chemotherapy.

GROUP 2

A group of 23 patients having both type of tumors were included in this group the tumor markers used for diagnostic purposes were LDH, β-hCG , CEA and AFP.

CONTROLS

In this study we have taken 15 controls which have a mean CA-125 levels of 13.9,we have selected these patients which were not diagnosed for ovarian cancer they were randomly selected from the population

AGE FACTOR

The first factor that is studied is the age of the patients in relation to CA-125 levels recorded at different stages.

The patients have been divided into 5 age groups:

Age group 1 ranges from 19-28 yrs

Age group 2 ranges from 29-38 yrs.

Age group 3 ranges from 39-48 yrs.

Age group 4 ranges from 49-58 yrs.

Age group 5 ranges from 59-68 yrs the mean age in our study is 42.8 years .The median and mode of age is 45 years with a standard deviation of $\pm$11.85.

In Table 1, The mean ±SD values of CA-125 levels i.e. pre chemotherapy, post chemotherapy, first follow up and last follow up are compared with the different age group (group 1A patients) who had surgery for removal of tumor before chemotherapy. The mean value of CA-125 at the start of chemotherapy shows that although the patients have been given chemotherapy but their Tumor Marker levels were below normal. In the first age group the mean basal levels of CA-125 pre chemotherapy group was 17.40±4.38 where as the CA post chemotherapy levels was 9.42±0.91 and the First follow up mean was 8.26±0.65 and the last follow up mean was 9.70±3.67.Like wise the mean value of all age groups are given in table 1 at these four stages of chemotherapy. They were all within normal levels. Table-2 shows the comparison of Age group with means values of CA-125 levels at four stages (Group 1B) The mean value of CA-125 pre chemotherapy were ,in age group 1, 130.73±43.32, in the second age group 242.58±170.54,in the third age group 345.12±180.85 it further increases to 375.76±196.71 in the last age group the mean value slightly falls to 339.28±156.85 CA-125 post Chemotherapy mean values also decreases accordingly in the first age group the values comes back to normal 12.50±2.62 In the second age group mean values were also within normal limits that is 25.82±11.70 in the third age group it was 76.85±97.58 the fourth and fifth age group also shows rise in the post chemotherapy levels of CA-125 values were 138.91±199.01 and 190.70±163.06. The mean values of First Follow In the first age group up was 24.90±27.49 and it shows a increases in the second third, forth age groups values being 67.50±99.70, 101.93±154.75 and 235.87±239.12 respectively, the follow up value shows a decline in progress as the age of the patient increase and in the last age group (59-68yrs) the CA-125 first follow up shows a return of the disease. Last Follow up mean value showed a normal level in the first age group just on the border line of cut-off value 30.56±34.49, the remaining 4 age group did not show a disease free profile the values of CA-125 in the last follow up were continuously on the rise the last value in 5th age group being 380.0-7±205.04 which shows that there was no effect of chemotherapy on the patient. The first two age groups show a decline in this CA-125 post chemotherapy levels and they fall in normal range. The rest of age groups did show a decline in the CA-125 level after chemotherapy but they did not return to normal. The first follow up shows that in only the first age group (19-28 years) CA-125 returns to the normal value. In all other

age groups the chemotherapy did not show any positive response, as is clear from the tumor marker CA-125 levels at these stages.

The Over all response of age groups with CA-125 at different levels of Chemotherapy (Group 1B) are shown in Figure-2

Table-1

Table showing mean ± S.D of age groups with CA-125 levels at four stages. (Group 1A)

NO.	Age (years)		CA-125 pre-ch/th	CA-125 post-ch/th	First Follow up	Last Follow up
1	19-28	N	2	2	2	2
		Mean	17.4000	9.4500	8.2600	9.7000
		S. D.	±4.384	±0.919	±0.650	±3.676
2	29-38	N	4	4	4	4
		Mean	20.6500	15.8750	15.6500	14.8250
		S. D	±7.763	±5.916	±9.457	±10.334
3	39-48	N	3	3	3	3
		Mean	15.2167	8.6000	8.9633	13.6000
		S. D	±9.313	±4.838	±3.940	±6.986
4	49-58	N	1	1	1	1
		Mean	15.5000	12.7000	9.4000	9.4700
		S. D	.	.	.	.
	Total	N	10	10	10	10
		Mean	17.8550	12.0900	11.5410	12.8970
		S. D	±6.925	±5.383	±6.778	±7.303

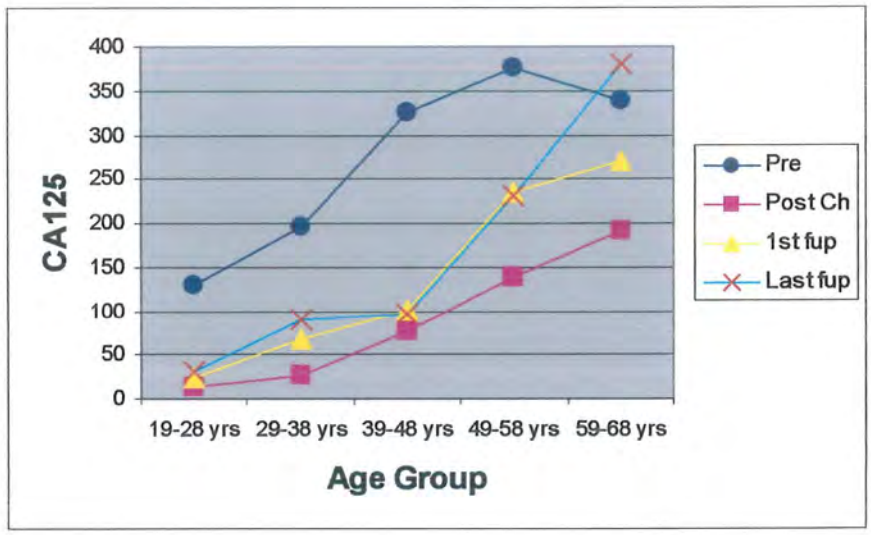
Table-2

Table showing, mean ±S.D age groups and CA-125 levels at four stages. (Group1B)

NO.	Age (years)		CA-125 pre-ch/th	CA-125 post-ch/th	First Follow up	Last Follow up
1	19-28	N	3	3	3	3
		Mean	130.733	12.5000	24.9000	30.5667
		S. D	±43.326	±2.622	±27.497	±34.492
2	29-38	N	3	3	3	3
		Mean	242.533	26.1333	85.4667	102.566
		S. D	±170.545	±14.321	±113.911	±138.42
3	39-48	N	12	12	12	12
		Mean	345.1275	82.2167	109.9000	103.265
		S. D	±180.855	±99.901	±158.823	±101.09
4	49-58	N	6	6	6	6
		Mean	375.766	138.9117	235.878	231.700
		S. D	±196.718	±199.018	±239.124	±230.95
5	59-68	N	7	7	7	7
		Mean	339.285	190.7029	270.271	380.071
		S. D	±156.853	±163.06	±151.239	±205.04
Total		N	31	31	31	31
		Mean	319.062	105.5126	159.905	183.525
		S.D	±174.843	±138.643	±177.884	±193.52

Figure-2

Over all response of age groups with CA-125 at different levels of Chemotherapy (Group 1B)



STAGE

The second variable of our study is the stage of the tumor. There are 4 stages of ovarian tumor.

STAGE 1: localized disease limited to the ovary only.

STAGE 2: Tumor is extended to the lymph nodes.

STAGE 3: Extensive involvement where the tissues are involved

STAGE 4:Tumor has Metastasized and spread to other organs.

