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REVIEW ARTICLE

TUMOUR MARKERS : AN OVERVIEW

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ABSTRACT

Tumor Markers comprise a wide spectrum of biomacromolecules synthesized in excess concentration by a wide variety of neoplastic cells. The markers could be endogenous products of highly active metabolic malignant cells or the products of newly switched on genes, which remained unexpressed in early life or newly acquired antigens at cellular and sub-cellular levels. The appearance of tumor marker and their concentration are related to the genesis and growth of malignant tumors in patients. An ideal tumor marker should be highly sensitive, specific, reliable with high prognostic value, organ specificity and it should correlate with tumor stages. However, none of the tumor markers reported to date has all these characteristics. Inspite of these limitations, many tumor markers have shown excellent clinical relevance in monitoring efficacy of different modes of therapies during entire course of illness in cancer patients. Additionally, determination of markers also helps in early detection of cancer recurrence and in prognostication.

KEY WORDS

Tumor Markers, Malignancy, Carcinogenesis, Cancer progression, Prognostic Value.

Tumor Markers are biochemical substances elaborated by tumor cells either due to the cause or effect of malignant process. These markers can be normal endogenous products that are produced at a greater rate in cancer cells or the products of newly switched on genes that remained quiescent in the normal cells. A tumor marker produced by the tumor and, when present in significant amounts, indicates the presence of a cancer. They may be present as intracellular substances in tissues or may be released into the circulation and appear in serum (1-4). Continuing search for suitable tumor markers in serum, tissue and body fluids during neoplastic process is of clinical value in the management of patients with various malignancies. The spectrum of biochemical tumor markers reported to date is very wide. Tumor markers can be broadly classified as

1. Oncofetal antigens (e.g. alpha-fetoprotein (AFP), Carcinoembryonic antigen (CEA), Pancreatic oncofetal antigen, fetal sulfoglycoprotein.
2. Tumor associated antigens /Cancer Antigens e.g. CA125, CA19-9, CA15-3, CA72-4 CA50 etc.
3. Hormones e.g. Beta human chorionic gonadotropin, calcitonin, placental lactogen etc.
4. Hormone receptors (e.g. estrogen and progesterone receptors)
5. Enzymes and Isoenzymes (e.g. prostate specific antigen (PSA), prostatic acid phosphatase (PAP), neuron specific enolase (NSE), glycosyl transferases, placental alkaline phosphatase (PALP), terminal deoxy nucleotidyl transferase (TDT), lysozyme, alpha amylase
6. Serum and tissue proteins (beta-2 microglobulin, monoclonal immunoglobulin/para proteins, glial fibrillary acidic protein (GFAP), protein S-100, ferritin, fibrinogen degradation products)
7. Other biomolecules e.g. polyamines

Quantitative as well as qualitative evaluation of these markers is possible through modern techniques of sensitive immunoassays (RIA and ELISA) using monoclonal or polyclonal antibodies immunoassays in majority of cases and biochemical and molecular biological techniques in other cases. The tumor associated antigens expressed on the

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malignant cells could be detected and characterized in depth only by using specific monoclonal antibodies against newer epitopes (5-10).

An ideal tumor marker theoretically should have the following criteria

1. It should be highly sensitive and should have low false negatives.
2. It should be highly specific and should have low false positive.
3. It should have high positive and negative predictive value.
4. 100% accuracy in differentiating between healthy individuals and tumor patients.
5. It should be able to differentiate between neoplastic and non-neoplastic disease and show positive correlation with tumor volume and extent.
6. It should predict early recurrence and have prognostic value.
7. It should be clinically sensitive i.e. detectable at early stage of tumor.
8. Its levels should be preceding the neoplastic process, so that it should be useful for screening early cancer.
9. It should be either a universal marker for all types of malignancies or specific to one type of malignancy.
10. It should be easily assayable and be able to indicate all changes in cancer patients receiving treatment.

Unfortunately none of the tumor markers reported to date have above ideal characteristics. It is not specific to single malignancy. Every tumor marker is specific to a group of malignancies or a single organ. Malignant process is known to elaborate a group of markers (11). Depending on the malignant cell type, a single organ can elaborate many cancer markers for e.g. adenocarcinoma of ovary could be frequently positive for CA 125 and rarely positive for carcinoembryonic antigen (CEA). The endodermal sinus tumors of ovary invariably show positivity for AFP and choriocarcinoma for βhCG (12-15). However, evaluation of tumor markers can be of valuable aid in diagnosis, prognosis, staging and in monitoring the growth of the tumor. Once the patient is positive for a particular marker before instituting therapy, the effective clinical use becomes evident only after its continued measurement throughout the patient's clinical course. The rising or declining value of marker concentration in majority of malignancies predicts progression or remission. The diagnostic efficiency of tumor markers depends on variety of factors such as sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). The sensitivity of tumor marker is the probability that the test results will be positive if a tumor is positive. Specificity of tumor marker as a screening parameter indicates whether it may be used for describing in what percentage of healthy individuals the test result is negative. The PPV describes the probability that the disease in question is actually present if the test result is positive. Negative Predictive Value (NPV) describes the probability that the disease is not actually present if the test result is negative.

ALPHA FETOPROTEIN (AFP)

AFP, a very popular and extensively studied carcinoembryonic glycoprotein / Oncofetal antigen, is a major fetal serum globulin with a molecular weight of approximately 65,000. The single chain glycoprotein has carbohydrate content of 3% and amino acid sequence similar to that of albumin. It is expressed either during malignancy or during intra uterine or early postnatal life. The AFP gene is almost completely repressed in fully matured fetus leading to disappearance of the protein soon after birth (16-17). Although abundant in fetal blood, its concentration in normal adults is below 15 ng/ml. The appearance of excess amount of serum AFP beyond 500 ng/ml indicates underlying malignancy except in cases of pregnancies. Fetal liver produces AFP during pregnancy from where it is secreted into the fetal blood stream. During fetal life, synthesis of AFP takes place mainly in the liver and yolk sac and to a lesser degree in the gastrointestinal tract. Fetal liver is capable of producing AFP at a rate of 30 mg/day. The first trimester amniotic fluid has a substantial amount of yolk sac derived AFP (Concanavalin – A non reactive). With advancing pregnancy, an increasing proportion of AFP is of liver origin which is concanavalin-A reactive. Even at the end of the second trimester as much as 25% of amniotic fluid AFP may be Con-A non reactive and presumably is of yolk sac origin. The AFP concentration increases above non-pregnant level at about tenth to twelfth week of pregnancy and reaches a peak between 30 and 32 weeks. Sudden decline in AFP level is noted shortly before term (18). Sensitive immunoassays reveal that small amounts of AFP (below 15 ng/ml) persist throughout adult life .The clinical significance of AFP measurement is well worked out for prenatal diagnosis of open spina bifida, anencephaly, atresia of esophagus and multiple pregnancy (19-20).

