RESEARCH ARTICLE

Comparative Study of Carcinoembryonic Antigen Tumor Marker in Stomach and Colon Cancer Patients in Khyber Pakhtunkhwa

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Abstract

Background: Due to the increase in morbidity and mortality rate, cancer has become an alarming threat to the human population worldwide. Since cancer is a progressive disorder, timely diagnosis would be helpful to prevent/stop cancer from progressing to severe stage. In Khyber Pakhtunkhwa, Pakistan, most of the time, tumors are diagnosed with endoscopy and biopsy; therefore rare studies exist regarding the diagnosis of gastrointestinal (GIT) carcinomas based on tumor markers, especially CEA. Objectives: This study made a comparative analysis of CEA in admitted hospitalized stomach and colon cancer patients diagnosed as GIT with biopsy. Materials and Methods: In this study, a total of 66 cases were included. The level of CEA was determined in the blood of these patients using ELISA technique. Results: Out of 66 patients, the level of CEA was high in 59.1% of the total, 60.7% in colon cancer patients and 57.9 % in stomach cancer patients. Moreover, the incidence of colorectal and stomach cancer was greater in males as compared to females. Patients were more of the age group of 40-60 and the level of CEA was comparatively higher in patients (51.5%) with histology which was moderately differentiated, than patients with well differentiated and poorly differentiated tumor histology. Conclusions: CEA level was high in more than 50% of the total patients. Moreover, CEA exhibited higher sensitivity for colon than stomach cancer.

Keywords: Gastrointestinal tumors - CEA - Khyber Pakhtunkhwa - enzyme linked immunosorbant assay

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Introduction

Cancer is a disorder in which cells divide abnormally and is an important health problem for human population, despite the advancement in medical and health technologies (Hanahan et al, 2000). An annual increase for GIT (gastrointestinal tract) carcinoma which is the most common among all the malignancies has been observed. Comparatively, this carcinoma is found mostly in younger age groups (Chaudhary et al, 2010) (Sisik et al, 2013).

In gastrointestinal system, colorectal and gastric cancers are common types of cancers (Sisik et al, 2013). Worldwide, colorectal cancer has been considered to be the second common cause of death. Colorectal cancer is named as disease of western world, due to the leading cause of death in western countries of the world (Gul et al, 2012). According to an evaluation in Europe, gender ratio of colon cancer with a survival rate of five years, ranges from 26-56 % in males and 29-59 % in females (Al- Shuneigat et al, 2011). Therefore, in a population based study conducted in Karachi (Pakistan) from 1995-1999, colorectal cancer has been reported to be the 6th and 9th prevailing cancer in male and female respectively (Hadi et al, 2009).

Among the gastrointestinal malignancies, stomach cancer is another common carcinoma and it has been observed to be wide spread mostly in Asia and Europe (Sisik et al, 2013) (Oue et al, 2004). It was estimated that 650,000 patients die annually due to this cancer.

For diagnosis of these cancers, tumor markers are valuable tools in the blood of the affected individuals. In serum, high concentration of these markers reflects the disease occurrence. For each particular organ carcinoma, a specific tumor marker was used to detect and diagnose the particular organ carcinoma (Al-Saady et al, 2012).Usually, these are soluble molecules in blood and can be used for screening, diagnosis, prognosis, measuring response to therapy and tracking of cancer recurrence (Attaullah et al, 2006). Among the tumor markers, CEA (Carcino Embryonic Antigen) is a glycoprotein with amolecular weight of about 200kDA (Dbouk et al, 2007). It is used for the diagnosis and prognosis of GIT carcinomas, i.e.

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stomach and colon. Since its discovery in 1965, it has been used as a tumor marker for colorectal cancer. Moreover, this is useful in identification of ovary, breast, pancreas and liver cancer (Al-Saady et al, 2012) (Ghoraishian et al, 2006) (Tan et al, 2009) (Liu et al, 2012) (Rezamansourian et al, 2011). Elevated CEA levels in serum indicate malignant condition while it can be present at low concentration in serum of healthy individuals (Moldrich et al, 2010). CEA can be measured through different techniques like Magnetic Particle Enzyme Immunoassay (MEIA) (Tan et al, 2009), Enzyme Linked Fluorescent Assay (ELFA) (Liu et al, 2012), Enzyme Linked Immunosorbent Assay (ELISA) (Al-Saady et al, 2012), Micro particle Enzyme Immunoassay (Abdel-Gawad et al, 2008), Radio Immuno Assay (RIA) (Wang et al, 1999) and Electroluminescence Immunoassay (ECLIA) (Herszenyi et al, 2008).