In Table 3 the stage of the Tumor markers are compared with the means of CA-125 pre, post chemotherapy 1st follow up last follow of group 1A.It was observed, that the group of patients which had surgery before chemotherapy, the tumor markers levels of CA-125 pre Chemotherapy, post chemotherapy levels the first and last follow up shows good response and the mean values in different age group falls within normal limit. The first age group mean value are16.67±7.66 rising slightly in the second age group to 21.40±6.27 and in the third age group it was 14.30±0.00 because here the bulk was removed by surgery and CA-125 was not released as a tumor marker by the tumor cells. The values remain within the cut-off value, and they did not show any signs of recurrence, complete recovery is observed. Further more normal values in the last follow up is an indicator of good prognosis. The levels did not rise beyond normal value of 30 U/ml in all cases, even in the fourth stage CA-125 remains within normal limits.

In Table 4 the stage of the Tumor markers are compared with the means of CA-125 pre, post chemotherapy 1st follow up last follow of group 1B The first stage-localized disease shows CA-125 pre chemotherapy of 386.315±123.05 and the mean values of last follow up was 6.10±3.83 indicating a very positive result. In the Stage 2 of the tumor the pre chemotherapy levels were also high 186.02±135.18 which did fall to 23.65±19.48 but again shows a rise in the first and last follow up with the mean values of 37.66±64.99 and 56.74±77.25.The Tumor marker levels in the third stage of the disease makes it even more clear that the patient is not responding to chemotherapy and Tumor marker levels are not dropping significantly, the mean value at last follow up was 222.26±209.88. Finally in the fourth stage (Metastasis) CA-125 pre chemotherapy were 161.37±152.70 kept rising up till the last follow up to 277.57±198.54 Figure 3 Showing the relation between stage of tumor, age groups and CA-125 levels

In the first and second age group the only 1st and 2nd stage of tumor is more common no 3rd stage or 4th stage of tumor is found. In the third age group all 4 stages of tumor are found. The 1st stage is in the highest %age because the number of patients is highest in this age group and the median age of patients in our study falls in this age group. In the fourth age group onwards the 1st stage of tumor is not found, 2nd stage showed a very high %age and 3rd and 4th stage shows equal presence. In the fifth age group only 2nd stage is found but in very low % age and 4th stage is shows highest abundance. In the last age group only patients are found in fourth stage. The values clearly indicate that the stage of the tumor is in direct relation to age. As the age of the patients increases the stage increases. Figure 4 showing the Overall response of age groups with stage with CA-125 levels (Group 1A).

Table-3

Table showing mean ±S.D 4 stages of tumor, CA-125 pre, post chemotherapy 1st follow up last follow up (Group 1A)

Stage		CA-125 pre ch/th	CA-125 post ch/th	First follow up	Last follow up
1	N	6	6	6	6
Localized	Mean	16.6750	9.9333	12.4350	15.4500
disease	S.D	±7.668	±4.998	±8.820	±8.577
2	N	3	3	3	3
Nodal	Mean	21.4000	17.0667	11.0000	9.7233
involvement	S.D	±6.279	±3.855	±1.552	±2.161
3	N	1	1	1	1
Extensive	Mean	14.3000	10.1000	7.8000	7.1000
disease	S.D	.	.	.	.
	N	10	10	10	10
Total	Mean	17.8550	12.0900	11.5410	12.8970
	S.D	±6.925	±5.383	±6.778	±7.303

Table-4

Table showing mean ±S.D, 4 stages of tumor, CA-125 pre, post chemotherapy, 1st follow up and last follow up (Group 1B)

Stage		CA-125 pre-ch/th	CA-125 post-ch/th	First Follow up	Last Follow up
1.00	N	2	2	2	2
	Mean	386.315	12.3000	11.7500	6.4000
	S. D	±123.057	±14.28356	±12.940	±5.37401
2.00	N	9	9	9	9
	Mean	186.0222	23.5211	40.3411	56.9111
	S. D	±135.189	±20.66687	±68.354	±81.9362
3.00	N	7	7	7	7
	Mean	349.0143	133.8143	211.571	222.2686
	S. D	±207.483	±167.9032	±229.77	±209.882
4.00	N	13	13	13	13
	Mean	384.6923	161.3769	237.653	277.5700
	S. D	±149.1204	±152.7062	±164.60	±198.5418
Total	N	31	31	31	31
	Mean	319.0623	105.5126	159.905	183.5255
	S. D	±174.8437	±138.6432	±177.88	±193.5268

Figure-3

Stage of tumor vs. age groups and CA-125 levels

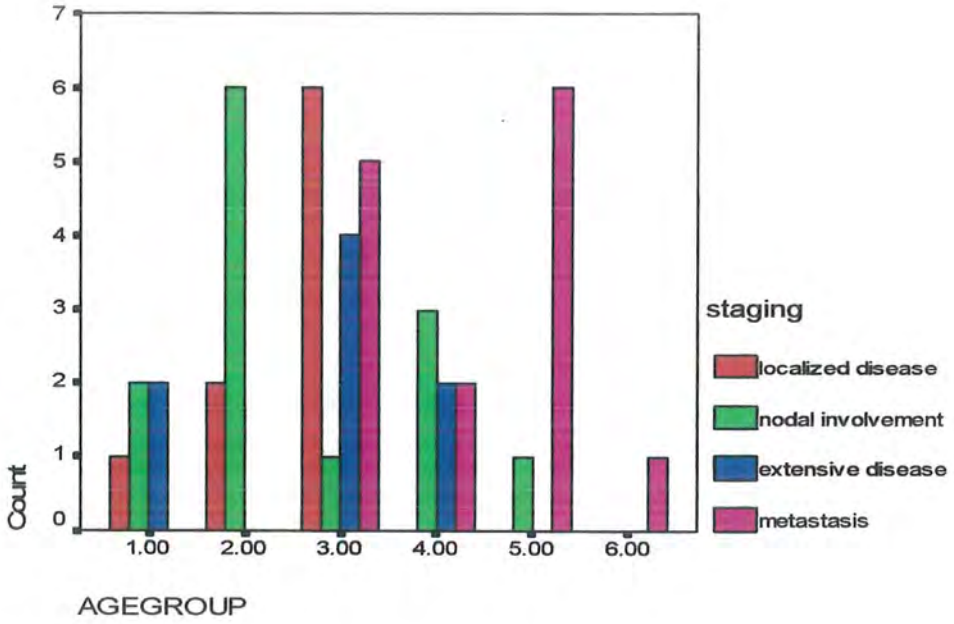


Figure-4

Overall response of age groups with stage with CA-125 levels (Group 1A)

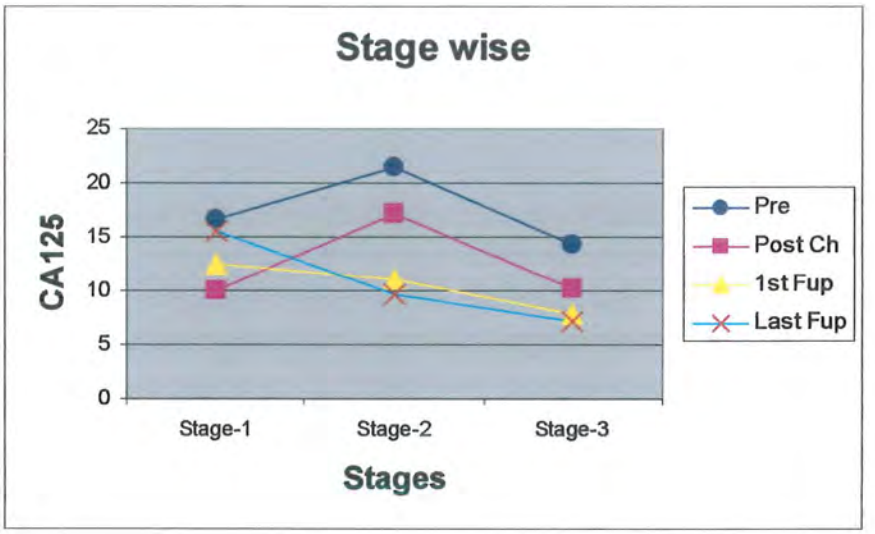


Table-5 shows the Paired Samples Statistics (Group 1A) where stage is compared to the means of CA-125 at 4 stages of chemotherapy

The mean values of the each two pairs are given which were used in Paired student T-Test to check the significance of our data. All the mean values are within normal limits. Results of the Paired student T- Test are given in Table 6 to check the significance of our data .It has highly significant value for all four pairs.