THE ROLE OF AFP IN MALIGNANCY

Serum AFP measurement is of valuable clinical aid in diagnosis, prognosis and monitoring primary hepatocellular carcinoma (21-22), hepatoblastoma, non-seminomatous

testicular germ cell tumors the embryonal carcinoma, teratomas, choriocarcinoma and yolk sac carcinoma etc. (23), germ cell tumors of ovary (24) and extragonadal germ cell tumors (25). Approximately 60-90% of patients with primary hepatocellular carcinoma have serum AFP concentrations more than 500 ng /ml. Hepatoblastoma; an embryonic form has always been found to be associated with grossly elevated serum AFP levels. The degree of fucosylation of AFP is reported to be a useful parameter for very early detection of hepatocellular carcinoma. The serum AFP measurements may be useful as a sensitive marker system for the early detection of recurring hepatocellular carcinoma, even before the clinical symptoms are evident. The most well differentiated and highly anaplastic hepatomas do not produce AFP, as the AFP synthesis is associated with degree of liver cell differentiation. Very significant elevation of serum AFP is documented rarely in malignancies of gastrointestinal tract, pancreas, lungs, kidney, and breast etc (26-29). Regenerated hepatic tissue following liver damage due to viral hepatitis, chemically induced necrosis and liver surgery are also associated with moderately raised AFP levels. An increase in serum AFP concentration below 400 ng /ml was also reported in 10-15% of cases of acute and chronic hepatitis, liver cirrhosis and secondary hepatic malignancies. Serial AFP estimations help in distinguishing nonmalignant and malignant conditions, as the steady and progressive rise of APF is observed in malignancies whereas non-malignant conditions show fluctuations and transient moderately elevated concentrations. More than 75% of patients with nonseminomatous testicular germ cell tumors have elevated serum concentration of AFP and βhCG. Medical Research council study (1985), after data compilation from 5 large centers using two assessments (HCG 1000 IU/l or AFP 500 KU/l, or both) could classify clinical stage with three categories viz., low, medium and high risk giving a five year survival rate as 95%, 85% and 54% respectively. From several studies it was concluded that serum AFP and βhCG levels revealed prevalence rate of 60% in untreated stage-I, 70% in untreated stage-II and 90% in untreated stage - III non-seminomatous testicular carcinoma. No decline of serum AFP following orchiectomy indicates either incomplete resection or residual tumor in retroperitoneal lymph nodes. The rapid rise in AFP indicates failure to respond to treatment and thereby indicating its usefulness in monitoring treatment. Pure seminomas are known to be non-secretor of AFP. Serum AFP and βhCG both together are valuable markers for nonseminomatous germ cell tumor and have contributed significantly in enhancing the cure rate. Among germ cell tumors of ovary (dysgerminomas, endodermal sinus yolk sac tumors, teratocarcinomas and choriocarcinoma) dysgerminomas are known to be AFP non-secretors. The rapidly growing and highly malignant endodermal sinus tumors of the ovary are invariably associated with elevated AFP. The concentration of AFP correlates very well with the quantity of endodermal sinus elements in yolk sac tumor of ovary and hence useful for monitoring the treatment and for early detection of recurrence. Depending on histological type, 30-70% of the patients of extragonadal germ cell tumors have an elevated serum AFP levels. Presence of AFP was reported in 25.8 % serum, 26.3% CSF of intracranial germ cell tumors. We have reported for the first time, elevated serum AFP concentration in a case of endodermal sinus tumor of Nasopharynx in a 4-year child (25). Measurement of serum AFP has been helpful aid in the diagnosis and prognosis of AFP secreting malignancies. It has also been useful in monitoring efficacy of chemotherapy, surgery and radiotherapy in primary hepatocellular carcinoma, hepatoblastoma, non-seminomatous testicular and other germ cell tumors.

MOLECULAR VARIANTS OF AFP

Yolk sac and liver AFP synthesized during fetal and adult life are immunologically cross-reactive. Molecular heterogeneity of AFP has been well documented with respect to its difference in charge, molecular weight, and affinity for fatty acids and lectins (30-33). Because of its affinity to the lectin, the AFP could be resolved into concanavalin A reactive (R Con A) and non-reactive (NR Con A) fractions. The AFP molecules synthesized by the yolk sac contain an additional sugar; N-acetyl glucosamine linked to the α-mannose blocking the Con A binding site on the AFP. Our study on qualitative and quantitative evaluation of AFP molecular variants in sera of patients with hepatocellular carcinoma (HCC), germ cell tumors at gonadal and extragonadal sites, and in suspected liver metastasis, has highlighted the clinical relevance of the variants in differential diagnosis of AFP-secreting tumors. Following the simple and noninvasive procedure the crossed immunoaffino electrophoresis (CIAE), AFP variants could be resolved into R Con A AFP and NR Con A AFP, because of differences in their carbohydrate moieties. Quantitative as well as qualitative evaluation of AFP molecular variants revealed two types of patterns, one specific to “liver” and the other to “yolk sac”. Remarkable consistency and reproducibility of each pattern was observed in many cases of HCC and in germ cell tumors occurring either in gonads or at extra-gonadal sites. As evident from our study, evaluation of AFP-secreting malignancies could be of relevance in assisting oncologists in searching the unknown primaries. The study of AFP micro heterogeneity is also helpful in discriminating between AFP-positive primary and secondary malignancies of liver where histopathological diagnosis is inconclusive or in cases when

biopsy could not be performed because of highly vascular tumors (34). Although earlier studies have dealt extensively with the structural micro heterogeneity of AFP, they have not been fully exploited from a clinical point of view. AFP variants as diagnostic aids may also help in providing better management and prognosis.

