IMMUNOBIOLOGICAL MONITORING OF VARIOUS GASTROINTESTINAL AND PRIMARY HEPATIC MALIGNANCIES

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ABSTRACT

Carcinoembryonic antigen (CEA) and alpha fetoprotein levels (AFP) were assayed by enzyme-labelled immunoassay in 75 cases of gastrointestinal (GI) and primary hepatic malignancies. Mean CEA levels were found to be significantly higher ($p < 0.005$) in metastatic non-operative group of GI malignancies (range 5.32 ng/mL to 175.2 ng/mL) as compared to early pre-operative cases (range 1.25 ng/mL to 33.2 ng/mL) and post-operative cases (range 1.41 ng/mL to 22.24 ng/mL). Variable levels of AFP were visualised in 12 cases of primary hepatic malignancies with eight cases having values less than 100 ng/mL. When both CEA and AFP were assayed simultaneously, the markers were helpful in differentiating cases of primary hepatic malignancies with low levels of CEA from 3 cases of secondaries in the liver where CEA levels were found to be highly raised (80.4 ng/mL to 146.4 ng/mL). To evaluate the variation of CEA and AFP levels in different patients having same stage of the disease, immunological monitoring for the functions of T and B cells was carried out by estimation of cytokine, i.e. interleukin-1 alpha (IL-1a), interleukin-2R (IL-2R) and various immunoglobulins. IL-1a and IL-2R levels were significantly higher ($p < 0.05$) in the groups of patients having higher CEA and AFP. This indicates an important T cell (TH1 and TH2) function in the tumour antigen production.

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KEYWORDS : Alpha fetoprotein; Carcinoembryonic antigen; Cytokines; Tumour antigens.

Introduction

Tumour markers are cell surface antigens, cytoplasmic proteins, enzymes and hormones, produced by a tumour or by the body in response to a tumour. These substances are released into body fluids and can be detected by sensitive analytical methods. The measurement of tumour markers should permit the detection of cancer and to identify the organ source of the tumour. The magnitude of tumour marker elevation should ideally correlate with the tumour mass and a fall in level should correspond to positive response to treatment. Thus a tumour marker should aid in the detection of tumours, prediction of prognosis, and in monitoring therapy [1]. However this is not the case with all tumour markers.

Carcinoembryonic antigen (CEA) and alpha fe-

toprotein (AFP) are widely use in clinical practice to support diagnosis [2]. A number of studies have been conducted worldwide to assess the functional importance of CEA and AFP in diagnosis, staging, and prediction of recurrent disease in gastrointestinal (GIT) and hepatic malignancies [3]. However their sensitivity and specificity have ranged widely. Although originally thought to be specific for GIT cancer elevations of CEA are also seen in malignancies of pancreas, liver, lung and breast. The elevation is also seen in benign polyposis and in heavy smokers [4]. In a recent study, 62 patients with colorectal cancer and 97 with benign GIT disease cases were studied [5]. It was observed that CEA was probably the best current test in diagnosing colorectal cancer. Other studies have shown that with cut-off values of 2.5 ng/mL the diagnostic sensitivity and specificity of CEA was 63 per

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cent and 90 per cent respectively [6]. In detection of recurrent disease and prediction of hepatic metastasis the sensitivity of CEA has been found to be 58 per cent and 80 per cent respectively [7].

Variable rise in levels of AFP are also visualized in various malignant and non-malignant conditions [8]. However, the cause of the variations in the rise of CEA and AFP is not clearly understood. The present study was undertaken to evaluate the importance of CEA and AFP in the diagnosis of various GIT and primary hepatic malignancies. An attempt was also made to correlate the findings of variable rise in tumour markers with the immune status of the patient by assaying T and B cell functions through evaluation of cytokines and immunoglobulins.