Stage and CA-125 pre Chemotherapy levels the P value is 0.000

Stage and CA-125 post Chemotherapy p value is 0.000

Stage and First follow up the p value is 0.001

Stage and Last follow up P=0.001

.Table 7 shows the Paired Samples Statistics (Group 1A) where age is compared to the means of CA-125 at 4 stages of chemotherapy.

Table 8 Gives the results of Paired Student T-test all the values of p were found highly significant P=0.000, 0.000, 0.002 and 0.001 between Age and CA-125 pre Chemotherapy CA-125 post Chemotherapy, first follow up and last follow up.

Table-5

Paired Samples Statistics (Group1A) stage vs. CA-125 levels

		Mean	N	S. D	Std. Error Mean
Pair 1	Staging	1.5000	10	±0.707	.22361
	CA-125 pre chemotherapy	17.8550	10	±6.925	2.18988
Pair 2	Staging	1.5000	10	±0.707	.22361
	CA-125 post chemotherapy	12.0900	10	±5.383	1.70238
Pair 3	Staging	1.5000	10	±0.707	.22361
	1st follow up	11.5410	10	±6.778	2.14350
Pair 4	Staging	1.5000	10	±0.707	.22361
	Last follow up	12.8970	10	±7.303	2.30965

Table-6

Paired Samples Test (group IA) stage vs. CA-125

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	S. D	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Staging - CA-125 pre chemotherapy	-16.355	±6.904	2.18340	-21.294	-11.415	-7.491	9	0.000
Pair 2	Staging - CA-125 post chemotherapy	-10.590	±5.200	1.64462	-14.310	-6.86962	-6.439	9	0.000
Pair 3	Staging - 1st follow up	-10.041	±6.961	2.20157	-15.021	-5.06069	-4.561	9	0.001
Pair 4	Staging - last follow up	-11.397	±7.650	2.41945	-16.870	-5.92383	-4.711	9	0.001

Table-7

Paired Samples Statistics (group 1A) age group vs. CA-125

		Mean	N	S. D	Std. Error Mean
Pair 1	Age group	2.3000	10	±0.948	0.30000
	CA-125 pre chemotherapy	17.8550	10	±6.925	2.18988
Pair 2	Age group	2.3000	10	±0.948	0.30000
	CA-125 post chemotherapy	12.0900	10	±5.383	1.70238
Pair 3	Age group	2.3000	10	±0.948	0.30000
	1st follow up	11.5410	10	±6.778	2.14350
Pair 4	Age group	2.3000	10	±0.948	0.30000
	Last follow up	12.8970	10	±7.303	2.30965

Table-8

Paired Samples Test (group 1A) age vs. CA-125

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	S. D	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Age group - CA-125 pre chemotherapy	-15.555	±7.173	2.26846	-20.6866	-10.4233	-6.857	9	0.000
Pair 2	Age group - CA-125 post chemotherapy	-9.790	±5.546	1.75394	-13.7577	-5.82230	-5.582	9	0.000
Pair 3	Age group - 1st follow up	-9.2410	±6.932	2.19221	-14.2001	-4.28188	-4.215	9	0.002
Pair 4	Age group - last follow up	-10.597	±7.340	2.32118	-15.847	-5.34614	-4.565	9	0.001

Table-9 gives the Paired Samples Statistics (Group 1B)Stage vs.. CA-125

It shows that the mean value of CA-125 pre chemotherapy is very high as compared to group 1A, which had surgery before chemotherapy the difference is clear between the two groups.In Table 10 Paired Samples Test (Group 1B) shows significant values for the means between

Stage andCA-125 pre chemotherapy levels p=0.000

Stage and CA-125 post chemotherapy levels p=0.000,

Stage and1st follow up p=0.000.

Stage and last follow up P value of 0.000

The p values here shows highly significant relation between stage of the tumor and the Tumor Marker values at different times at and after treatment.

In Table 11 paired sample statistics (group 1B) age vs.. CA-125 levels are given

Table 12 paired samples tests group 1B age vs.. CA-125 all the CA-125 levels at pre chemotherapy, post chemotherapy, first and Last follow up show that their relation with age plays a highly significance role in monitoring the response to treatment, The p values are indicative of this fact they all are =0.000.

Table-9

Paired Samples Statistics (Group 1B) Stage vs. CA-125

		Mean	N	S. D	Std. Error Mean
Pair 1	Staging	2.9091	33	±1.041	0.181
	CA-125 pre chemotherapy	303.6403	33	±180.182	31.365
Pair 2	Staging	2.9091	33	±1.041	0.181
	CA-125 post chemotherapy	100.2512	33	±135.887	23.655
Pair 3	Staging	2.9091	33	±1.041	0.181
	1st follow up	150.8173	33	±176.029	30.642
Pair 4	Staging	2.9091	33	±1.041	0.181
	Last follow up	174.2421	33	±191.123	33.270

Table-10

Paired Samples Test (Group 1B) Stage vs. CA-125

		Paired Differences					t	df	Sig. 2-tailed
		Mean	S. D	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Staging - CA-125 pre ch/th	-300.731	±179.753	31.29	-364.46	-236.99	-9.611	32	0.000
Pair 2	Staging - CA-125 post ch/th	-97.342	±135.410	23.57	-145.35	-49.327	-4.130	32	0.000
Pair 3	Staging - 1st follow up	-147.908	±175.480	30.54	-210.13	-85.685	-4.842	32	0.000
Pair 4	Staging - last follow up	-171.333	±190.548	33.17	-238.89	-103.76	-5.165	32	0.000

Table-11

Paired Samples Statistics Group 1B Age vs. CA-125

		Mean	N	S. D	Std. Error Mean
Pair 1	Age group	3.303	33	±1.211	0.2109
	CA-125 pre chemotherapy	303.6403	33	±180.182	31.36576
Pair 2	Age group	3.303	33	±1.211	0.2109
	CA-125 post chemotherapy	100.2512	33	±135.887	23.65501
Pair 3	Age group	3.303	33	±1.211	0.2109
	1st follow up	150.8173	33	±176.029	30.64274
Pair 4	Age group	3.303	33	±1.211	0.2109
	Last follow up	174.2421	33	±191.123	33.27025