HUMAN CHORIONIC GONADOTROPIN (βHCG)

HCG, A marker of germ cell tumors and trophoblastic disease, is 45KD glycoprotein, composed of two dissimilar subunits the alpha chain (14 KD) and beta chain (24KD). It contains 30 % carbohydrate. The beta subunit determines the immunological and hormone specificity. HCG is synthesized by the synctiotrophoblasts of the placenta during pregnancy. The peak HCG concentration is reached between 10^{th} & 12^{th} weeks of gestation. The reference values in serum of healthy men and non-pregnant women are less than 5 IU /ml and post-menopausal women are less than 10 IU /ml (35). HCG is a marker of first choice for gonadal (testes and ovary) choriocarcinoma and extragonadal choriocarcinoma. HCG shows 100 % sensitivity for choriocarcinoma irrespective of their site in addition to hydatidiform mole. In testicular tumors, the detection of HCG and AFP correlates with the histological findings, and is therefore crucial for the therapeutic procedures with the use of serial determination of βhCG, the biochemical recurrence precedes by 3 months before the patient has symptoms of clinical recurrence / metastases. The marker also helps in monitoring high-risk group of testicular tumors especially individual with undescended testicle or the healthy monozygotic twin of a testicular tumor patient. High levels of βhCG indicate poor prognosis and frequent assays during therapy level correlated to the clinical response. Serum HCG levels are rarely elevated in nontrophoblastic tumors such as lung, breast, pancreas and bladder cancers (36).

CARCINO-EMBYRIONIC ANTIGEN (CEA)

Carcinoembryonic antigen (CEA), first described in 1965 by Gold and Freedman, was characterized as a glycoprotein of 200 KD. Subsequent development of a radioimmunoassay (RIA) made it possible to detect very low concentrations of CEA in blood, other body fluids, and also in normal and diseased tissues. It is excreted by certain embryonic and adult tissues in addition to adenocarcinoma of the digestive organs. Extensive studies of patients bearing primary and metastatic colorectal neoplasms have determined that its primary use is in the detection of local and metastatic cancer recurrence after initial resection of the primary tumor, through periodic postoperative analysis of CEA in serum or plasma. The notion that fluids bathing tumors in metastatic sites might contain higher levels of CEA than those found in the blood pool led to analysis of CEA levels in gallbladder bile from patients bearing colorectal liver metastases. It was observed that CEA levels in gallbladder bile were strikingly higher than those in serum. Furthermore, linear regression analysis of tumor volume versus gallbladder bile CEA levels in patients with liver metastases predicted that tumors as small as 1 cm would produce easily measurable gallbladder bile CEA levels as high as 41 ng /ml. This data suggested that measuring biliary CEA levels in patients with primary colorectal lesions might permit detection of small, occult colorectal liver metastases earlier than is now possible through conventional methods (computed tomography liver scanning, ultrasound, and intraoperative exploration).The results of clinical studies that CEA, although originally thought to be specific for digestive tract cancers, may also be elevated in other malignancies and in some nonmalignant disorders. CEA testing is of significant value in the monitoring of patients with diagnosed malignancies in whom changing concentrations of CEA are observed. A persistent elevation in circulating CEA following treatment is strongly indicative of occult metastatic and / or residual disease. A persistently rising CEA value may be associated with progressive malignant disease and a poor therapeutic response. A declining CEA value is generally indicative of a favorable prognosis and a good response to treatment. Patients who have low pre therapy CEA levels may later show elevations in the CEA level as an indication of progressive disease. Clinical relevance of the CEA assay has been shown in the follow-up management of patients with colorectal, breast, lung, prostatic, pancreatic, and ovarian carcinoma. Follow-up studies of patients with colorectal, breast, and lung carcinoma suggest that the preoperative CEA level has prognostic significance. CEA testing is not recommended as a screening procedure to detect cancer in the general population; however, use of the CEA test as an adjunctive test in predicting prognosis and as a aid in the management of cancer patients has been widely accepted (37- 41).

PROSTATE SPECIFIC ANTIGEN (PSA)

Prostate specific antigen (PSA) termed earlier as gamma-seminoprotein due to its presence in seminal plasma. PSA is 34 KD single chain glycoprotein consisting of 93% amino acids and 7% carbohydrate. It is a monomer made up of 240 amino acid residues. PSA, a neutral serine protease, having chymotrypsin and trypsin like activities belongs to glandular kallikrein family. Prostate epithelium synthesizes PSA and efficiently prevents the escape of the protease into the circulation. However, minor amount PSA does enter into the

