Evaluation of Ca19-9 Tumor Marker in Gastric, Pancreatic and Colorectal 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 risk to the human population all over the world. As cancer is a progressive disorder, hence timely diagnosis would be helpful to prevent cancer from further progressive severe stage. In Pakistan and especially in Khyber Pakhtunkhwa (KPK), most of the time, tumor is diagnosed with endoscopy and biopsy. Although studies exist regarding diagnosis of gastrointestinal tract (GIT) carcinoma based on tumor marker, especially CA19-9, but rare is known about its upmost importance in diagnosis and screening as best tumor marker in pancreatic, stomach and colorectal cancer.

Objectives: The objectives of current study were to evaluate the serum level of CA19-9 as best tumor marker in stomach, pancreatic and colorectal cancer patients admitted in hospitals, who

have been diagnosed as GIT carcinoma patients with biopsy.

Methodology: In this study, a total of 73 admitted patients having gastric, pancreatic and colorectal cancer were included. The level of CA19-9 was determined in the blood of these patients using Enzyme Linked Immunosorbant Assay (ELISA) technique.

Results: Out of 73 patients, CA19-9 level was high in about 47.94% of the patients. CA19-9 level was high in pancreatic patients (81%) followed by colorectal (52%) and least was in stomach (23%). Moreover, the incidence of gastric, pancreatic and colorectal cancer was more in males as compared to females and patients were more of the age group of 40-60.

Conclusion: CA-19-9 level was high in pancreatic cancer patients followed by colorectal and least was in stomach cancer patients. Moreover male were affected and age group of 40-60 had high cancer incidence.

Key words: Cancer, Khyber Pakhtunkwa, CA19-9, ELISA

Introduction

Cancer is an unusual cells growth and causes a serious health problem in affected individuals. Cancer results when cells divide out of control and eventually acquire the ability to spread beyond to their prescribed boundaries. Cancer is sometime enhanced in the body parts by different factors or individuals depended actions/habits like smoking can cause throat, mouth and lungs cancer whereas the use of alcohol is the reason of liver cancer (1). Disclosure to emission, sunlight and ultra-violet rays can cause skin cancer. The broad symptoms of cancer are relentless, weight loss, fatigue, fever, and change of metabolism, blood clots, weakness, dizziness, and wounds that do not cure. All over the world, every year round about 11 million human beings are diagnosed with cancer (2). In 2020, the figure will exceed from 16 million cases (3). The mortality and morbidity rates in Pakistan are unknown till date. As with increase in the rate of cancer related mortality and morbidity, it is considered as the third most important cause of death, worldwide. In cancer patients, the major cause of death is metastatic lesion, rather than primary tumor. About 14.1 million new cases were reported in 2012 (4). Amongst different carcinomas, the most concession and contributor's carcinomas are colorectal, pancreatic, lung, breast and prostate. Worldwide, all these may account for 18-50% of the

individual loss per year (5). Among these, contribution of lung cancer is about 1.825 million, breast cancer 1.677 million, stomach 0.952 million and colorectal cancer 1.36 million (6).

Gastric cancer is noticed generally in Asia and particularly in Eastern Europe while continuing to be a principal cause of cancer-related deaths (2). A lot of studies have projected that gastric and colorectal cancers show a major correlation with geographic location, socioeconomic structure and cultural habits of a population (7). Pancreatic cancer is an aggressive disease. Most cases and most deaths occur in developed countries (approximately two thirds) (8). Among all of the various types of cancer, the survival rate of pancreatic cancer patients is very much less and are the most terrible of any solid cancer, is the fourth most important cause of cancer related mortality and morbidity in Western countries (9). About 227, 000 deaths occur per year globally and the five-year survival rate is only 4.6 percent (10). Most patients of pancreatic cancer with screening methods are quite hard to be diagnosed properly, when already they are at the late stage of the cancer (11). In pancreas some growth is non-cancerous (benign), while the untreated by the passage of time may become cancerous**.**

For diagnoses and treatment of cancer patient, the best tool is tumor marker selection. Factors for tumor selection would be easy availability, time saving and non-subjective (12). In previous decade various biomarkers have been studied in the hope of finding an easy evaluation method for physicians. Initially to detect cancer patients at an early stage of tumor, and laterally to detect the treatment effectiveness, so that current treatment strategies can be altered.

An important serum marker, carbohydrate antigen 19-9 (CA19-9) (13), for pancreatic cancer is being noticed that it should be serum specific and sensitive (14). This tumor marker, CA19-9, has its screening applications in colorectal, gastric, pancreatic, hepatic and as well as in splenic carcinomas.