This study was conducted using ELISA technique due to high sensitivity, easy availability of kits in market and rapid results. In Pakistan and especially in KPK, the evaluation of CEA, as tumor markers in diagnosis of Colon and gastric cancer has not been done; thus this was the reason we conducted this study to know about the importance of serum CEA level in colon and gastric cancer patients and also to compare its level in both cases.

Materials and Methods

Clinical data

This study included 66 patients already diagnosed by biopsy as GIT carcinoma patients and six healthy subjects as control group. None of the patient has taken anticancer therapy. The patients and healthy subjects were selected from IRNUM Peshawar, KPK. In addition, written informed consent was obtained from each patient.

Ethical approval

This study was approved by the ethics and research committee of the centre of Biotechnology and Microbiology, University of Peshawar.

Inclusion criteria

Patients who have been diagnosed recently by endoscopic examination and biopsy, having stomach and colon cancer were included in the study. Moreover, none of the patient has taken any anticancer therapy. Healthy subjects were screened for any abnormality and also their habits of smoking and alcohol consumption were observed.

Exclusion criteria

Patients who have been admitted in hospital within the past two years and had taken anti-cancer therapy were not included in the study. Also, the healthy subjects who were addicted to smoking and alcohol consumption were excluded from the study population.

Study design

Blood collection: Study population were divided into

Table 1. Colon Cancer Patients' Demographics

Age group	CEA conc. (ng/ml)				Gender		Histological tumor grade	
	Patients ID	Result	Patients ID	Result	M	F		
S20-40	+	-						
	C1	259	C8	4.9			Poorly differentiated	2
	C2	36.2	C9	4.8			Moderately differentiated	10
	C3	25	C10	2.4			Well differentiated	2
	C4	5.4	C11	2.2	8	6		
	C5	5.2	C12	1.6				
	C6	6.9	C13	1.1				
	C7	6.0	C14	0.7				
Total	7		7					
40-60	C15	198	C23	5.0			Unknown	1
	C16	124	C24	2.8			Poorly differentiated	4
	C17	104	C25	2.5			Moderately differentiated	5
	C18	7.6	C26	0.6			Well differentiated	2
	C19	7.5			5	7		
	C20	6.1						
	C21	6.5						
	C22	6.2						
Total	8		4					
60-80	C27	114	0				Poorly differentiated	1
	C28	6.3	0		0	2	Moderately differentiated	1
Total	2		0					

*C stands for colon cancer, Ng/ml for nanogram per mili litre, C1 to C28 for number of individuals having colon cancer

Table 2. Stomach Cancer Patient Demographics

Age group	CEA conc. (ng/ml)				Gender		Histological tumor grade
	Patients ID	result	Patients ID	result	M	F	
20-40	+		-				
	S1	77.9	S6	4.5			Un known 1
	S2	63.1	S7	1.9			Poorly differentiated 3
	S3	11.7	S8	1.79	5	4	Moderately differentiated 5
	S4	65.7	S9	1.4			Well differentiated 0
	S5	5.1					
Total	5	4					
40-60	S10	289	S23	2.3			Poorly differentiated 12
	S11	280	S24	1.5			Moderately differentiated 7
	S12	190	S25	1.6			Well differentiated 0
	S13	151	S26	0.6			
	S14	47.5	S27	0.5			
	S15	25	S28	0.4			
	S16	15.8			12	7	
	S17	13.7					
	S18	5.9					
	S19	5.5					
	S20	5.4					
	S21	5.3					
	S22	5.3					
Total	13		6				
60-80	S29	133	S33	4.1			Un known 1
	S30	9.8	S34	3.7			Poorly differentiated 2
	S31	8.6	S35	3.3			Moderately differentiated 6
	S32	6.5	S36	2.4	8	2	Well differentiated 1
	4		S37	1.5			
			S38	1.4			
Total	4		6				

*S stands for Stomach cancer, Ng/ml for nanogram per mili litre, S1 to S38 for number of individuals having Stomach cancer

three groups; 1st group included 28 colon cancer patients, 2nd group included 38 stomach cancer patients and 3rd group included 6 healthy subjects. Blood samples were collected from all of the patients and healthy subjects followed by serum separation by centrifugation. Also, isolated serum was stored in eppendorf tubes at - 20 0C till further use.

