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Dr. Kruti P Ladani
Third Year Resident, Department of Pathology, Shri M.P. Shah Medical College, Jamnagar, Gujarat, India

Dr. Dhara P Trivedi
Associate Professor, Department of Pathology, Shri M.P. Shah Medical College, Jamnagar, Gujarat, India

Dr. Amar R Chapla
Senior Resident, Department of Pathology, Shri M.P. Shah Medical College, Jamnagar, Gujarat, India

Corresponding Author:
Dr. Kruti P Ladani
Third Year Resident, Department of Pathology, Shri M.P. Shah Medical College, Jamnagar, Gujarat, India

Study of carcinoembryonic antigen tumor marker in gastrointestinal pathology

Dr. Kruti P Ladani, Dr. Dhara P Trivedi and Dr. Amar R Chapla

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Abstract
Background: Cancer mortality and morbidity has been increased in recent years, with gastrointestinal cancers comprising the majority of overall malignant conditions. All Indian cancer registries identify the digestive system as the most common cancer site in males. In women, breast cancer exhibits the highest occurrence, followed by cancers of the genital organs and the digestive system. Tumor markers are usually proteins or glycoproteins produced by the body in response to cancerous growth or by the cancer tissue that are possible to detect in serum, urine, or tissue samples. CEA assay is an inexpensive and easy to perform test; however, repeated measurements to monitor disease, follow up and the response to therapy contribute to higher costs and increase laboratory workload.
Materials and Methods: The present study was carried out on 100 patients. Type of sample includes blood sample in plain vacutte received in tumor marker section of the pathology department.
Objective:
- To make a comparative analysis of CEA in various gastrointestinal lesions.
- Pre and post operative study of Serum CEA in known case of gastrointestinal carcinoma.
- To study the factors affecting serum CEA concentrations.

Results: The present study was conducted from June 2018 to December2020 in the Department of Pathology, at tertiary care hospital, 100 cases were studied. Preoperative and postoperative mean CEA value, Mean CEA value in inflammatory, Benign and Malignant lesions as well as in well, moderately and poorly differentiated carcinoma were measured.
Conclusion: In general, this study showed that serum CEA levels are increased in people with habit of cigarette smoking and in benign and inflammatory lesions of GIT along with Malignant GI lesions. Carcinoembryonic antigen (CEA) usually normalizes after surgery. After curative resection of primary CRC (colorectal carcinoma), a large number of patients' CEA levels declined to normal values within 4-6 weeks. Amongst many contradictory results, the measurement of CEA is a useful method for screening, diagnosis, follow-up and treatment of CRC but Physiological influences that need to be considered in interpreting the results include effects of aging and smoking. Serum CEA values fails to decline in patients with distant metastasis and advanced disease even after complete surgical removal of the tumour. Serum concentrations of CEA tend to be higher in patients with well-differentiated tumors compared with those with poorly differentiated tumors.

Keywords: Carcinoemryonic antigen (CEA), gastrointestinal lesion, pre and post operative

Introduction
Cancer mortality and morbidity has been increased in recent years, with gastrointestinal cancers comprising the majority of overall malignant conditions All Indian cancer registries identify the digestive system as the most common cancer site in males. In women, breast cancer exhibits the highest occurrence, followed by cancers of the genital organs and the digestive system [1].
Tumor markers are usually proteins or glycoproteins produced by the body in response to cancerous growth or by the cancer tissue that are possible to detect in serum, urine, or tissue samples. This group of protein is also observed in many healthy individuals, so it is not their presence in serum but their quantity that makes tumor markers useful [2].
CEA is glycoprotein with a molecular weight of approximately 200 KD. It is the first of the so called carcinoembryonic proteins and was discovered by Gold and Freedom in 1965 [3]. CEA is a glycosylphosphatidylinositol-cell surface anchored glycoprotein with specialized sialofucosylated glycoforms that act as functional colon carcinoma L-selectin and E-selectin ligands, which may significantly affect the metastatic dissemination of colon carcinoma [4].

It is expressed in normal mucosal cells and it is over expressed in adenocarcinoma, especially in colorectal Cancer. Elevated levels of CEA is also seen in other malignancies such as salivary gland tumors i.e. carcinomas with glandular differentiation and can also be observed in squamous cell carcinomas [5].

CEA assay is an inexpensive and easy to perform test; however, repeated measurements to monitor disease, follow up and the response to therapy contribute to higher costs and increase laboratory workload. The aim of this study is to investigate the status of serum CEA values and evaluation of cigarette smoking, age and sex effects on carcinoembryonic antigen levels among gastrointestinal lesions.