blood circulation. PSA is known to form complexes with different protease inhibitors in serum as well as in seminal fluid and hence various molecular forms are known to circulate in blood. The 100 KD PSA-ACT complex remains the major immunoreactive form of PSA constituting 80-90% of total PSA in serum. The complexes of PSA with other serine protease inhibitors (SERPINES) are PSA-AT (alpha-1 antitrypsin) PSA-PCI (protease C inhibitor). The PSA-AT and PSA-PCI occur at much lower concentrations in serum accounting for less than 1% of total complexes. Alpha-2 macroglobulin (α_2M) also forms complex with PSA. Due to very large molecular weight the α_2M encapsulates the whole PSA molecule resulting in masking of all epitopes on PSA molecule. The masked epitopes prevent immunodetection of PSA in this complex during routine enzyme immunoassays. The remaining free PSA constituted approximately 5-15% of the total immunoreactive forms. PSA has emerged as the most useful and clinically relevant tumor marker for carcinoma prostate. Histology alone could give final diagnosis of carcinoma prostrate in approximately in 67% of cases whereas 33% are diagnosed with the help of other modalities. At the time of presentation at the hospital, 42.3% of patients already had extensive multiple bone metastases, where 31% had loco regional spread and rest 27% had localized tumor. Hence, prevention and early detection of prostate cancer is a valuable life saving and cost effective health strategy. For early detection of prostate cancer, the American Urological Association (AUA) and Food and Drug Administration (FDA) have recommended combined use of digital rectal examination and serum PSA estimation annually in all men at the age 50 years without any family history of cancer and at the age of 40 years with family history of prostate cancer. Our earlier report has clearly documented that healthy Indian males have lowest concentrations compared to western and oriental populations. PSA remains a suitable marker as a diagnostic aid and an excellent marker for monitoring the efficacy of any treatment modality. It is known from many studies that PSA by itself is not a very effective screening tool for the early diagnosis of carcinoma prostate as it is reported to be prostate tissue specific and not prostate cancer specific. Several studies have demonstrated an association between BPH and mildly elevated serum PSA concentrations. PSA is synthesized in very low quantity by normal healthy prostate, in moderate quantity by inflamed or hyperplastic prostate and in excess amount by the malignant prostate. The overlapping serum PSA concentrations between patients of BPH and patients with early, organ confined prostate adenocarcinoma limits the ability of serum total PSA in detecting early prostate cancer in appreciable number of patients associated with gray zone serum PSA concentrations. The serum PSA value of many patients with histologically confirmed BPH has been reported to be within the 4-10 ng/ml range. A dilemma arises when the clinician is faced with mild to moderate elevations in serum total PSA in a patient with prostatism, who by virtue of age alone, is also at risk for prostate cancer. In spite of these limitations and advantages, serum PSA alone exhibited the highest diagnostic sensitivity for pretherapy adenocarcinoma prostate. However, if combined with digital rectal examination (DRE), Transrectal ultrasound (TRUS), PSA proves very useful in identifying early and curable adenocarcinoma prostate. Additionally with multidisciplinary approach unnecessary biopsies could easily be avoided. In our earlier report we have documented the role of serum total PSA, free PSA, free to total PSA ratio, TRUS and biopsy in screening high risk Indian males for benign prostate hyperplasia (BPH) and in detecting early carcinoma prostate. (Malati et. al. 2003). Our study on stability of total prostate specific antigen (PSA) and free prostate specific antigen (FPSA) in serum of healthy males as well as in patients of benign and malignant disorders of prostate at various freezing and nonfreezing temperatures and at different duration of time have indicated long-term stability of both the analytes in frozen serum. Total and free PSA were stable only for three to four days in regular refrigerators in unfrozen states. Clotted blood kept at room temperature (25°C-30°C) did not cause change in concentrations of both the analytes for twenty-four hours (Rajani & Malati, 2004). Our study with healthy subjects of different age groups ranging from 19 years to 89 years has shown maximum concentration of 6.1 ng /ml. The mean, median and 95 percentile PSA value for all age groups were 1.4 ng /ml, 1.0 ng/ml and 1.5 ng /ml respectively. The mean PSA value increased from 0.664 ng / ml for men younger than 30 years to 2.02 ng /ml for men older than 80 years. The 95 percentile value (the upper limit of normal range) rose from 1.07ng/ml for 20-29 years age group to 2.47 ng /ml for the older group of more than 80 years. An interesting point to note was progressively significant increase in mean and median PSA concentration with advancing age right from 20 to 89 years. The maximum concentrations of serum PSA also increased progressively from1.7 ng/ml to 6.1 ng /ml for age groups 20-29 to 70-79 years. Comparative data from world's literature on serum PSA reference values in healthy subjects of different populations have clearly shown slight but striking variations. International multicentre study coordinated by M/s Boerhinger Mannheim Germany in 1996 covered more than 70 centers (including ours) from18 countries (Austria, Canada, Chechen, France, Germany, India, Israel, Italy, Japan, Saudi Arabia, Netherlands, New Zealand, Poland, Portugal, South Africa, Spain, Sweden, Turkey and UK) on estimation of serum PSA in 1063 asymptomatic healthy men revealed a minimum serum PSA value as 0.001 and

maximum as 14.8 ng /ml. Surprisingly this study did not document mean or 95 percentile value. In this study, the median total PSA concentration for all ages was 0.77 ng /ml. However, the average median PSA concentration for all age groups in our study was 1.0 ng/ml for 583 healthy men contrary to BM multicentre study. Interestingly, Indian males had very low concentrations of PSA compared to healthy males from other Asia Pacific countries and America. The cut off value of 4 ng /ml as upper limit of normal reference interval has been widely used by many groups, but this value does not take into account the age related increase in serum PSA. Our results on Indian men substantiated that there is an increase of PSA with advancing age. Hence, the routine use of age specific reference intervals for PSA will improve the diagnostic efficiency in prostate cancer patients from India and will help in segregating individuals carrying high risk of harboring very early focus of prostate malignancy. Compared to free PSA, the PSA.ACT complex concentration in serum is reported to rise in adenocarcinoma prostate patients compared to benign diseases of prostate. Hence the clinical analysis of molecular forms of the PSA, the free PSA or free PSA/total PSA ratio becomes important to evaluate their role in differentiating BPH with early malignant disease of prostate, in particular the adenocarcinoma prostate. In our study total PSA; free PSA and Free PSA/Total PSA ratios were determined in serum. The 95th percentile value for healthy Indian men were very useful for interpreting the PSA results in benign as well as malignant disorders of prostate in Indian patients (42-46). The other clinically important tumor markers reported for prostate cancer are prostate acid phosphatase (PAP) and alkaline phosphatase (ALP), prostate-specific membrane antigen (PSMA), Zn-alpha 2-glycoprotein, Leucine amino peptidase and lactic dehydrogenase.

PROSTATE ACID PHOSPHATASE (PAP)

Acid phosphatase activity is 200 times more abundant in prostate tissue than in any other tissue. Acid phosphatase prostatic fraction is useful only in staging apparently localized disease i.e., primary prostate cancer before definitive therapy such as radical prostatectomy. Its activity in serum can be estimated by several synthetic substrates, but now specific antibodies are available for immunoassays. The enzymatic assay appears superior to the immunoassay in this context. Interest in acid phosphatase assays in serum as a measure of prostatic cancer staging has decreased with the availability of the more sensitive and specific PSA assay.

TUMOR ASSOCIATED ANTIGENS

Tumor associated antigens CA 125, CA 19.9, CA 15.3, CA 72.4 etc are defined by highly specific monoclonal antibodies produced against tumor tissue or cell lines of a histologically well-defined primary tumors.