CEA Determination

CEA level in serum was determined using ELISA kit (Monobind Inc. USA), according to the manufacturer instructions. Simply, 25ul (Microlitre) of patient`s sample and 100ul of enzyme labeled antibody (Antibody used was mouse IgG) were added to a 96-well microplate, coated with antigen and incubated for an hour at room temperature. Washing was performed, followed by the addition of substrate. The reaction was stopped by adding a stop solution and finally florescent was measured for each sample.

Results

To evaluate CEA tumor marker for diagnosis of colon and stomach cancer, different age groups population was selected. The age groups were, 20-40, 40-60 and 60-80. Therefore, the results of the three groups are shown below:

Results of the 1st groups: A total of 28 colon cancer patients were included in the study and out of 28, 17 (60.7%) patients showed positivity for CEA. Among different age groups, level of CEA was high in 7, 8 and 2 individuals, in 20-40, 40-60 and 60-80 age groups respectively.

Regarding histological tumor grade, different age groups, had different histology like among the age group of 20-40; 2 patients were found with poorly differentiated histological tumor grade,10 with moderately differentiated and 2 with well differentiated tumor histology. In the age group of 40-60, 4 patients were found with poorly differentiated, 5 moderately differentiated, 2

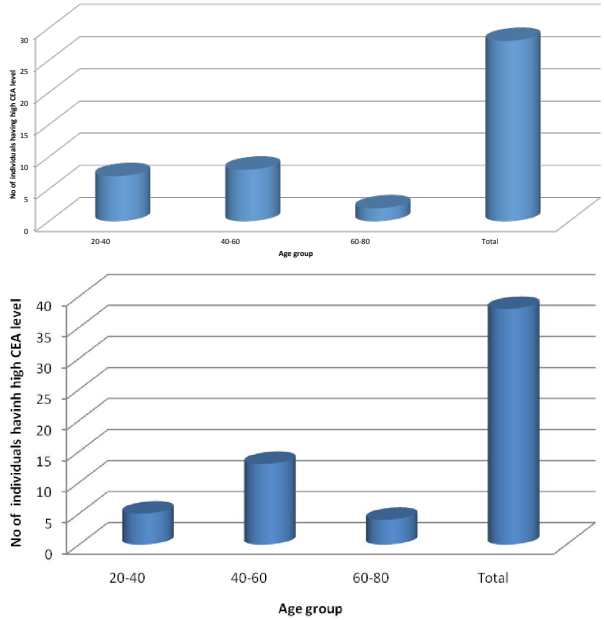

Figure 1. (a): Level of CEA in Different Age Groups of Colon Cancer Patients. (b): Level of CEA in Different Age Groups of Stomach Cancer Patients

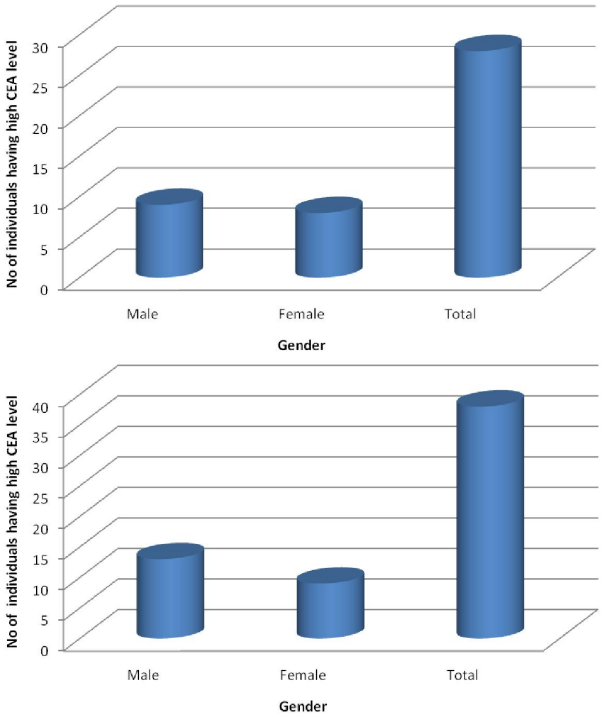

Figure 2. (a): Level of CEA in male and female individuals of colon cancer. (b): Level of CEA in male and female individuals of stomach cancer

well differentiated histological tumor grade and 1 with unknown results for tumor grade. For patients with age group of 60-80, 1 patient was found with poorly differentiated and 1 with moderately differentiated tumor grade (Table 1).