Aims & objectives

- To make a comparative analysis of CEA in various gastrointestinal lesions.
- Pre and post operative study of Serum CEA in known case of gastrointestinal carcinoma.
- To study the factors affecting serum CEA concentrations.

Type of study: Prospective study.

Materials & methods

- **Place of study:** The present study was carried out at the department of pathology in collaboration with the various departments in our hospital.
- **Design of study:** A diagnostic prospective study.
- **Duration of study**: 2.5 year
- **Sample size:** Total 100 cases.
- **Sample types:** Pre and post operative blood samples received in tumor marker section of the pathology department.

Principle of the assay

The CEA test is a solid phase sandwich ELISA method. The samples and anti- CEA-HRP/Biotin conjugate are added to the wells coated with streptavidin. CEA in the patient's sample forms a sandwich between two specific antidotes to CEA. Unbound protein and HRP conjugate are washes off by wash buffer. Upon the addition of the substrate, the intensity of color is proportional to concentration of CEA in the sample. A standard curve is prepared relating to color intensity to the concentration of the CEA.

1. Materials provided

- Micro well strip (96 wells): streptavidin coated wells.
- CEA standard: 7 vials
- CEA enzyme conjugate: TMB Substrate
- Stop Solution
- 20X Wash concentrate

2. Material and Equipment Needed

- Distilled water or deionized water
- Precision pipettes
- Disposable pipette tips
- ELISA reader capable of reading absorbance at 450nm
- Absorbance paper or paper towel
- Graph Paper
- Timer
- Manual or automatic equipment for rinsing well

3. Storage and Stability

- Store the kit at 2-8 c.
- Keep microwells sealed in a dry bag with dessicants.
- The reagents are stable until the expiration of the kit.
- Do not expose test reagents to heat, sun or strong light.

4. Preparation Of Assay

- All reagents and samples are brought to room temperature and gently mixed
- Once the test has begun it must be performed without any interruptions to get the most reliable and consistent results
- New disposable tips were used for each specimen

5. Procedure

- Place the desired number of coated strips into the holder
- Pipet 25 ul of CEA standards, control and patient's sample into designated wells
- Add 100 ul of ready to use enzyme conjugate to all wells. Shake for 10-30 seconds.
- Cover the plate and incubate for 60 minutes at room temperature
- Remove liquid from all wells. Wash wells three times with 300ul of wash buffer. Blot on absorbent paper towels.
- Add 100 ul of TMB substrate to all wells.
- Incubate for 15 minutes at room temperature.
- Remove liquid from all wells. Wash wells three times with 300 ul of 1x wash buffer. Blot on absorbant paper towels.
- Add 100 ul of TMB substrate to all wells.
- Incubate for 15 minutes at room temperature.
- Add 50 ul of stop solution to all wells. Shake the plate gently to mix the solution.
- Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stopping solution.

6. Warnings and Precautions

- For Research Use Only.
- Not for use in diagnostic procedures.
- For Laboratory use.
- Not for Internal or External Use in Humans or Animals.
- There should be no eating or drinking within work area.
- Always wear gloves and a protective lab coat.
- No pipetting should be done by mouth.
- Handle all specimens and reagents as potentially infectious and biohazardous.
- Do not add sodium azide to samples as preservative. • Do not use external controls containing sodium azide.
- Use disposable pipette tips to avoid contaminating chromogenic substrate reagent.
- Do not freeze reagents.
- Do not mix reagents from different kit lot numbers.
- Keep reagents out of direct sunlight.
- Handle stop reagent with care, since it is corrosive.
- Viscous forensic samples should always be diluted in phosphate buffered saline or distilled water prior to pipetting.
- Ensure the bag containing the micro-plate strips and desiccant is sealed well, if only a few strips are used.

Inclusion criteria

- All patients suffering from various gastrointestinal lesions from various departments.
- All cases with relevant clinical history of gastrointestinal lesion which is proven radiologically or

pathologically,

Exclusion criteria

All patients with a clinical history of abdominal pain and other symptoms which are not due to gastrointestinal lesions.

Results

The present study was conducted from June 2018 to December2020 in the Department of Pathology, at tertiary care hospital, 100 cases were studied and following observations were made.