CANCER ANTIGEN 125 (CA 125)

CA 125, a tumor associated glycoprotein of more than 200 KD, was detected by using murine monoclonal antibody OC 125 generated by immunization against histologically well defined ovarian adenocarcinoma cell line. OC 125 monoclonal antibody recognizes multiple repeating antigenic determinants on a high molecular weight glycoprotein. These epitopes are also detected in fetal coelomic epithelium, mullerian duct remnants, amnion and amniotic fluid, fetal and adult normal tracheal, bronchial, bronchiolar and terminal bronchiolar epithelium (47-50). CA 125 antigenic determinants are not found in normal adult ovarian tissues but are associated with epithelial ovarian carcinoma. Elevated CA 125 concentrations are found in the serum, milk and cervical secretions of pregnant women. Apparently healthy women without any ovarian mass (benign or malignant) show CA 125 levels less than 35 u /ml in their serum and globally, this value was chosen as the cut off value, i.e. upper limit of normal range. CA 125 was proved to be marker of first choice for epithelial carcinoma of ovary in particular the adenocarcinoma ovary. As per literature, the marker shows higher sensitivity for non-mucinous epithelial ovarian carcinoma compared to mucinous epithelial ovarian carcinoma. The sensitivity of serum CA 125 for pretreatment ovarian carcinoma varied from 43%-97% depending on the stage of ovarian malignancy. The progressive increase of sensitivity was observed from stage I to stage IV (stage 1:43%, stage II: 85%, stage III: 93%, stage IV: 97%, overall sensitivity for all stages: 82%) thus reflecting that the sensitivity increases with advancing stage of malignancy. In addition, this marker is also reported to be elevated in malignancies of breast (17.6%), colorectal (15.1%), gastric (30.9%), esophagus (10.5%), liver (15.1%), biliary tract (45.8%), pancreas (52.6%), lung (29.5%), and endometrium (31.8%). An increase of more than 35 u /ml CA 125 was also reported in almost 10.4% of benign ovarian tumors. In our study (51-53) 95% of healthy women had values ranging from 13 to 16.9 u /ml. CA 125 values rose to 1.7 times in 352 women with benign ovarian masses. The lower and upper limits of confidence intervals ranged from 23 to 28 u /ml. The highest concentration of 110 u /ml was observed in this group. Significantly, the pretherapy adenocarcinoma group consisting of 366 subjects revealed 99.66 fold elevation of CA 125

compared to healthy subjects and 57.56 fold elevation compared to benign group. Highly significant 83.75 fold increase in CA 125 levels was observed in-group of 172 patients having recurrent disease. The highest CA 125 concentrations of 25600 u /ml and 11940 u /ml were observed in pretherapy and recurrence respectively. Three hundred and ninety patients during stable course of disease showed nearly normal mean concentration of 15.2 u /ml as against 14.9 u / ml value in healthy subject. In one of our study, tumor marker CA 125, CEA and CA 19-9 were evaluated to assess the best tumor marker for epithelial ovarian carcinomas in diagnosis, prognosis and management. The results indicated that CA 125 is the best and the most superior marker today for the epithelial ovarian malignancies irrespective of the histological type. CA 125 has the highest positivity rate in mucinous tumors compared to CEA and CA 19-9. Combined assay of either CEA or CA 19-9 or both along with CA 125 did not increase diagnostic sensitivity compared to sensitivity achieved by CA 125 alone for epithelial tumors of the ovary. Serum CA 125 concentration correlated well with pretherapy, stable course and recurrence of ovarian carcinoma. CA 125 could be used effectively for diagnosis, prognosis and monitoring of the ovarian malignancies as evident from long term follow up of Adenocarcinoma ovary. At the time of diagnosis and prior to therapy, CA 125 has 80% sensitivity for serous adenocarcinoma and slightly lower sensitivity for mucinous adenocarcinoma. After removal of the tumor there is a rapid decline in the concentration within one week and normalization of the values by about three weeks after therapy indicating good correlation to the clinical response to treatment. However, normal CA125 level does not exclude residual peritoneal micro metastases. The rising value of marker concentration precedes the clinical diagnosis or recurrence by fourth months. CA 125 indicated good prognostic value as remission or relapse was predicted in about 90% of the cases. Elevated CA 125 levels were also observed with varying degree of sensitivity in carcinoma of the neck of the womb, cervical carcinoma, gastrointestinal carcinoma. Non-specific mild increase is observed in benign ovarian cysts (follicular cysts), endometrioses, coelomic epithelium pathologies, ascitic cirrhosis, pleural effusion, ascitis, peritonitis, pericarditis, during menstruation and during last 3 months of pregnancy (54-60). Our earlier report and case studies has highlighted the clinical importance of CA 125 in a) supporting the clinical and histopathological diagnosis, b) monitoring the efficacy of treatment and c) predicting not only the stable course of disease, but also tumor regression, progression and in prognostications. A comparative study of the tumor markers, CA 125, CA 19-9 and CEA reported by our group clearly indicated superiority of CA 125 which proved to be the best and the most superior marker today for epithelial ovarian tumors. This was best indicated by its highest sensitivity (80%) compared to CA 19-9 (36.4%) and CEA (8.1%). A specificity of 96.7%, positive predictive value of 92.3% and negative predictive value of 90.9% was reported for CA 125 in pretherapy samples. Combination of CA 125 with other two markers studied did not further enhance the sensitivity CA 125 highlighting that in case of epithelial ovarian tumors CA 125 alone could be used effectively and efficiently (54-60).