Results of the 2nd group

Beside colon cancer patients, 38 patients having stomach cancer were also included in the study and out

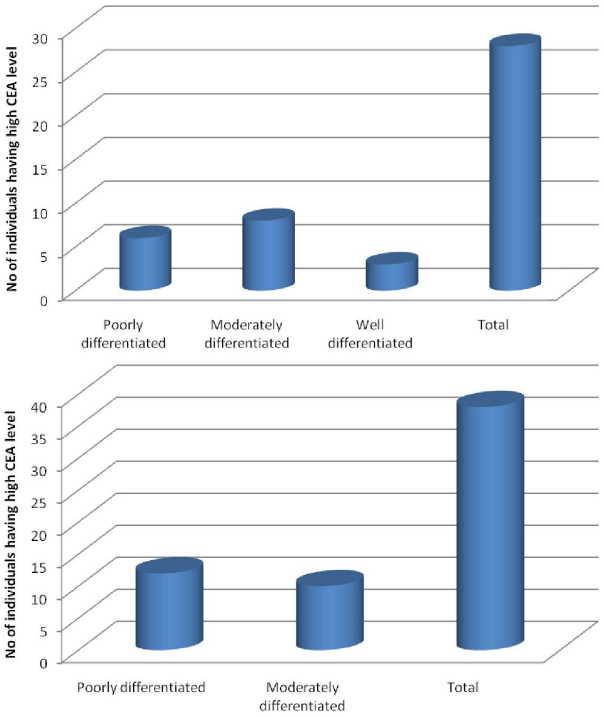

Figure 3. (a): Level of CEA in Patients of Colon Cancer Having Different Histology. (b): Level of CEA in Patients of Stomach Cancer Having Different Histology

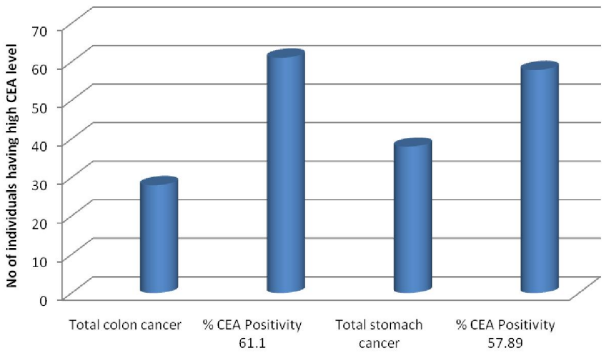

Figure 4. CEA Positivity in Colon and Stomach Cancer Patients

of 38, 22 (57.89%) patients showed positivity for CEA. In this, the number of individuals having high CEA level in different age groups of 20-40, 40-60 and 60-80 were 5, 13 and 4 respectively. While tumor histology among these age groups were also different, like in age group of 20-40, 3 patients were found with poorly differentiated, 5 moderately differentiated tumor histology and 1 with unknown results for tumor grade . For patients with age group of 40-60, 12 patients were found with poorly

Table 3. Detail of Normal Healthy Controls

Controls	CEA conc. (ng/ml) Cut off value for CEA=5
Control 1	4
Control 2	3.9
Control 3	1.3
Control 4	2.9
Control 5	0.8
Control 6	2.9

differentiated histological tumor grade and 7 with moderately differentiated tumor histology. In another group of age 60-80, 2 patients were found with poorly differentiated, 6 with moderately differentiated tumor histology, and 1 with well differentiated histological tumor grade and 1 with unknown results for tumor grade (Table 2).

Results of the 3rd group

In this study, one control group was also included and the sera of all the 6 healthy individuals showed negative results for CEA i.e.; below the cut off value of 5ng/ml (Table 3).

Moreover, Comparative study in respect to age group, gender based tumor histology and role of CEA as diagnostic tool in colon and stomach cancer patients has also been done (Figure 1, 2, 3 and 4).