The aim of the current study was to determine the level of CA19-9 in different cancer patients like in gastric, colorectal, pancreatic using ELISA, the ratio of male, female and age wise among the affected individuals. Although, studies are designed based on extensive investigations like endoscopy and biopsy even for the simple screening and this a common practice here in KPK, that caused too much stress and burden over the affected persons, this

study will be helpful to highlight the importance of using simple ELISA based technique for screening of the persons. This in turn will be useful for the affected individuals to save time and money.

Materials and Methods

Ethical Approval

This study was approved by the ethical committee of the department of Biotechnology, Abdul Wali Khan University Mardan with the reference number, Awkum IRB # 129.

Study area

This study included 73 patients, already diagnosed by biopsy as GIT carcinoma patients, six healthy subjects as control group and none of the patient has taken anticancer therapy. The patients and healthy subjects were selected from Institute of Radiotherapy and Nuclear Medicine (IRNUM) Peshawar.

Collection of Blood

Study population were divided into three groups; 1stgroup included 41 colorectal cancer patients, 2nd group included 21 gastric cancer patients and 3rd group included 11 pancreatic cancer patients. Blood samples were collected from all of the patients and healthy subjects followed by serums separation by centrifugation.

Assay Procedure

The assay was carried out according to manufacturer's instructions (Biotech, USA). Simply, secured the desired number of coated wells in the holder. Dispensed 50μL of CA19-9 standards, specimens, and Controls into appropriate wells. Dispensed 100μL of assay buffer to each well mixed gently for 30 seconds and incubated at 37 °C for 60 minutes. Removed the incubation mixture by emptying the plate content into a waste container, rinsed and did empty the microtiter plate 5 times with washing buffer. Did strike the Microtiter plate sharply onto absorbent paper to remove all residual water droplets. Dispensed 100μL of enzyme conjugate reagent into each well and mixed and incubated at 37 °C for another 60 minutes.Dispensed

100μL of the TMB substrate reagent into each well and gently mixed for 10 seconds. Incubated at room temperature in the dark for 20 minutes without shaking. Stopped the reaction by adding 100μL of stop solution to each well.Gently mixed for 10 seconds, it is very important that the blue color completely changes to yellow and read the optical density at 450nm with a microtiter plate reader within 20 minutes.

Results

Study Background

To evaluate CA19-9 tumor marker as serological marker for the diagnosis of gastric, pancreatic and colorectal cancer, the population selected were divided into three different age groups (20-40, 40-60 and 60-70), as shown below.

Level of CA19-9 Enzyme in Gastric, Pancreatic and Colorectal cancer

A total 21 gastric cancer patients were included in the study and out of 21, 5(23%) patients showed positivity for CA19-9. Beside gastric cancer patients, 11 patients having pancreatic cancer and out of 11, 9 (81%) showed positivity for CA19-9. The colorectal cancer were 41 and out of 41, 21 (51%) patients showed high positivity for CA19-9 (**Table. 1**).

Tble.1: Percent positivity of different cancer patients in different carcinomas.

Nature of Patients	Total	Percent positivity
Gastric	21	5 (23%)
Pancreatic	11	9 (81%)
Colorectal	41	21 (51%)
Total	73	35 (48%)

Level of CA19-9 in different age groups

Level of CA19-9 was calculated in different age group. For gastric cancer its percentage was 14.28% in age group of 20-40, 80.95% in age group of 40-60 and 4.7% in 60-70 age

group. In pancreatic cancer patients, level of CA19-9 was, 18.18% in age group of 20-40, 72.72% in age group of 40-60 and 9.09% in age group of 60-70. In colorectal cancer, the level of CA19-9 was 48.78% in age group of 20-40, 43.90% in age group of 40-60 and 7.31% in age group of 60.70 (**Table. 2**).

Table.2: Percent positivity of CA19-9 in different age groups

Nature of patients	Age groups		
	20-40	40-60	60-70
Gastric	14.28%	80.95%	4.7%
Pancreatic	18.18%	72.72%	9.09%
Colorectal	48.78%	43.90%	7.31%