Table-12

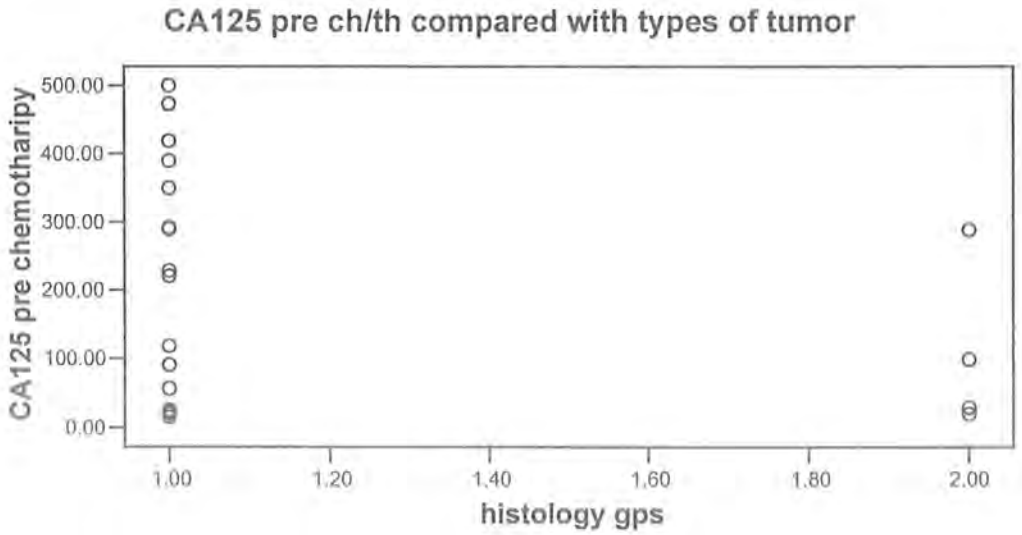
Paired Samples Test Group 1B Age vs. CA-125

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	S. D	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Age group CA-125 pre ch/th	-300.337	±179.769	31.293	-364.080	-236.593	-9.597	32	0.000
Pair 2	Age group CA-125 post ch/th	-96.948	±135.357	23.562	-144.944	-48.952	-4.114	32	0.000
Pair 3	Age group 1st follow up	-147.514	±175.451	30.542	-209.726	-85.301	-4.830	32	0.000
Pair 4	Age group last follow up	-170.939	±190.402	33.144	-238.452	-103.425	-5.157	32	0.000

GROUP 2

The comparison of all the different Tumor markers with the types of tumor (epithelial and germ cell) are given in Figures 5-9