Discussion

Cancer, an uncontrolled growth of the cells has become an alarming threat for the human population. It has been estimated that 12.7 million people are suffering from cancer and has been the major cause of the mortality of about 7.6 million, worldwide (Jemal et al, 2011). Of all the malignancies, GIT carcinoma is the most common, worldwide. (Sisik et al, 2013). Among GIT carcinoma, colorectal and stomach cancer, has affected over 1 million and 933,900 individuals respectively (Haggar et al, 2009) (Wang et al, 2009). Most commonly, these cancers are present in Western countries, Asia and Eastern Europe (Sisik et al, 2013) (Gul et al, 2012). Beside its prevalence in other parts of the world, in Asia, especially in Pakistan, its prevalence has also been reported (Ahmad et al, 2005) (Khan et al, 2011).

This study, based on determination of CEA level in sera of admitted patients, revealed the presence of cancer. In this study, out of 66 admitted patients, CEA level was high in 59.09% of the patients, 57.89% stomach and 60.7% colon respectively [Table 1and 2]. Before, no study has been conducted to screen out cancer on the basis of CEA in KPK, but for the first time, we have reported this.

CEA based diagnosis of GIT carcinomas

CEA level based cancer proportion was different in different age groups. In this study, level of CEA was high in colorectal and stomach cancer patients having age group of 40-60 [Figure 1]. Other studies have also showed similar relationship of the level of CEA with age (Hadi et al, 2009) (Laishram et al, 2010). However, comparative study has shown contradictory results in comparison with the current study of patients' age group, when sensitivity test of CEA, CA19-9 and CA72-4 in patients with upper gastrointestinal cancers was performed.

Hence, it has also been reported that the incidence of gastric carcinoma increases with the increase in age. Like a study about gastric adenocarcinoma, has shown that with the increase in age, the chances of occurrence of carcinoma are more (Mcgrath et al, 2007).

Current study indicated that the incidence of colorectal and stomach cancer based on the level of CEA was more in males as compared to females [Figure.2]. Such results have also been shown by different studies, conducted nationally as well as internationally (Hadi et al, 2009) (Ahmad et al, 2005) (Khan et al, 2011). Correlation of CEA with histology has also been observed in the present study. Like level of CEA was comparatively higher in patients (51.5%) having histology of moderately differentiated than patients with well differentiated and poorly differentiated tumor histology [Figure 3]. Similar results have also been shown by other studies too, like a retrospective study for colorectal carcinoma reported tumor histology of 37% well differentiated, 52% moderately differentiated and 34% poorly differentiated (Shaikh et al, 2009) (Fazeli et al, 2007). In one of the studies, most of the patients with colon and stomach cancer were found with moderately differentiated histology [3]. Serum CEA production has also been investigated to be higher in well differentiated adenocarcinoma than moderately and poorly differentiated adenocarcinoma for colorectal cancer (Suwanagool et al, 1990). In our study, CEA level in well differentiated tumor was near the cut-off level and it was high in moderately and poorly differentiated tumors in colon and stomach cancer respectively.

In the present study, CEA showed positivity for both colon and stomach cancer; and high positivity of CEA was observed for colon (60.7%) than stomach (57.89%) cancer [Figure 4]. In a comparative study of CA24-2 and CEA, sensitivity of CA24-2 for both colon and rectal cancer was 39%, while the sensitivity of CEA was 40% for colon and 47% for rectal cancer respectively (Carpelan et al, 1996). In other studies too, sensitivity of 74% and 48% for CEA in colon cancer was reported (Attaullah et al, 2006) (Bagaria et al, 2013). Similarly, role of CEA in gastric cancer has also been shown by different studies. Like, a study conducted on the role of serum and gastric juice levels of CEA, CA19-9 and CA72-4 in patients affected by gastric cancer, CEA showed a positivity rate of 57.6%, indicating CEA to be a good prognostic factor in gastric cancer (Tocchi et al, 1998). Patients with elevated CEA levels have high recurrence risks for gastric cancer. In gastric cancer patients, serum CEA levels can be considered as an independent prognostic factor (Kochi et al 2000).

The ultimate purpose of diagnosis is the cure. Tumor marker concentration is primarily determined to monitor the success of treatment. The remaining procedure used for treatment assessment like x-ray, magnetic resonance and scanning are comparatively costly and also involves human health risks. Increased levels of CEA during the initial diagnosis provide greater prognostic significance and can benefit clinical practice. With the advancement in screening, diagnostic and targeted therapeutic techniques, the survival rates may be increased. Hence, the need exist to have some molecular studies for actual diagnosis of tumor.

This study concluded that CEA can be used as a diagnostic marker for the screening of both colon and stomach cancer and CEA exhibited the highest sensitivity for colon than stomach cancer.

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