CA 19-9 (CANCER ANTIGEN 19-9)

CA 19-9 is a tumor marker of first choice for cancer pancreas and cancer gall bladder. The marker is 210 KD tumor associated glycoprotein antigen present as carbohydrate determinant on glycolipid and glycoprotein. CA 19-9 is characterized by monoclonal antibody 1116 - NS 19-9 by immunizing BALB/c mice with human colorectal cancer line. This antibody reacts with a carbohydrate antigenic determinant (CA 19-9) which has been identified as a sialylated lacto-N-fucopentaose II, an oligosaccharide sharing structural features with Lewis blood group substances. The antigen was localized immunohistologically on fetal epithelia of the colon, small intestine, stomach, pancreas and liver and very small concentrations on adult gastrointestinal tract and lung tissue. Appreciable concentration of CA 19-9 is also present in mucin rich saliva, seminal fluid, gastric juice, amniotic fluid, urine, ovarian cyst fluid, pancreatic, gall bladder and duodenal secretions. In 99.6% of healthy adults, serum CA 19-9 levels are lower than 37 u /ml. Value less than 100 u /ml considered as grey zone values in which malignant and benign diseases may overlap. In malignant tumors values above 100,000 U/L may be observed. CA-19-9 is neither tumor specific nor organ specific. However, the diagnostic sensitivity (85 %) and specificity (95 %) of CA-19-9 are highest for the adenocarcinoma pancreas. Sensitivity of 70 % is observed in cholangiocarcinomas and gallbladder carcinomas. Very low sensitivity was seen for colorectal, stomach, primary liver, bronchial, mucinous ovarian, uterus and mammary carcinoma. The 19-9 concentration correlated well with the clinical response to treatment. In addition to its use as a diagnostic tool, CA19-9 appears to be a promising marker that can predict recurrence of tumor after pancreatectomy prior to clinical or radiographic evidence of disease. CA 19-9 increases very early during recurrence in patients with a mean lead-time of 4-6 months before the clinical diagnosis. Non-specific increase of marker is reported in 8% acute and chronic Pancreatitis (61-64).

CA 15-3

Ca 15-3 is heterogeneous 300 KD glycoprotein antigen was defined by using two monoclonal antibodies 115D8 and DF3 raised against breast carcinoma cells. The diagnostic sensitivity of the CA15.3 for breast carcinoma is low as its elevated levels are also observed in benign breast diseases and in liver cirrhosis, acute and chronic hepatitis. The marker concentrations is also elevated in metastatic cancers of pancreas, ovary, colorectal, lung, stomach, uterus. However, recent report from India on pretherapy breast cancer patients have shown better correlation with CA15.3 compared to CEA (61, 65-67).

CA 72-4

CA 72-4 is mucin like high molecular weight tumor associated antigen (TAG-72). Its molecular weight is more than 10^6 KD. This antigen was characterized by using two murine monoclonal antibodies the CC-49 and the B 72-3 both recognizing tumor associated glycoprotein TAG-72 in human serum (Colcher et al 1981). This antigen was detected in fetal epithelium and also in serum of patients of various adenocarcinomas. CA 72-4 once emerged as the tumor marker of first choice for gastric carcinoma and is thereby superior to CA 19-9 and CEA The sensitivity of CA 72-4 was found to be 38%, 33% for CA 19-9, 31% for CEA and 21% for CA 125. Hence, CA 72-4 was considered to be the multiple markers for epithelial cell derived tumors (68-69).

CA 19-5 AND CA 50

These two markers have been characterized by using monoclonal antibodies. CA 19-5 was detected by using mouse monoclonal antibodies, CC-3, C-19-5 reacting with epitopes on sialylated Lewis blood group antigen. CA-50 antigen was detected in gangliosides and glycoproteins CA –50. CA 19-5 was found to be associated with colon, pancreatic and hepatocellularcarcinoma. Individually both antigens have low sensitivity. However use of both together improves sensitivity in detecting pancreatic and other carcinomas (62, 70).

CA 549

CA 549 is a high molecular weight circulating glycoprotein antigen associated with breast cancer. This antigen was characterized by murine monoclonal antibodies (T417 and BC4 N154) immunized against human breast tumor and fat globule membrane present in human milk. Elevated levels of CA 549 are observed in serum of advanced breast cancer by using sensitive immunoassays. An over all sensitivity of 50% and specificity of 98% are observed with immunoradiometricassays. However, it has very low sensitivity, very low negative predictive value and high positive predict value for early breast cancer (71).

BETA -2 MICROGLOBULIN (β2M)

β2M is 11 KD light chain constituent of HLA antigen. The Beta 2 M is used clinically as a marker of first choice for B-cell leukemia, lymphomas and multiple Myeloma. However, due to its non-specificity its moderate elevation is observed in cases of solid tumors and also in various inflammatory diseases, benign infectious disorders, and primary biliary cirrhosis and in acquired immune deficiency syndrome. It is used routinely for evaluating tumor cell load, disease activity and prognosis. It is also used to monitor efficacy of patient's response to treatment. Elevated levels of Beta 2 – M are also reported in cerebrospinal fluid (CSF), central nerves system (CNS) mets, acute lymphobalstic leukemia, Lymphoma and other Lymphoproliferative disorders/diseases. Hence, the determination of β2M in CSF helps in identifying and managing CNS metastases. Review of literature indicates that human myeloma cell lines and plasma cells from patients of multiple myeloma, lymphoma and leukemia synthesize β_2 microglobulin at higher rate compared to resting lymphoid cells. Serum β_2M could be also used as a marker of both the malignant plasma cell mass and also the disease activity. Serum β_2M could be clinically relevant marker for Waldenstrom's macroglobulinemia, secretary and non-secretary multiple myeloma, leukemia and lymphoma. Like other tumor markers, β_2M has proves to be the best marker for monitoring therapeutic course, as it is a useful serum parameter to monitor tumor progression as well as early biochemical relapse. Serum β_2M is the most powerful prognostic marker of monoclonal gammapathies. Pretreatment serum β_2M concentrations greater than 6 mg/L correlated well with low response rate and were the most important variable that predicted a short survival time. (72-73)