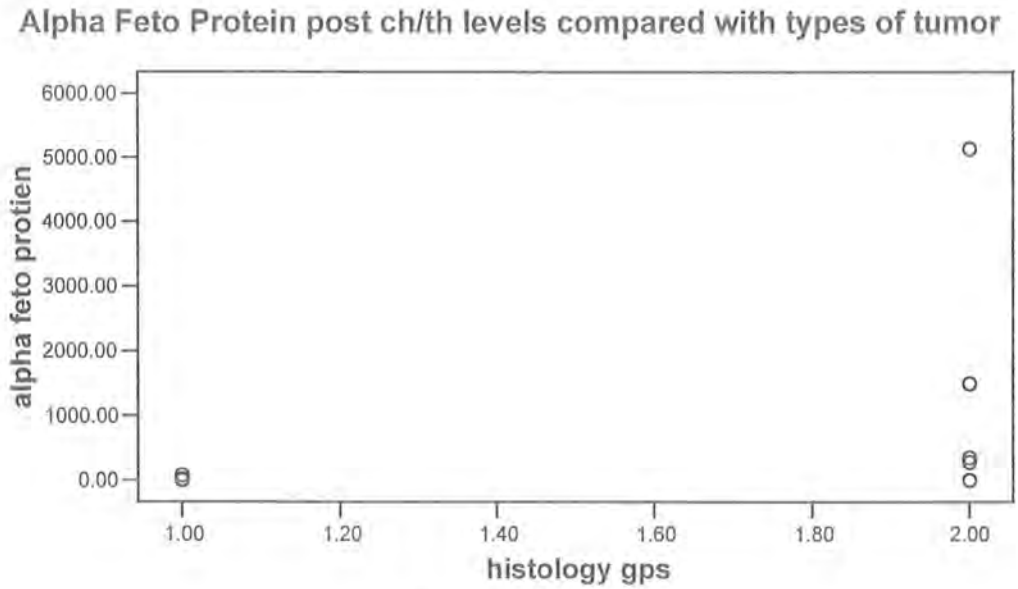
Figure 5



Type 1=epithelial

Type 2=germ cell

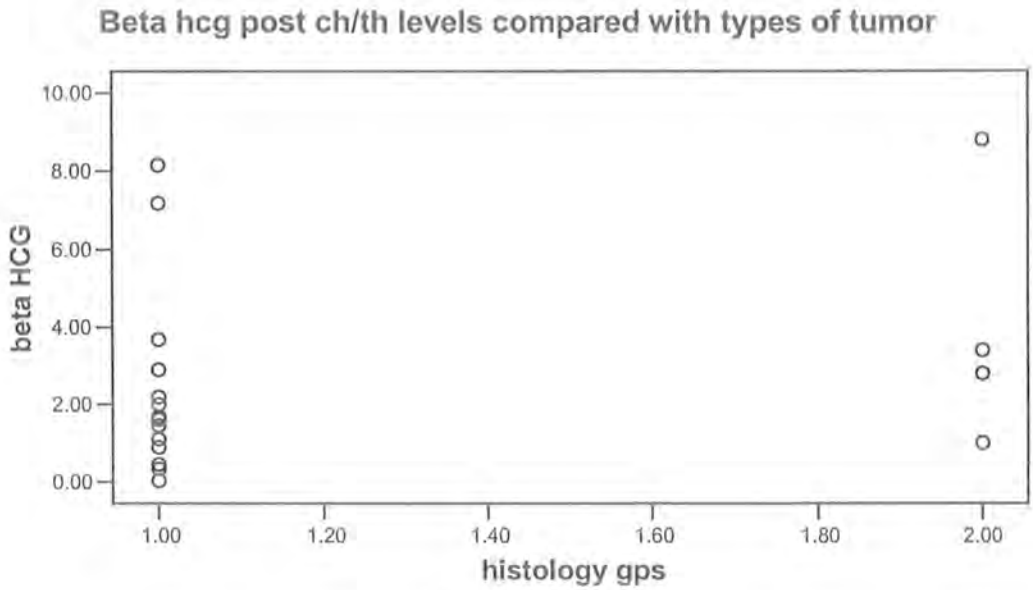
Figure 6



Type 1=epithelial

Type 2=germ cell

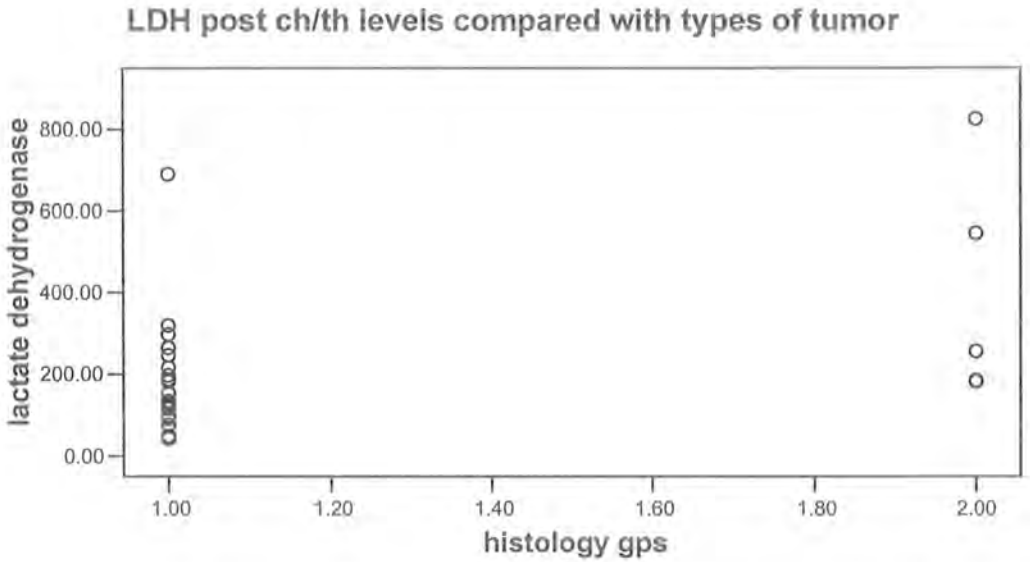
Figure 7



Type 1=epithelial

Type 2=germ cell

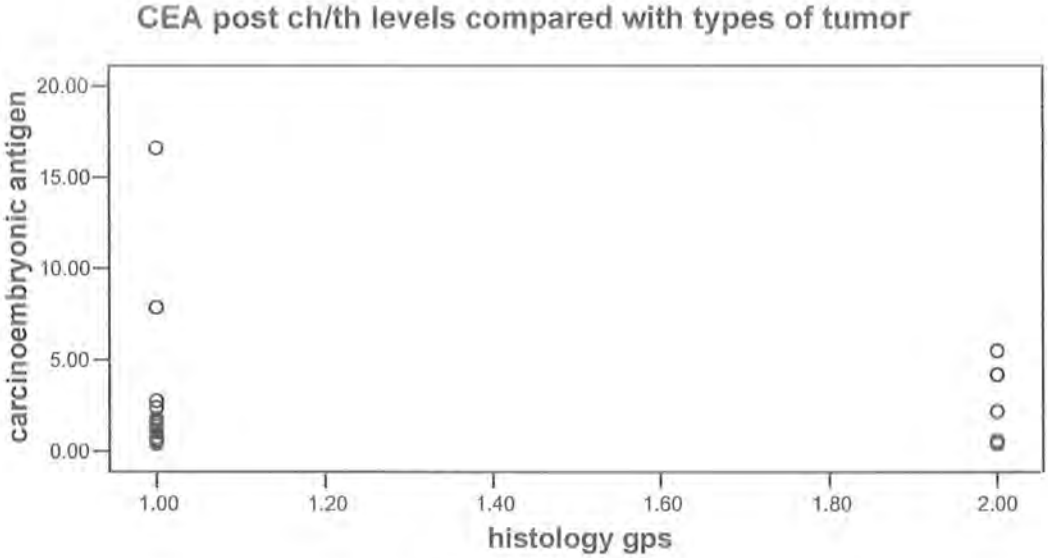
Figure 8



Type 1=epithelial

Type 2=germ cell

Figure 9



Type 1=epithelial

Type 2=germ cell

Table 13 shows the independent sample T-TEST Group Statistics of AFP, LDH, β- hCG and CEA with type of tumor marker. The mean value of the germ cell tumor markers is checked against the histology of the tumor. Mean value of all the tumor markers taken before chemotherapy were given for the two types of tumor with standard deviation. CA-125 shows high means only in epithelial type of tumors AFP shows a mean value of only 8.37 ± 17.25(cut-off value of AFP=0.82-9.12 IU/ml) for Epithelial type of tumor where as it was 1453.06 ± 2136.11 in case of germ cell tumor a very significant difference making AFP a suitable marker for the diagnosis and response of germ cell tumor. The beta hCG does not show any difference in the two types of tumor 2.29±2.08 for Epithelial Tumor and 3.4±3.2 for Germ Cell Tumor, hardly rising above its normal value in either case (<5IU/ml). it was not a very good marker for following a germ cell tumor response because β- hCG is also found high in many other conditions (Understanding Tumor Markers-Grades/Prognosis)

LDH shows a mean value of 189.85±145.09 for Epithelial Tumor and elevated mean value of 397.92±281.71 for germ cell tumor. (Normal range 120-240,and up to 320 in some cases) CEA also is of no real value to distinguish between two types of tumor. CA-125 again shows a drastic difference for Epithelial Tumor the value was 240.55±186.41 and for germ cell tumor it was 93.25±113.87.once again it becomes clear that AFP, LDH and CA-125 can be used as tumor markers for monitoring the response of germ cell tumors. Table 14 shows Independent Samples Test for CA-125, LDH, AFP, β-hCG and CEA.In the independent sample test significant P values are observed for AFP and LDH and CA-125 levels the pre chemotherapy levels are significant they are 0.050 for CA-125, 0.005 for AFP, 0.030 for LDH these two markers AFP and LDH are helpful in diagnosis of germ cell tumor from those of epithelial. CA-125 also can be relied upon in both cases it has a P value just on the borderline of both probabilities.

Table- 13

T-TEST Group Statistics

	Histology groups	N	Mean	S.D	Std. Error Mean
AFP	1.00	19	8.3737	±17.252	3.957
	2.00	5	1453.06	±36.114	955.299
β-hCG	1.00	19	2.296	±2.085	0.478
	2.00	5	3.400	±3.203	1.432
LDH	1.00	19	189.857	±145.096	33.287
	2.00	5	397.920	±281.710	125.984
CEA	1.00	19	2.474	±3.786	0.868
	2.00	5	2.580	±2.232	0.998
CA-125	1.00	19	240.559	±186.412	42.766
	2.00	5	93.258	±113.871	50.924

Histology group1-epithelial tumor
Histology group2-Germcell tumor

Table-14
Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
AFP	Equal variances assumed	26.82	0.000	-3.15	22	0.005	-1444.68	457.87	-2394.26	-495.103
	Equal variances not assumed			-1.51	4.0	0.205	-1444.68	955.30	-4097.00	1207.63
β-hCG	Equal variances assumed	0.947	0.341	-.942	22	0.356	-1.103	1.170	-3.531	1.324
	Equal variances not assumed			-0.73	4.9	0.498	-1.103	1.510	-5.002	2.796
LDH	Equal variances assumed	6.048	0.022	-2.32	22	0.030	-208.062	89.425	-393.518	-22.605
	Equal variances not assumed			-1.59	4.5	0.177	-208.062	130.30	-552.658	136.534
CEA	Equal variances assumed	0.035	0.853	-0.05	22	0.953	-0.105	1.786	-3.811	3.599
	Equal variances not assumed			-0.08	10.9	0.938	-0.105	1.323	-3.019	2.808
CA-125	Equal variances assumed	4.262	0.051	1.67	22	0.109	147.301	88.194	-35.603	330.206
	Equal variances not assumed			2.21	10.4	0.050	147.301	66.500	0.0337	294.569

Mean Values of each tumor markers are also compared with the age groups.

Figure 10 gives the CA-125 pre chemotherapy levels compared with different age groups.

Figure 11 gives the AFP pre chemotherapy levels compared with different age groups.

Figure 12 gives theβ-hCG pre chemotherapy levels compared with different age groups.

Figure 13 gives the LDH pre chemotherapy levels compared with different age groups.

Figure 14 gives the CEA pre chemotherapy levels compared with different age groups