Material and Methods

Patients

A total of 75 consecutive patients suffering from gastrointestinal and primary hepatic malignancies were included in this prospective study. The diagnosis in all was subsequently confirmed by histopathological examination and guided fine needle aspiration cytology (FNAC). All the patients were managed at the Malignant Disease Treatment Centre of our institution.

CEA and AFP estimation

Patient's serum samples were collected and aliquated at -20°C for future immunological evaluations. CEA and anti-AFP estimations were carried out by enzyme linked immunoassay (ELISA) using commercially available monoclonals [9]. Briefly, the serum samples were allowed to react with separate plates already coated with anti-CEA and AFP monoclonal antibodies. In the second reaction the treated plates were allowed to form a complex with the help of a rabbit antibody conjugate. Finally the colour was developed with addition of enzyme-labelled substrate. The assay estimation was completed by using serial standards and computerized laboratory data system of Anthos HT-2 ELISA reader.

Interleukin-1 alpha and interleukin-2R assay

IL-1 alpha and IL-2R assay was also carried out by ELISA method [9] using commercially available monoclonal kits.

Immunoglobulin assay

Serum immunoglobulins G, A and M levels were estimated by immunoturbidity method [10]. Briefly, the serum samples diluted to 1 in 20 concentrations were allowed to react with antibody to the specific immunoglobulin for 30 minutes. The precipitating immunocomplexes formed in the presence of polyethylene glycol were photometrically measured at wavelength of 340 nm and final concentrations calculated by using different standards.

Results

The patients' age ranged from 32 years to 68 years (mean 45.2 ± 12.6 years). Sixty three patients (84%) were suffering from GI malignancies and only 12 (16%) were diagnosed as primary hepatic malignancy cases. Out of 75 patients, 15 were operated earlier and were on follow-up treatment. Ten healthy subjects in the age group of 35-45 years served as controls.

Mean CEA levels were found to be significantly higher ($p < 0.005$) in metastatic group of gastrointestinal malignancies as compared to early pre- and post-operative cases (Table 1). Highest levels were however seen in metastatic group of carcinoma colon (mean 68.92 ± 68.30 ng/mL), as compared to stomach (mean 25.48 ± 21.96 ng/mL) and rectal carcinoma cases (mean 78.4 ± 24.8 ng/mL) (Table 1). Interestingly, in few early operated and metastatic group of GI malignancies, CEA levels were found to be quite low and were comparable to levels found in normal healthy controls (mean 1.25 ± 0.45 ng/mL). AFP levels were found to be variable in primary hepatic malignancies and ranged from (50.4 to 700.0 ng/mL) with mean of 190.4 ± 63.5 ng/mL. Combined CEA and AFP assay was found to be useful in differentiating primary hepatic malignancies from 3 cases of secondaries in the liver due to metastatic GIT malignancy. In these cases, where CEA levels were very high, AFP was found to be comparatively lower.

IL-1 alpha and IL-2R assay showed higher mean levels ($p < 0.05$) in patients with greater CEA (> 5 ng/mL) and AFP (> 100 ng/mL) values (Table 2). In contrast, immunoglobulin assay showed lower values except mild to moderate increase in IgG levels (1938 ± 1170 ng/mL) in higher CEA

TABLE 1
Carcinoembryonic antigen levels in GI malignancies (63 cases)

	Ca colon			Ca stomach			Ca rectum		
	No.	Mean $\pm$ SD ng/mL	Range	No.	Mean $\pm$ SD ng/mL	Range	No.	Mean $\pm$ SD ng/mL	Range
Preoperative early values	11	6.96 $\pm$ 8.61	1.2 - 15.8	8	16.79 $\pm$ 13.9	1.8 - 33.2	12	7.02 $\pm$ 6.35	2.05 - 25.36
Post-operative values	7	3.01 $\pm$ 0.97	1.8 - 5.32	5	1.80 $\pm$ 0.31	1.41 - 2.8	5	8.60 $\pm$ 9.73	1.73 - 22.24
Metastatic non-operative cases	8	68.92 $\pm$ 68.30	10.6 - 175.2	4	25.48 $\pm$ 21.96	5.32 - 52.24	3	78.4 $\pm$ 24.8	34.31 - 110.52
Total	26			17			20		