Table 1: Information regarding studied cases (Distribution of cases according to preop/post op CEA values)

Sr. No.	Studied Cases	No.
1	Total cases studied	100
2	Pre-op CEA value measured	100
3	Post-op CEA value measured	60
4	Duration of post op value measured after surgical removal	3 months
5	Postoperative value not measured	40
6	Reasons for not measuring	
	Inflammatory and benign condition	38
	Patient died during followup	02

Age Group: In our study we observed that most patients with gastrointestinal tract lesion were from 4th - 8th decade. Out of 100 patients, majority (61%) of the patients were belonging to 41 to 60 years age group, followed by 61 to 80 years age group (24%) and 14% in the age group of 21 to 40 years of age while only 1 patient was below the age of 20 years. There was no case reported above the 80 years of age during the present study.

Gender: In present study we observed mal preponderance (67%) in cases of GI lesions, females being 33%.

Association with smoking: Out of 100 cases, history of smoking is present in 57 patients in whom serum CEA levels were higher than those with non-smokers. This shows that smoking has some role for elevation of CEA level as well as pathogenesis of carcinoma.

Table 2: Organ involvement by different condition and mean CEA values

Name of organ	Inflammatory cases	Benign Condition	Malignant condition
Esophagus	-	-	04(6.45%)
Stomach	1(3.70%)	2(18.18%)	06(9.68%)
Small Intestine	12(44.44%)	3(27.27%)	11(17.74%)
Large Intestine	6(22.22%)	4(36.36%)	18(29.03%)
Anorectal	-	2(18.18%)	21(33.87%)
Pancreas	2(7.40%)	-	1(1.61%)
Gall Bladder	5(18.52%)	-	1(1.61%)
Liver	1(3.70%)	-	-
Total	27	11	62
Mean CEA value	8.01	10.41	95.92

It is observed that Serum CEA levels are at lower side < 5 ng/ml in inflammatory lesions while serum CEA levels markedly raised >20 ng/ml, are seen in malignant cases. While CEA levels are midway between 5-20 ng/ml in benign cases.

There is consistently higher levels of CEA in malignant lesions and therefore, serum CEA levels can be used as screening test for carcinomas of GI tract.

It is observed that majority of inflammatory cases are seen in small intestine (44.44%) and large intestine (22.22%), followed by gall bladder involvement (18.52%) in the form of cholecystitis, while the rest of cases are in liver, pancreas and stomach involvement.

There is predominantly involvement of small and large intestine in benign cases while the stomach and rectum shows the same incidence of benign cases (18.18%).

In present study out of 62 cases of malignancy, 33.87% were anorectal carcinoma, 29.03% were colon carcinoma while small intestinal malignancy (17.74%) and carcinoma of stomach were 9.68% and other malignant cases included pancreatic carcinoma and gall bladder carcinoma.

Table 3: Number of adenocarcinoma cases according to site of involvement with mean CEA value (n=60)

Sr. No.	Malignancy site	No. of cases	Mean CEA value (ng/ml)	Percentage (%)
1	Esophagus	03	195.58	5
2	Stomach	06	63.49	10
3	Small intestine and ileocecal junction	10	82.99	16.67
4	Colon	18	109.60	30
5	Rectum and Anal canal	21	99.11	35
6	Pancreas	01	17.73	1.67
7	Gall bladder	01	87.25	1.67
	Total No. of cases	60 cases	95.92	100

Patients with tumors in left side of the colon (61.11%) were more than right side of the colon (38.89%).

In present study, among total 60 cases of Adenocarcinoma, 32 cases (53.33%) were well differentiated type, 24 cases

(40%) were moderately differentiated while 4 cases (6.67%) were poorly differentiated type. Mean value of CEA is maximum (111.07) in well differentiated type while moderately differentiated explain low CEA value (87.46), while it is minimum (64.88) in case of poorly differentiated gastrointestinal carcinoma.

There are two cases were identified other than adenocarcinoma of gastrointestinal tract. One was of lymphoma which showed the CEA value of 17.78 ng/ml while the other case was of Squamous cell carcinoma of esophagus which had the CEA value of 16.28 ng/ml.

Mean preoperative CEA values were higher (>20 ng/ml) in majority of malignant cases (95.16%) while it was in the range of 5-20 ng/ml in 4.84% of cases.

In present study after noting the postoperative serum CEA values, it is observed that there is marked decline in serum CEA value in cases with complete surgical resection of the tumor (52 cases-83.87%). While 7 cases (11.29%) had no decline in CEA values rather few of them showed some increase in CEA value. Most of these cases were having widely metastatic disease or had recurrent disease.2 patients died during follow up after surgery while follow up of 1 patient was not available.