Figure -10

CA-125 pre chemotherapy levels compared with different age groups

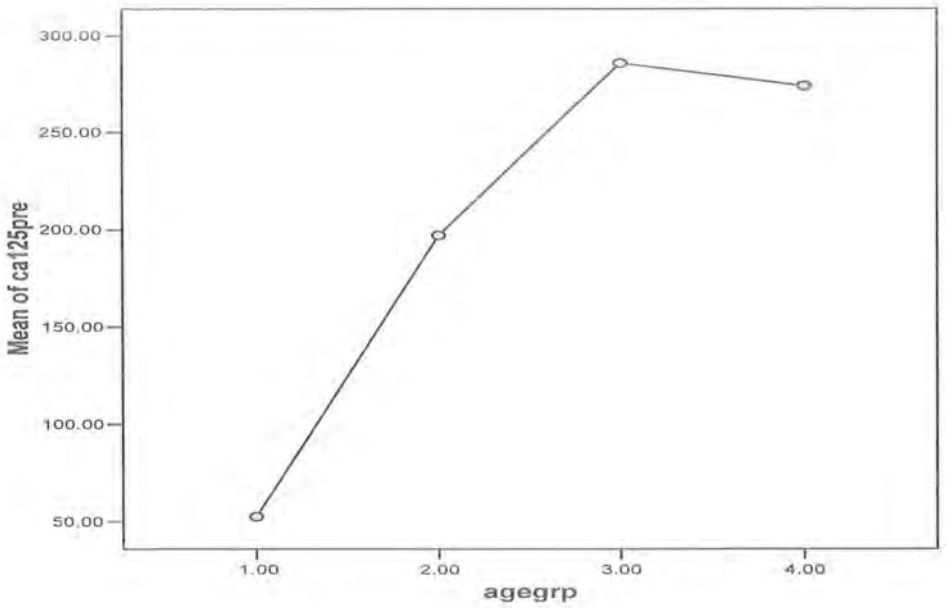


Figure -11

AFP pre chemotherapy levels compared with different age groups

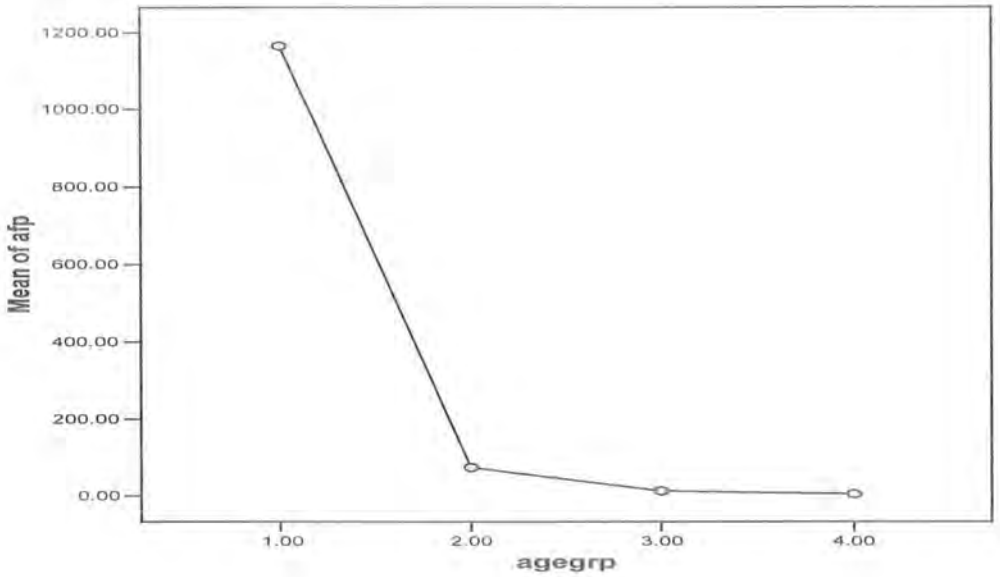


Figure- 12

β- hCG pre chemotherapy levels compared with different age groups

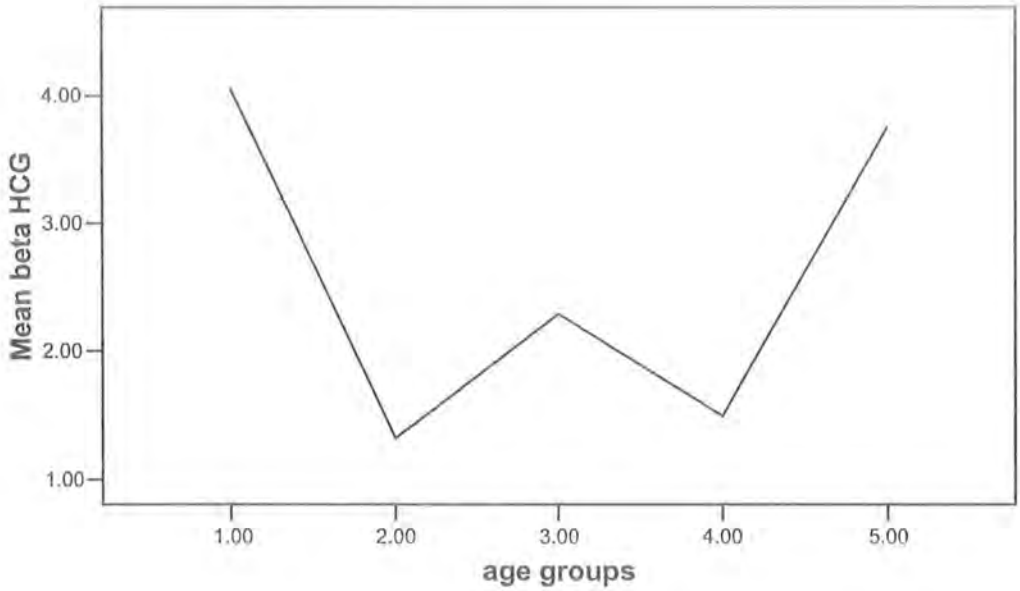


Figure-13

LDH pre chemotherapy levels compared with different age group

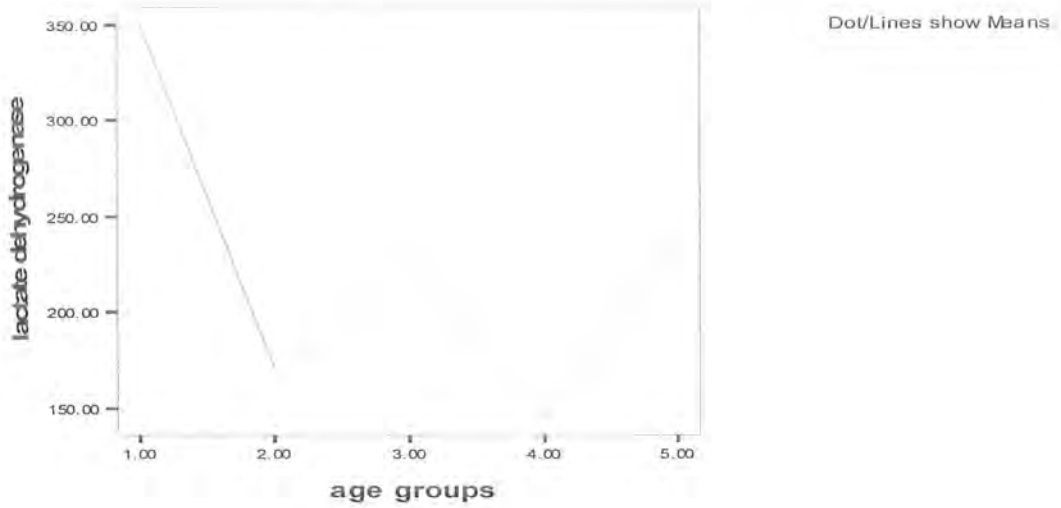


Figure -14

CEA pre chemotherapy levels compared with different age group

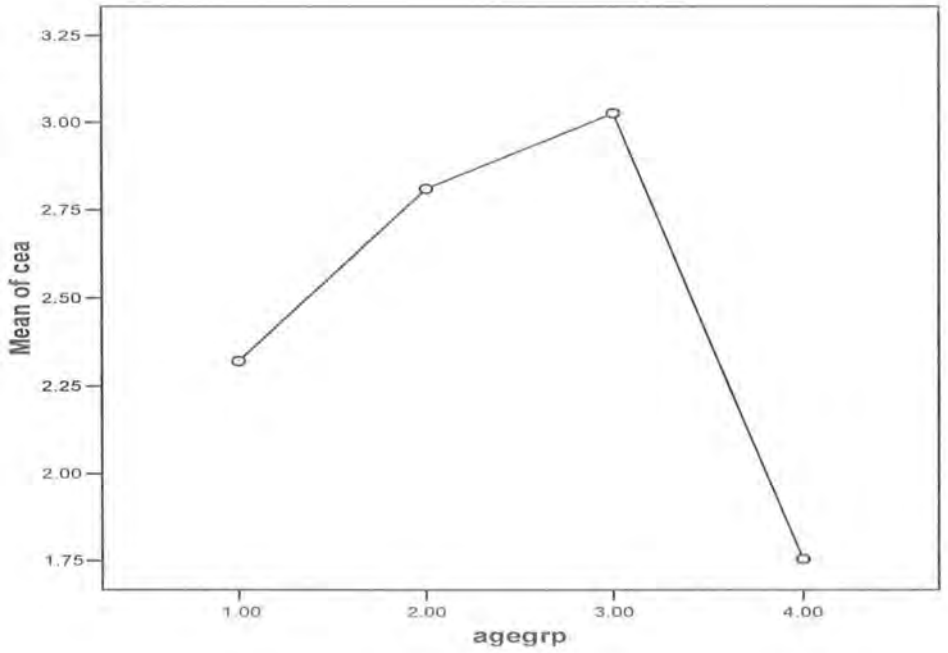


Table-15 Age compared with post chemotherapy levels of all tumor markers.The means of different TM compared with the age groups show that Germ cell Tm shows a high mean value in younger age groups as the germ cells tumor is more common in young girls and teen ages, the mean value of AFP was189.98±216.51 in the youngest age group and it keeps falling till the last age group to 4.63±1.83 .Beta hCG does not show any particular pattern .LDH shows the same trend as AFP showing high mean values in first age group 351.16±275.91 and the last age group has a mean value of 236.30±116.95 ,the last but not the least TM CEA shows no ascending pattern with age groups making it unreliable as a diagnostic marker all the values are tabulated in Table 15. CA-125 as usual shows low value where the AFP and LDH are showing a high value when the age is increasing and epithelial type of tumor are increasing in number.

Table 16 shows the Stage of the tumor compared with post chemotherapy levels of all tumor markers. The stage of the Tumor is also compared with the response of all these Tumor Markers Except for CA-125 which gives low mean value in Stage 1 and 2, 12.14±9.34 and 27.62±20.78 and high values in stage 3 tumor 114.04±131.28 and in the 4th stage it was 88.58±103.18 the reason for low mean in 4^{th} stage is that not many patients sample could be collected in the fourth stage as many patients expired .AFP shows the same pattern as CA-125 with a mean value of 15.63±30.76 rising through the second and third stage with mean value of 916.78±2070.63 and 256.55±557.93 to 6.80±4.30. β-hCG gives the same result as in the comparison with age no significant pattern seen and LDH follows AFP in its response curve all the mean values are given in Table 16

Table-15

Age compared with post chemotherapy levels of all tumor markers

Age groups		CA-125	AFP	β -hCG	LDH	CEA
1.00	N	6	6	6	6	6
	Mean	17.40	189.981	4.0583	351.166	2.3217
	S. D	±8.498	±216.511	±3.538	±275.91	±2.08543
2.00	N	4	4	4	4	4
	Mean	85.70	73.922	1.320	170.975	2.8100
	S. D	±129.566	±137.449	±1.30	±105.92	±3.41948
3.00	N	8	8	8	8	8
	Mean	99.212	12.953	2.205	228.237	3.0263
	S. D	±114.280	±26.571	±0.732	±191.061	±5.50605
4.00	N	4	4	4	4	4
	Mean	13.257	5.480	1.4650	126.875	1.6900
	S. D	±4.054	±4.508	±0.58757	±115.224	±0.88325
5.00	N	2	2	2	2	2
	Mean	92.9500	4.635	3.7550	236.3000	1.8850
	S. D	±119.288	±1.831	±4.84368	±116.955	±0.72832
Total	N	24	24	24	24	24
	Mean	61.659	65.433	2.5267	233.2042	2.4962
	S. D	±91.627	±137.245	±2.32358	±194.237	±3.47714

Table-16

Stage compared with post chemotherapy levels of all tumor markers

staging		CA-125	AFP	β-hCG	CEA	LDH
Localized disease	N	6	6	6	6	6
	Mean	12.1450	15.6350	1.7600	1.8500	274.1500
	S. D	±9.34101	±30.76016	±1.08630	±1.22608	±220.1539
Nodal involvement	N	6	6	6	6	6
	Mean	27.6267	916.7867	2.7083	3.1800	264.3667
	S. D	±20.78046	±2070.632	±3.10651	±2.86747	±282.4118
Extensive disease	N	7	7	7	7	7