TABLE 2
Correlation of carcinoembryonic antigen and alphafetoprotein with different cytokines

Tumour marker	No of cases	IL- α (OD)	IL-2R (pg/mL)
CEA levels			
< 5 ng/mL	18	360.7 $\pm$ 30.9	2788.8 $\pm$ 1885.5 ^a
> 5 ng/mL	34	396.3 $\pm$ 42.88	4321.9 $\pm$ 3173.2 ^b
AFP levels			
< 100 ng/mL	8	348.0 $\pm$ 34.8	3703.0 $\pm$ 1590.2 ^c
> 100 ng/mL	4	395.5 $\pm$ 73.0	4973.0 $\pm$ 1250.0 ^d

(p value a vs b, c vs d < 0.05)

group as compared to the group having lower CEA and AFP values (Table 3).

Discussion

In 1965 Gold and Freedman described a tumour-associated antigen found in adenocarcinoma of colon and digestive system epithelium of embryonic tissue. This antigen was subsequently labelled as carcinoembryonic antigen (CEA). This is

a heterogenous group of glycoproteins with a molecular weight of approximately 200,000 daltons and beta electrophoretic in mobility. Although originally thought to be specific for digestive tract cancers, CEA is also found to be elevated in other malignancies like those of breast, lungs, and prostate [4]. Similarly its levels are also found to be raised in nonmalignant conditions like ulcerative colitis, benign polyps and in heavy smokers [4]. Various studies conducted have found sensitivity and specificity of CEA to be variable in detection of various malignant conditions. Recently CEA levels were determined in 100 gastric cancer and 77 relative benign diseases [11]. The sensitivity and specificity of CEA with cut-off levels of 3 ng/mL for gastric cancer was found to be 33 per cent and 73 per cent respectively. However, it was observed that high pre-operative CEA levels predicted higher stage of disease [11]. In another study of 125 cases of colorectal cancer, a single CEA level of more than 100 μ L-1 (normal range less than 60 μ L-1), was found to be highly sensitive (87%), specific (89%), and accurate (88%) in-

TABLE 3
Correlation of carcinoembryonic antigen and alphafetoprotein levels with immunoglobulins

	No of cases	IgG (mg/mL)	IgA (mg/mL)	IgM (mg/mL)
CEA levels				
< 5 ng/mL	18	1873 $\pm$ 659	291 $\pm$ 113	160 $\pm$ 114 ^a
> 5 ng/mL	34	1938 $\pm$ 1170	207 $\pm$ 112	113 $\pm$ 68 ^b
AFP levels				
< 100 ng/mL	8	2122 $\pm$ 841	364 $\pm$ 220	107 $\pm$ 44.3 ^c
> 100 ng/mL	4	1712 $\pm$ 430	195 $\pm$ 114	82 $\pm$ 27.0 ^d

(p value a vs b, c vs d < 0.05)

indicator of recurrent disease [12]. Raised CEA levels were preceded in 72 per cent of patient with recurrence of colorectal cancer [12]. Persistent rise in the CEA values have also been associated with progression of the disease [3]. Overall sensitivity of CEA has been found to be 43 per cent. The sensitivity was 26, 32, 38 and 77 per cent in Dukes A, B, C and D colorectal cancers. Takahashi [13] has found that CEA doubling time in gastric cancers ranged from 12 to 105 days with median of 32 days. However, this was found to be significantly shorter in papillary adenocarcinoma of stomach. Sensitivity of CEA for predicting hepatic metastases has been found to be very high (80%) as compared to 46 per cent for other sites [7].