CYTOKERATINS / KERATINS

Keratins are proteins ranging from 40 to 68 KD are known to form intermediate filaments of 8-10 nm in diameter. Keratins are remarkably diverse, highly resistant and the most conserved cytoskeletal proteins present in all types of epithelial cells. The composition of keratin filaments ranges from a few polypeptides to 19 different polypeptides ranging from 40 to 68 KD. In epithelial tumors some keratin polypeptides are either not expressed or are over expressed. Therefore, keratins have

gained importance as marker proteins useful in diagnosis of tumors of epithelial origin. In malignancy, epithelial tissues may lose features to varying degree resulting in the absence of a recognizable epithelial morphology. There may be variations in keratin expression compared to that in normal tissue, depending on the degree of differentiation of epithelial tumors. This property of keratins allows their effective use, in combination with other changes, as tumor markers for malignant transformation in epithelioid tumors. Keratins have acquired great importance in tumor biology and have been designated as epithelial differentiation markers. Keratins as tumor markers have two main applications: (i) in distinguishing epithelial from nonepithelial tumors, and (ii) in distinguishing the type of epithelial tumor. The degree of keratin expression in tumors is remarkably high. Therefore keratin is also a reliable marker for (i) undifferentiated and anaplastic carcinomas, (ii) disparately growing infiltrating carcinoma cells, and (iii) metastasizing single carcinoma cells in suspension. Keratins have been used effectively as markers for epithelial carcinomas, especially those of stratified and squamous-cell origin, e.g. lung carcinomas, breast carcinomas, urinary bladder carcinomas, thymomas, and cervical carcinomas. Since the gastrointestinal-tract lining, from the buccal cavity to the rectum, including the pancreas and gall bladder, is of epithelial origin, keratin serves as a useful marker for gastrointestinal tumors. Keratin has been used as a differential marker in thyroid, gastrointestinal, prostate, lung and breast tumors. We compared the pattern of expression of keratin polypeptides between control and malignant gastric tissues by employing different morphological and biochemical techniques. The results indicated preferential decrease of acidic keratin polypeptides in malignant gastric tissue. The nonmalignant gastric tissues expressed four keratin polypeptides, two acidic and two basic, whereas in the gastric adenocarcinoma the expression of only two basic polypeptides was observed. Marked variation of keratin elucidated the underlying changes due to malignant transformation. (74-76).

CYFRA 21-1

Cyfra 21-1 is an antigenic determinant present on 40 KD protein the cytokeratin 19. This antigen is defined by using two mouse monoclonal antibodies the KS 19-1 and BM 19-21 by immunization against MCF – 7cell line. This antigen is expressed in normal, simple epithelium as well as in proliferating epithelium. It is also defined by using monoclonal antibody, which detects specific epitopes on cytokeratin 19. Cyfra 21-1 is used as a tumor marker for non-small cell lung cancer (NSCLC), such as squamous cell carcinoma (SCC), adenocarcinoma and large cell carcinomas. This marker shows highest sensitivity for SCC in lung. Both Cyfra 21-1 and CA 19-9 have improved the sensitivity for the detection of adenocarcinoma for lung. (77-78)

TISSUE POLYPEPTIDE ANTIGEN (TPA)

TPA, which is regarded as a marker of cell proliferation, is a mixture of proteolytic fragments containing the relatively stable α-helical rod domains of simple epithelium-type cytokeratins. These fragments are probably released during necrosis and lysis of the carcinoma cells. Thus TPA should be regarded as a broad-spectrum epithelial tumor marker and not as a specific molecular marker for epithelial neoplasms. TPA is a constituent of intermediary filament proteins. It has a mixture of low molecular weight cytokeratins 8, 18 and 19. The moderate elevation in TPA occurs in many diseases and in pregnancy. The marked elevation of serum TPA is reported in variety of cancers such as breast, lung, gastrointestinal, urological, gynecological cancer. TPA is known to be sensitive but non-specific tumor marker. However TPA along with CEA is of some help for monitoring lung, bladder, and breast, colorectal and ovarian carcinomas. TPA is also reported to differentiate between cholangiocarcinomas and hepatocarcinomas (79-80).

CALCITONIN

Calcitonin, a low molecular weight circulating peptide hormone, synthesized by C cells of the thyroid is used as tumor markers as its increased concentration is reported in malignancies with skeletal metastases. Serum calcitonin concentrations are also reported to increase in medullary carcinoma of the thyroid, Bronchogenic carcinoma, small cell lung cancer, breast, liver, lung, renal cancers and carcinoid tumors (81)

CATECHOLAMINES

Plasma and urinary norepinephrin and epinephrine levels are increased markedly in Pheochromocytoma (82-83).

CATHEPSIN D

Lysosomal aspartyl protease of lysosomes is considered a potential tumor marker for metastases arising out of primary breast cancer. Cathepsin D predicts early recurrence. It has high prognostic value in node-negative breast cancer compared to node-positive breast cancer. The patients with low cathepsin D are known to have better over all survival compared to patients with high cathepsin D. The high level of cathepsin D enhances the process of metastases in patients of carcinoma breast (84-85).

CHROMOGRANIN A

Chromogranin A (secretogranin I) belonging to group of closely related secretary acidic proteins is used as a tumor marker to assess exocytotic sympathoadrenal activity in Pheochromocytoma patients. Its concentrations in plasma are elevated in peptide producing tumors (86-87).

EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR)

EGFR a 170 KD, glycoprotein binds to epidermal growth factor (EGF) with high affinity and shows significant sequence homology with V-erbD oncogene product. EGFR gene over expression is observed in SCC. EGFR levels are raised in breast cancer, gliomas, lung cancer, blood cancer, SCC and tumors of female genital tract. The breast cancer patients with positive EGFR for reported to have reduced relapse free survival. Absence of EGFR indicates a good response to tamoxifan therapy (88).

ESTROGEN RECEPTOR (ER), PROGESTERON RECEPTOR (PR)

ER a 70 KD protein is present in nuclei of mammary and uterine tissues. ER & PR belong to receptor super gene family including receptors for thyroid hormone, vitamin D_3 and retinoic acid. In breast tumor patients the ER and PR measurements help in identifying the patients likely to achieve benefit from endocrine therapy, 55% to 60% ER positive primary malignancies of breasts show good response to hormone therapy. Following mastectomy, the patient with high concentrations of ER and PR positive malignant tumors have longer disease free survival compared to patients with low levels of both receptors. PR is a more sensitive indicator than ER in predicting effective responsiveness to endocrine therapy in breast tumor patients (89-90).