	Mean	114.0429	256.5529	2.6914	2.9914	205.3571
	S. D	±131.2897	±557.9303	2.49493	±6.01377	±160.0982
Metastasis	N	5	5	5	5	5
	Mean	88.5800	6.8000	2.9980	1.7580	185.6600
	S. D	103.18666	4.30042	2.62925	.82284	100.28065
Total	N	24	24	24	24	24
	Mean	61.6596	309.3500	2.5267	2.4963	233.2042
	S. D	±91.62745	±1073.774	±2.32358	±3.47714	±194.2377

DISCUSSION

DISCUSSION

CA-125 is the most reliable marker in the diagnosis and observing the response to treatment and follow up of ovarian cancer (Karem *et al* 1997). The correlation of CA-125 levels and removal/reduction of tumor bulk, process of cancer disease and histopathological types of cancer were studied. Data showed that the reduction of tumor mass decreases marker level in the serum. The low level of CA-125 in postoperative patients indicates that the tumor is removed; tumor marker is not produced and not found in the sample. The marker level is the highest in the tumor and decreases in the peritoneal fluid and serum which proves that the marker is produced by tumor cells (Markowska and Wilkoszarska 1996) CA-125 tests for stage 2, 3 and 4 ovarian cancer patients during chemotherapy helps to determine the activity of the cancer, and the effect of chemotherapy on cancer. The levels of CA-125 rise when the disease is at a higher stage and with the increase in age. Although patients who have surgery before chemotherapy showed a better results and prognosis, This study confirms that those patients in which the tumor marker level falls within normal levels and showed no signs of disease even after one year. It was also observed that is that early diagnosis at stage 1 or 2 shows better response in post chemotherapy CA-125 levels and better chances of survival. The patients who have had tumor removed before chemotherapy showed disease free profile and the mean values of CA-125 post chemotherapy level further goes down and remain stable in the follow up . Initial surgical staging procedures consisting of radical hysterectomy achieved excellent survival and minimal morbidity in stage 2 cancer (Ayhan *et al*, 2004) CA-125 does not appear to be an independent risk factor for survival but in a study of stage 1 patients (Nagele *et al* 1995) it was concluded that the preoperative CA-125 level was the most powerful prognostic factor for survival.

The same pattern is observed in patients who did not have surgery; pre chemotherapy levels of CA-125 were elevated well beyond the cut-off value (30 U/ml). The post chemotherapy levels also show an increasing pattern. The first age groups show a decline in CA-125 post chemotherapy levels but they did return to normal range. In the age groups 2, 3, 4 and 5, CA-125 did not return to normal. The response was not good because in these age groups women One reason for poor response to treatment in older

age groups is that women around 35-40 years are near menopause (although it varies for each individual case), body is under going hormonal changes and ovaries are no longer the normal ovaries they are also undergoing changes that is why new cells are not being produced. Ovaries are not functioning properly and degeneration process has started in the ovaries as well as other organs the response to treatment was not as good as in pre-menopausal age group 1. The post chemotherapy levels of CA-125, first and the last follow up did not show any positive response. CA-125 gives reliable prediction of progressive disease during postoperative chemotherapy (Tuxen *et al* 2001).