Level of CA19-9 positivity on the basis of gender

On basis of gender, CA19-9 showed high positivity in gastric, pancreatic and colorectal cancer in male. In gastric cancer, CA19-9 level was 85.71% in male and 14.28% in female. Level of CA19-9 in pancreatic cancer was high in 81.81% in male while in female it was 18.18%. Also the level of CA19-9 in case of colorectal cancer was more in 68.29% in male while 31.70% in female (**Figure. 1**).
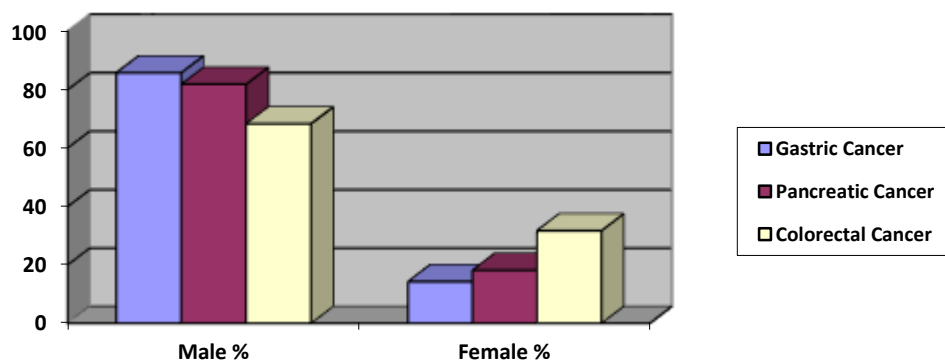

Figure.1: level of CA19-9 in male and female

Discussion

Globally, among all lethal diseases cancer has still high morbidity and mortality. As we know that cancer is an advancing disorder and can be transformed into severe state, therefore, at last it causes death of a person. There are so many different components involved in causing and development of cancer. Besides these, there is a significant purpose of chronological background including environmental, genetics, socioeconomic culture and habits of the people play a major part in incidence of cancer. Gastric cancer is the 2nd frequent source of cancer related deaths and the 4th most commonly diagnosed cancer globally (15). Pakistan, the seventh-most heavily populated country in the world, is a republic in south central Asia.

Only 33% of the tumors were resettable and 66.7% of the patients were treated with analgesics. Overall mortality was 13.3% due to late adventitiousness (16). Similar findings were noticed by (17) in his study reported 30-60% of patients with advanced gastric cancer and also reported that the most reliable marker was CA19-9 marker for the prediction of PR (82%) to CEA for the prediction for PD (93%).

In the present study, the analysis of the level CA19-9 in sera of admitted patients was carried out, who had cancer. In the current study, CA19-9 level was high in about 47.94% of the patients. CA19-9 level was high in pancreatic patients (81%) followed by colorectal (52%) and least was in stomach (23%), respectively (Table. 1). In KPK, such studies have not been conducted before and we for the first time comparatively evaluated the level of CA19-9 in gastric, pancreatic and colorectal cancerous patients. Although CA19-9 was found to be elevated in many different malignancies like in many cancers of the upper gastrointestinal, in ovarian cancer, hepatocellular cancer, and in benign conditions of the hepatobiliary system (1). Beside this, its values are found elevated only in patients with advanced stages of pancreatic adenocarcinoma and gastric carcinoma. CA19-9 is the most useful tumor marker for tracking the progression of disease, but the sensitivity of pancreatic cancer patients is 80% and the specificity is 90% (18).

CA 19-9 is considered a standard for monitoring chemotherapy and response to recurrence after surgical resection of patients with pancreatic cancer, but is not suitable for early disease diagnosis. CA19-9 is the most useful diagnostic tool to monitor patients after

gastric cancer surgery as well as to distinguish acute pancreatitis from chronic pancreatitis (1).

Regarding age groups, the most affected age group was 40-60 followed by 20-40 and the least was 60 and above (Table.2). Similar findings were also suggested by many studies (19), the reasons behind involvement of such groups may be the active involvement of such young populations in different activities to be performed.

The current study revealed that male subjects were more affected as compared to female (Figure.1). Similar findings were also suggested by one study, showing that out of 485 patients, the cancer was found in 26.8% and 73.2% in female and male respectively (20). Another study has the same agreement like Parkin et al, suggested that Stomach Carcinoma was generally found in men (17).

Diagnosis success is the first step in successful treatment. Tumor marker concentrations are primarily determined to monitor the success of treatment. Other procedures used to treat patients, such as X-rays, magnetic resonance, and scanning, are costly and have the disadvantage of affecting human health. Initially, if CA19-9 is present in large quantities at the time of diagnosis, prognosis is better and can help clinical practice. Recent survival rates have been increasing with highly diagnostic techniques and therapies used for screening and molecular targeting.

Conclusion: The concern study aimed to evaluate the importance and explore comparative analysis of CA-19-9 as best tumor marker for three cancer conditions, that is pancreatic, colorectal and gastric cancers. The study concluded that CA-19-9 was the best screening and diagnostic marker in case of pancreatic cancer patients as compared to gastric and colorectal cancer patients.

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