RESEARCH ARTICLE

Level and Evaluation of Tumor Marker CA-125 in Ovarian Cancer Patients in Khyber Pakhtunkhwa, Pakistan

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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 is necessary to prevent/stop cancer from progressing to a severe stage. In Khyber Paktunkhwa, Pakistan, many tumors are diagnosed with endoscopy and biopsy; rare studies exist regarding the diagnosis and evaluation of ovarian cancer, based on tumor markers like CA-125. Objectives: The objectives of this study were to investigate and evaluate levels of CA-125 in hospitalized ovarian cancer patients. Materials and Methods: In this study, a total of 63 admitted patients having ovarian cancer by biopsy were included. The level of CA-125 was determined in the blood of these patients using ELISA technique. Results: Out of 63 patients, the level of CA-125 was high in 52% . The affected individuals were more in the group of 40-60 and the level of CA-125 was comparatively higher in patients having moderately differentiated histology than those having well differentiated and poorly differentiated tumor histology. Moreover, the highest level of CA-125 was present among the patients having serous subtype of carcinoma and the common stage of carcinoma was stage II followed by stage III, I and IV. Conclusions: CA-125 level was high in more than 50% of the total patients. Moreover, CA-125 elevation was more common in serous subtype and stage II cancer patients.

Keywords: Ovarian cancer - CA-125 - ELISA - subtypes - Kyber, Pakistan

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Introduction

Cancer is a pathological condition, characterized by an uncontrolled growth of the cells. Worldwide, about 11 million people are diagnosed with cancer every year. By 2020, this number may exceed to 16 million cases (Kanavos, 2006). The risk factors for cancer may be environmental and genetic. Environmental factors may include, tobacco smoking, intake of alcohol, infections, like *Helicobacter pylori*, Hepatitis B and C, Sun/ Ultra Violet (UV) exposure, environmental pollutants. Genetically, cancer is caused by mutation in cell and for that it is estimated that about three hundred oncogenes are responsible (Futreal et al., 2004). Cancer has become a serious health threat and the leading cause of death worldwide and especially in Asian countries. In Pakistan, exact morbidity and mortality rates are still not known (Perk et al., 2008).

Among all the gynecologic malignancies, ovarian carcinoma is the most lethal, with more than 0.24 Million (M) new cases and 0.125M deaths every year (Fritsche et al, 1998). This may contribute about 4.2% of all the deaths due to cancer in females worldwide (Gallagar et al., 2011). In the United States (US), ovarian cancer is the fifth leading cause of death, causing 15520 deaths every year (Muazzam et al., 2010). While it ranks fourth among the cancers in Scottish, Chinese and in Pakistani women (Xu et al., 2013).

Epithelial ovarian cancer (EOC), a silent killer, is asymptomatic even it continues to progress (Bhatt et al., 2010). Due to unavailability of diagnostic protocols and untimely diagnosis, disease occurrence is more. Although the survival chances for stage 1 ovarian cancer is more than 90%. Ovarian cancer is found as either an epithelial or non-epithelial tumor. Non epithelial tumor is 90% of all ovarian carcinoma's (Sarwar et al., 2006). This disease is mostly present in post menopausal women and upto 75% of the women are diagnosed in later stage even at that instant metastasis has occurred (Skates et al., 2003). Those who are diagnosed at early stage of disease, has 90% of survival rate as compared to diagnosed at a later stage, having less than 20 % of survival rate (Fritsche et al, 1998). In one study conducted in Pakistan, EOC is 63.5% of all ovarian malignancies (Sarwar et al., 2006).

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Table 1. Demographics of Cancer Patients

Age group	Number of positive/ negative patients		Histological tumor grade		Cancer stages		Histological sub types	
20-40	+	8	Poorly differentiated	8	I	3	Serous cystadeno Carcinoma	8
	-	11	Moderately differentiated	8	II	8	Endometrioid	6
	Total=	19	Well differentiated	3	III	8	Mucinous	2
					IV	0	Clear cell carcinoma	0
							Mixed carcinoma	3
							Transitional cell carcinoma	0
40-50	Positive=	11	Poorly differentiated	3	I	1	Serous cystadeno Carcinoma	9
	Negative=	11	Moderately differentiated	7	II	12	Endometrioid	4
	Total=	22	Well differentiated	11	III	8	Mucinous	3
			Not stated	1	IV	1	Clear cell carcinoma	2
							Mixed carcinoma	2
							Transitional cell carcinoma	2
50-60	Positive=	13	Poorly differentiated	6	I	0	Serous cystadeno Carcinoma	10
	Negative=	5	Moderately differentiated	8	II	9	Endometrioid	4
	Total=	18	Well differentiated	4	III	6	Mucinous	2
					IV	3	Clear cell carcinoma	0
							Mixed carcinoma	1
							Transitional cell carcinoma	0
60-70	Positive=	2	Poorly differentiated	1	I	0	Serous cystadeno Carcinoma	2
	Negative=	2	Moderately differentiated	0	II	2	Endometrioid	0
	Total=	4	Well differentiated	3	III	2	Mucinous	1
					IV	0	Transitional cell carcinoma	1
							Clear cell carcinoma	0

EOC, representing diverse group of diseases, is diagnosed at early stage through transvaginal sonography but serum biomarker testing is preferred (Jacobs & Menon, 2004).