The relation between age groups and CA-125 levels measured at different stages of treatment showed that age plays a major role in response to treatment. ($p<0.002$)Younger patients are more receptive to chemotherapy and recurrences are low. As the age of the patient increases CA-125 levels did not come back to normal and usually the disease extends to 3rd or 4th stage. Age specific survival varied from a high of 90% at age 40 to a low of 55% at age 80, survival decreases at age 50. (Farley *et al* 2000)

The stage of the tumor also plays a very pivotal role in expressing the response to Chemotherapy In the group of 10 patients who have surgery before chemotherapy, showed good response to Chemotherapy because CA-125 is no longer being released and tumor growth has also stopped. And no recurrence is found till the end of this study period normal values were found in the last follow up which is an indicator of good prognosis. Ayhan *et al* 2005 stated that age and disease grade were significant factors for assessing metastasis both in univariate and multivariate analysis. Rates of long-term survival among patients with early-stage disease (stage I or II) can be as high as 80 to 95 percent, whereas patients with advanced disease (stage III or IV) have lower survival rates 10 to 30 percent. (Stephen 2005) The stage of the tumor is also reflected in the CA-125 levels taken at different times after chemotherapy has been given, The higher the stage of the tumor the lesser is the probability of the tumor marker level coming back to normal and when such patients are checked by other clinical methods it was found that the tumor has spread since diagnosis.

There was a higher percentage of borderline significance of women from the study group that developed ovarian cancer between 39 and 48 years of age. In the study group, ovarian cancer was significantly more often found at Stage I, although the groups did not

differ in detection procedures.Paired student T- Test is applied to see the significance of and stage on response to Chemotherapy (P=0.000, at all 4 times). This shows a very significant relation of stage to chemotherapy. Because the tumor marker level present in the serum is a measure of the stage of the tumor in the body and its response to treatment. Paired Student T- Test also gave statistically significant values when it was applied to age factor and CA-125 levels taken at specific intervals after chemotherapy (P <0.002).

When age is compared to the means of different tumor markers, it is seen that since germ cell is more common in younger ages, germ cell tumor marker also showed high level in this young patients. In germ cell tumors stage and tumor marker levels shows no particular relation. The germ cell tumors are 5-10% of total percentage of the tumors of the ovary, in this study only 9 germ cells cases were found out of which 4 cases had to be dropped as their sample could not be collected, only 5 samples are included in the group 2 of our study. The level of the four tumor markers AFP, LDH, CEA and β-hCG were taken after Chemotherapy. Independent sample test applied to this group show significance in case of AFP and LDH in the treatment and follow up of germ cell tumors. An elevated level of AFP strongly suggests the presence of either primary liver cancer or germ cell cancer. (Understanding Tumor Markers-Grades/Prognosis) Ovarian dysgerminomas with elevated serum LDH levels have been reported (Yoshimura *et al* 1988).Beta subunit of human chorionic gonadotrophin (β-hCG) is useful as markers of early stage disease and as monitors of therapy in germ cell tumors of the ovary, but these tumors only comprise a small proportion of ovarian malignant disease (Gadduci *et al* 2004). β-hCG levels did not show any difference in the two types of tumors. CEA is positive in epithelial cancer of the ovary it did not show elevated values in germ cell tumors in this study. CA-125 is also valuable in germ cell tumors of the ovary (Altaras *et al* 1986).

Although the serum CA-125 level is elevated in more than 80 percent of patients with advanced epithelial ovarian cancer, this measurement alone is neither sufficiently sensitive nor specific enough to be diagnostic. Sometimes abnormal levels of CA-125 before treatment, returned to normal range after treatment and an increase in the other tumor markers was observed with a relapse of disease. In the absence of an increase in

CA-125 this suggest that in addition to CA- 125, a combination assay of several tumor markers is necessary for monitoring and treatment of ovarian cancer. Tumor markers are specific to histological type of the tumor. The level of CA-125 with the combination of other Tumor Markers like β-hCG, LDH and Alpha Feto protein in all the samples showed that CEA is also positive in epithelial cancer, AFP is positive in endodermal sinus tumor, LDH is positive in Dysgerminoma and Solid germ cell tumor of the ovary. β-hCG is also found elevated in choriocarcinoma (a rare cancer of the uterus) and other germ cell tumors of the ovary (Konishi *et al* 1986).

While a basal level of circulating CA-125 may be expected, there exist many conditions in which the level of the antigen may be elevated. These conditions can be classified into gynecological and non- gynecological types, including cirrhosis of liver and tuberculosis. Cancers of the pancreas, breast, colon and Lung also expresses higher CA-125 levels. Elevated serum CA-125 levels may be associated with various conditions, such as pregnancy, endometriosis, adenomyosis, uterine fibroids, pelvic inflammatory disease, menstruation, and benign cysts. While these non-gynecological and gynecological conditions have been associated with increased levels of CA-125, the highest serum level of the antigen are found in ovarian cancer patients. Serial measurements of a combination of markers is very helpful in monitoring response to treatment A raised level of antigen is found in 82% of women in epithelial malignancies but only 1% in of healthy donors (Bast *et al* 1983). Thus, measurement of the CA-125 level is not usually helpful in the preoperative evaluation of a complex ovarian cyst, and surgery is generally necessary for definitive diagnosis. Complete tumor resection should always be attempted, since residual tumor disease is associated with poor prognosis (Sehouli *et al* 2004). The use of tumor marker to monitor response to treatment is particularly helpful in ovarian cancer where there is often a lack of clinically or radiologically measurable disease, a fall in tumor marker level correlates well with a decrease in disease level and shows a better response to treatment. Failure of CA-125 to fall with chemotherapy indicates drug resistance, a poor response and a need for change in therapy .The rate of fall of CA-125 in response to chemotherapy, particularly during early treatment does provide prognostic information, the rate of decline in CA-125 during primary chemotherapy has been an important independent prognostic factor in various multivariate analyses. A deviation

CONCLUSIONS

Although not 100% accurate and still under a lot of ongoing research and controversy, CA-125 and five variables Age, FIGO stage, Histopathology, Tumor grade and bulk of residual tumor showed that the CA-125 value was the most significant prognostic parameter. CA-125 is one of the best tools available for management of tumors of the ovary. CT scan and other diagnostic modalities are very expensive, has a greater radiation hazard to the patient. The damage to the organs is high as compared to its cheaper alternative.CA-125, as a diagnostic tool, is cheaper and cost effective in maintaining a disease profile and later in prognosis. CA-125 is also an effective tumor marker to assess response to treatment and can be efficiently utilized to detect early recurrence in epithelial ovarian cancer. CA-125 estimation is of clinical value in the pre-operative diagnosis and monitoring of ovarian malignancies, data collected suggests that CA-125 is elevated in the majority of epithelial ovarian malignancies prior to clinical presentation. The best established application of the CA-125 assay is in monitoring ovarian cancer, increase and decrease in CA-125 serum values correlated with tumor progression or regression, respectively .The rate of decline in CA-125 during primary chemotherapy is an independent prognostic factoring many cases, 95% cases in the study are of epithelial type and only 5-6% comprises of germ cell malignancies, this finding also correlates with other studies. All the epithelial tumor cases showed elevated CA-125 levels and low levels of all other markers, which are diagnostic in germ cell malignancies. Some cases in our study showed low levels of CA-125 although they are diagnosed cases of epithelial tumors because they are non-secreting tumors where the level of CA-125 does not rise visibly in such cases CA-125 is non diagnostic but still important marker for prognosis.

In all the cases of germ cells, teratomas, yolk sac, dysgerminomas the level of tumor markers like AFP and LDH were found to be high indicating clearly that other than CA-125 these markers are also helpful in assessing the response of treatment.

This study had its limitations due to small sample size, and non availability of complete data at all four stages no definite pattern for these germ cell tumor markers could be established.

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