Out of total 62 cases 10 cases had distant metastasis and the mean serum CEA value for these patients were 181.27 ng/ml while the remaining 52 cases without metastatic disease had mean serum CEA values of 71.89 ng/ml.

Discussion

The present study was conducted from June 2018 to December 2020 in Department of Pathology, at tertiary care hospital. 100 cases were studied.

Carcinoembryonic antigen (CEA) describes a set of highly related glycoproteins involved in cell adhesion. CEA is normally produced in gastro-intestinal tissue during fetal development, but the production stops before birth. Therefore, CEA is usually present only at very low levels in the blood of healthy adults. However, the serum levels are raised in some types of cancer, which means that it can be used as a tumor marker in clinical tests.

The result obtained from present study are discussed as follows.

Table 4: (Comparative study of total number of cases studied)

No	Author	No of cases
1	Bhawna bagraria *et al* (2013) [6]	150
2	Mohammad reza younesi *et al* (2016) [7]	125
3	Konishi *et al* (2017) [8]	1027
4	Weiqiang you *et al*) (2020) [9]	1008
5	Present study (2020)	100

Sample size is an important parameter that can affect the results of the study. A Larger study can find many confounding factors.

Most of the studies had predominant male patients with 67% in the present study. Weiqiang you *et al*. (2020) [9] and Bhawna bagraria *et al*. (2013) [6] had 60% and 61.34% male patients, respectively. While Konishi *et al*. (2017) [8] had 50.4% male patients among their cases.

Weiqiang *et al*. [9] have noted that 794 (78.8%) of patients were well or moderately differentiated, while 214 colorectal carcinoma (21.2%) were poorly differentiated. In present study, we have divided all cases of adenocarcinoma into three subcategories. i.e. well differentiated, moderately differentiated and poorly differentiated. Of which well differentiated accounts for 53.34%, moderately differentiated accounts for 40.0% and poorly differentiated accounts for 6.67%

Several studies have shown that well-differentiated colorectal cancers produce more CEA per gram of total protein than poorly differentiated specimens. A lack of differentiation or poor differentiation may explain why some patients with advanced colorectal cancer do not have increased serum CEA values.

C. Aggarwal *et al*. [10] conducted the study in 2013 and it showed that cases without metastatic disease had lower mean CEA values than cases with metastatic disease.

Present study also showed the same results of higher mean CEA values in patients with metastatic disease and lower mean CEA values in patients without metastatic diseases.

Afshin Shafaghi *et al*. [11] noted that mean pre-operative CEA value was in range 110.18 ng/ml. In present study we also noted mean preoperative CEA value in Malignant cases were 95.92 ng/ml.

Gota saito *et al*. [12] noted that patients with tumors in the left side of the colon were more than right side of the colon. In present study, left side cases were more than right side of colon.

Wnebo H J *et al*. [13] and Slater G *et al*. [14] noted that patients with tumours in left side of colon generally have a higher incidence of increased CEA concentrations than those with malignancy on the right side of colon. In present study, we also noted that from cases of colon carcinoma, left side preference is seen over right side of colon.

Conclusion

The present study was aimed at making a comparative analysis of CEA in various gastro intestinal lesions.

In general, this study showed that serum CEA levels are increased in people with habit of cigarette smoking and in benign and inflammatory lesions of GIT along with Malignant GI lesions.

Carcinoembryonic antigen (CEA) usually normalizes after surgery. After curative resection of primary CRC (colorectal carcinoma), a large number of patients' CEA levels declined to normal values within 4-6 weeks. Residual disease should be suspected if the CEA level does not return to normal. Care was taken to allow sufficient time following surgery before measuring CEA to allow CEA normalization (the half-life of CEA is 3-7 days.)

Amongst many contradictory results, the measurement of CEA is a useful method for screening, diagnosis, follow-up and treatment of CRC but Physiological influences that need to be considered in interpreting the results include effects of aging and smoking.

Serum CEA values fails to decline in patients with distant metastasis and advanced disease even after complete surgical removal of the tumour. Hence, persistent elevated serum CEA levels should alert the pathologist and the surgeon about either residual disease or distant spread of the disease.

Other diagnostic tools, should be considered for patients with elevated levels of CEA, such as colonoscopy, chest, abdominal and pelvic CT scans and physical examination before treatment, because in our findings it is indicated that many non-neoplastic lesion and benign conditions are associated with elevated CEA values, which may lead to misinterpretation of the CEA levels.

Serum concentrations of CEA tend to be higher in patients

with well-differentiated tumors compared with those with poorly differentiated tumors.

Conflict of Interest
Not available

Financial Support
Not available

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