FERRITIN

Serum Ferritin, one of the acute phase reactant is an intracellular protein playing a role in sequestration and storage of iron. Increased level of serum ferritin is reported in cancer patients in the absence of iron overload. Ferritin levels are also increased in advanced cancers of breast, ovaries, lungs, colon and esophagus. Elevated levels are also reported in acute myelocytic leukemia, teratoblastoma and SCC of head and neck.

HOMOVANILLIC ACID (HVA) AND VANILLYMANDELIC ACID (VMA)

HVA and VMA are acidic metabolites of catecholamine. Their increased excretion is observed in patients with neural crest tumors. Determination of VMA and HVA also helps in detecting and monitoring therapy in patients of Pheochromocytoma. Their measurements are also relevant for neuroblastomas (91).

HYDROXY INDOLE ACETIC ACID (5-HIAA)

Urinary HIAA measurement is used clinically and helps in diagnosis of indole secreting tumors. Its concentration also increased in patients with carcinoid tumors. HIAA is routinely used for monitoring efficacy of carcinoid syndrome therapy.

INTERLEUKIN-2 RECEPTOR / TAC ANTIGEN (IL-2R)

IL-2 α matured receptor, a 55 KD glycosylated protein is over expressed in some types of lymphoid malignancies. Serum levels of IL-2 are elevated in adult T-cell leukemia and the marker also helps in monitoring therapy in these patients.

LIPID-ASSOCIATED SIALIC ACID IN PLASMA (LASA-P)

Increased concentration of LASA-P is reported in many malignancies such as breast, gastrointestinal tract, lung, leukemia, lymphoma, Hodgkin's diseases and melanoma. The slight increase of this marker is also observed in several inflammatory diseases indicating its poor specificity. The sensitivity of this marker varies from 77% to 97% for different tumors.

NEURON-SPECIFIC ENOLASE (NSE)

NSE, the gamma subunit of enolase enzyme, is present predominantly in neurons and neuroendocrine cells. Elevated concentrations of NSE are observed frequently in glucagonomas, insulinomas, carcinoid tumor, pheochromocytoma, medullary carcinoma of the thyroid, oat cell carcinomas and small cell lung carcinoma and rarely in other cancers of lung. It is a marker of first choice for SCLC. Monitoring of NSE concentrations is also utilized in assessing prognosis and monitoring therapy in 85% of neuroblastomas and SCLC (92).

ONCOGENE P21 RAS

RAS, one of the transformation-inducing gene, belonging to

family of cellular oncogenes (c-ras) frequently identified in human solid tumors. C-ras encodes for 21 KD proteins called P-21. The mutations in P21 gene are frequently identified in approximately 15% of solid tumors. Point mutations of ras proto-oncogene, in codons 12 & 13 are detected in colorectal carcinomas and large adenomas. Increased concentrations of ras mRNA and ras P-21 are reported in bladder, lung and colon carcinomas. Ras gene types helped in prognostication of colorectal carcinomas and adenomas (93).

TUMOR SUPPRESSOR GENE P53

P53, a 53 KD nuclear phosphoprotein functions as tumor suppressor by inhibiting cell proliferation. P53 plays a dominant role in cellular apoptosis. P53 gene mutations are reported in approximately 50% of all types of cancers. Commonly occurring P53 gene mutations are reported in primary breast, colon, ovarian, lung, and esophageal carcinomas (94-95).

BRCA 1 AND BRCA 2

BRCA 1 and BRCA 2 belong to few tumor suppressor susceptibility genes conferring the high risk to individual for few cancers. BRCA 1 gene mutations predict very high risk for breast, ovary, colon and prostate cancer. BRCA 2 gene mutations are reported in high frequencies (70%) in inherited breast cancer in females. This second susceptibility gene also imposes an increased risk of cancer breast in men. More than hundreds germ line mutations are reported in BRCA genes by employing current molecular technologies. The types of mutations are framshift, nonsense and splice site including deletions and duplications in the BRCA1 and BRCA 2 genes. Three very common mutations observed in many populations are the 185delAG and the538insC in BRCA1 gene. The BRCA2 gene mutation the 999del 5 is common in Iceland whereas the 617delT mutation in Ashkenazi Jews. All the women with BRCA mutations do not develop cancer and hence there is need to evaluate exact risk to provide appropriate benefits in these women (96-97).

PARTHYROID HORMONE-RELEATED PEPTIDE (PTH-RP)

Elevated plasma concentrations of PTH-RP are found in many patients of cancers having hypercalcemia. PTH-RP determination in plasma helps in differential diagnosis of hypercalcemia associated with primary hyperparathyroidism, sarcoidosis, vitamin D toxicity, squamous cell carcinoma of renal, bladder and ovarian carcinoma.

PS2

PS2, a low molecular weight cysteine rich protein is elevated in 50% of breast tumors. PS2 protein seems to be a marker for hormone dependent breast tumors and its expression seems to indicate a better prognostication compared to ER or PR positivity in breast carcinomas patients. This protein is also expressed in normal stomach mucosa and ulcerative diseases of the GI tract (98).

SQUAMOUS CELL CARCINOMA (SCC) ANTIGEN

SCC antigen, a 48 KD protein, is purified from uterine cervix. The antigen concentration is elevated or in squamous cell carcinomas of head and neck, lung, esophagus and anal canal. The highest concentration of SCC antigen is found in patients with metastases. The marker elevation is also observed in 70% patients with advanced cervical cancers. Serial serum SCC antigen determination helps in determining progression and regression of cervical cancer following chemotherapy. The antigen levels are also raised in some patients of extensive liver disease. The combined use of CEA, NSE and SCC antigen has helped in increasing sensitivity for detection and monitoring of lung tumors (99).

MONOCLONAL IMMUNOGLOBULIN / PARAPROTEIN

Monoclonal immunoglobulin content is of great value in diagnosis as well as for monitoring efficacy of therapeutic management of plasma cell neoplasms namely multiple Myeloma, Waldenstrom's macroglobulinemia, plasmacytoma, B cell leukaemias and lymphomas(100-104).
Recent publication by Yilmaz and his team from Turkey have emphasized the need for judicious use of tumor markers following the practice of evidence-based medicine. It is needless to state that inspite of non-specificity of wide spectrum of tumor markers available today, their potential role in monitoring entire cancer therapeutic course is very relevant clinically (105).

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