Alpha fetoprotein is a glycoprotein with a molecular weight of approximately 700,000 daltons and is normally produced during fetal and neonatal development by the liver, yolk sac and in small concentrations by the gastrointestinal tract. Elevation of AFP levels is most notably seen in non-seminomatous testicular cancers and primary hepatocellular carcinoma. The levels may also be raised in ataxia telangiectasia, hereditary tyrosinaemia, viral hepatitis or cirrhosis [8]. Similarly raised AFP levels have been found in some gastric cancers which are predominantly either papillary clear carcinomas or hepatoid carcinomas [14].

Interleukin-1 was originally described as a factor which activated phytohemagglutinin-stimulated thymocytes. Later on many other effects of IL-1 on immunocompetent cells have been observed *in vitro* including the induction of high affinity receptor for interleukin-2 and potentiation of synthesis of various cytokines by activated T cells [15]. Its elevated serum levels have been observed in many cases of autoimmune, inflammatory and certain malignancies [16]. In advanced gastric carcinoma, levels of IL-1 beta have been found to be raised [17]. However, in another study, IL-1 levels were not raised in patients with colorectal carcinoma while IL-6 and IL-8 were raised [18].

Interleukin-2 receptor is unique among growth factor receptors in that it is made up of at least three distinct membrane components, i.e. alpha, beta and gamma chain. Soluble form of IL-2R has been studied as a marker for T cell involvement in a number of disease states including autoimmune

disorders, malignancies, transplant rejection, and infections [19]. T helper 1 (Th1) cells produce cytokines like IL-2, tumour necrosis factor B (TNF-B), and interferon γ [20]. In contrast, T helper 2 (Th2) cells produce cytokines like IL-4, 5, 6 and 10. IL-1 production has however not been linked with Th1 and Th2 responses till recently [21]. Immunoglobulins production by activated B cells after differentiation into plasma cells require help from activated T cells by release of cytokines like IL-2 [22]. Thus production of immunoglobulins and release of cytokines is interdependent.

In our study, patients having GI malignancies with metastases showed significant higher mean levels of CEA ($p < 0.005$). Other studies have also observed this finding [2,3]. Using 5 ng/mL as cut-off level, sensitivity of the assay was found to be raised and it reached 67 per cent for Duke's stage D in colorectal cancers. A very similar phenomenon has been seen in our study, where higher (> 5 ng/mL) CEA levels were found in metastatic group of colorectal cancers (Table 1). AFP levels in primary hepatic malignancies have been found to be variable in our study, ranging from 50.4 ng/mL to 700 ng/mL with mean of 190.4 ± 63.5 ng/mL. This is probably attributed to initial disease and less tumour load in few cases. We found combined CEA and AFP estimation very useful in differentiating primary hepatic malignancies from secondaries in liver due to GI malignancies. In these 3 cases of hepatic malignancy, CEA levels were found to be markedly increased whereas there was marginal increase in AFP levels. On further investigations primary malignancy was detected to be in the GIT necessitating different treatment modality. However, this observation may not be useful in all cases of secondaries in liver due to low production of CEA in some cases of colorectal cancer. Significant higher levels of IL-1a and IL-2R were observed in patients showing higher CEA and AFP levels (Table 2) ($p < 0.05$). However most of the immunoglobulin levels were reduced in this group of patients. This is probably due to activation of complement cascade and ultimately immune complex formation. Different studies have shown higher cytokine levels, e.g. IL-1, IL-4, IL-6 in different advanced stages of GI malignancies [15,18]. However to the best of our knowledge, no national and international study has

correlated production of tumour antigens (CEA and AFP), with immunological state of the patient by cytokine (IL-1a, IL-2R) and immunoglobulin assays. It is concluded that immunological state of the patient may have some relevance in production of tumour antigens and may be helpful in the diagnosis of these malignancies. In future, treatment with anticytokine therapy may have to be regulated in different low and high tumour antigen producing malignancies. The finding also may lead to the cause of immunological behaviour of various malignancies. Keeping this thought process in mind, further studies are however recommended.

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