Ideally, a tumor marker is the best tool for screening and to monitor the response to treatment and also for cancer diagnosis, patient prognosis and in treatment selection. Many factors are contributing in selection of tumor marker, like it is invasive, time saving, easily available and non subjective (Yin et al., 2001). For detection of ovarian cancer, CA-125 is considered as an ideal tumor marker. It is secreted by abnormal mullerian epithelial cells as it has been found in 83% of the EOC patients and only in one healthy individual (Markman, 1997). CA-125 is used to localize the tumor and to determine its stage, subtype, and response to therapy (Nair et al., 2008). Its level varies among the normal and affected individuals, like in healthy women its level is less than 35 U/mL but is more than 50% in stage 1 EOC patients, while up to 90% in stage III or IV. Increase level of CA-125 is in direct proportion to serous ovarian tumor which is the most fatal subtype of ovarian malignancies (Xu et al., 2013). Persistent abnormal level of CA-125 confirms > 95% certainty that the disease come back or is still present (Kobel et al., 2008).

This study aimed to determine and evaluate level of CA-125 in ovarian cancer patients, already admitted at IRNUM (Institute of Radiotherapy and Nuclear Medicine) Peshawar, using enzyme linked immunosorbent assay (ELISA) . Moreover, to invesitigate CA-125 level in different EOC stages and in different age group of patients.

Materials and Methods

Ethical approval

This study was approved by the ethical committee of the centre of Biotechnology and Microbiology, university of Peshawar.

Clinical presentation

Patients were selected from the IRNUM Peshawar. These patients were already diagnosed with biopsy and have not taken any anti-cancer therapy. Along with patients, some healthy individuals were also selected as control group. These healthy individuals were free of any physical abnormalities, from smoking and alcohol consumption.

Blood collection

5 ml blood sample was collected from EOC patients as well as from control healthy individuals with written informed consent. Blood was centrifuged and serum was isolated and kept at -4˚C till further use.

CA-125 Determination

CA-125 concentration in patients and healthy individuals was determined using commercial ELISA based CA-125 kit (Monobind, USA). Before proceeding with the assay, all the reagents, serum references, and

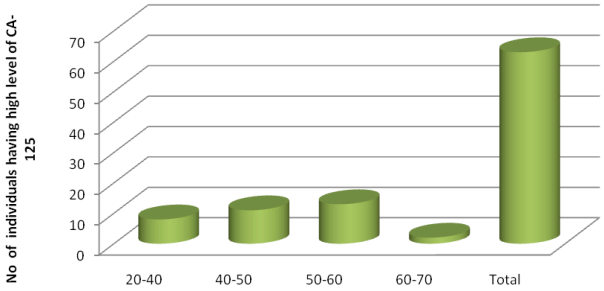

Figure 1. Age-wise Distribution of Affected Individuals of Ovarian Cancer

controls were taken to room temperature (20-30 ˚C). Test was performed according to the manufacturer's instruction. Simply 10 ul serum was added to coated micro well, after incubation, washing and adding labeled antibody, florescence was measured.

Quantitative analysis and comparison of circulating tumor markers in serum of EOC patients along with differentiation of different stages of ovarian cancer was done.

Results

To evaluate CA-125, tumor marker for diagnosis of ovarian cancer, different age groups population was selected. The characteristics of patients are listed in Table 1. The age groups were, 20-40, 40-50, 50-60 and 60-70. A total of 63 ovarian cancer patients were included in the study. Among different age groups, the number of affected individuals based on elevated serum CA-125 level, were 8, 11, 13 and 2 in 20-40, 40-50, 50-60 and 60-70 age groups respectively (Table 1). Beside this, the level of CA-125 was high in age group of 50-60 (Figure 1).

Regarding histological tumor grade, different age groups had different histology, like among the age group of 20-40, 8 patients were found with poorly differentiated histological tumor grade,8 with moderately differentiated and 3 with well differentiated tumor histology (Table 1). CA-125 level was high in moderately differentiated histology (Figure 2). In the age group of 40-50, 3 patients were found with poorly differentiated, 7 with moderately differentiated, 11 with well differentiated histological tumor grade and 1 with unknown results for tumor grade. Patients with age group of 50-60, 6 patients were found with poorly differentiated and 8 with moderately differentiated, 4 with well differentiated tumor histology. While in the age group of 60-70, 1 patient was found with poorly differentiated and 3 with well differentiated tumor grade (Table 1) (Figure 2).

Distribution of patients according to the International Federation of Gynecology and Obstetrics (FIGO) stages (I, II, III, IV), in age group of 20-40, 3, 8 and 8 patients had stage I, II and III respectively while there was no patient of stage IV. In age group of 40-50, 1, 12 and 8 patients had stage I, II and III respectively while only 1 patient had stage IV. In age group of 50-60, 9 and 6 patients had stage II and III respectively while only 3 patients had stage IV. In age group of 60-70, only 2 patients had stage I and 2 patients had stage III while stage I and IV were not found (Table 1). In these, the level of CA-125 was high in stage II patients compared to satge III, IV and I (Figure 3)

Histological sub typing (serous, endometroid, mucinous, clear cell and malignant Brenner, mixed epithelial, undifferentiated, unclassified epithelial, sex-cord stromal, germ cell, unclassified non epithelial), showed that in age group of 20-40, 8 were serous, 6 endometriod, 2 mucinous and 3 clear cell carcinoma patients. In age group of 40-50, 9 were serous, 4 endometrioid, 3 mucinious, 2 transitional cell carcinoma, 2 Clear cell and 2 patients of mix carcinoma's. In age group of 50-60, 10 were serous, 4 endometrioid, 2 mucinous and 1 of mix carcinoma's patient. While in age group of 60-70, 2 were serous, 1 mucinous and 1 Transitional cell carcinoma patient (Table 1). In all of these level of CA-125 was comparartively high in serous sub type of carcinoma,s patients (Figure 4).

Table 2. Detail of Normal Healthy Controls

Controls	CA-125 conc. (U/ml) Cut off value for CA-125=25
Control 1	11.735
Control 2	15.036
Control 3	10.315
Control 4	12.413
Control 5	3.24
Control 6	2.653

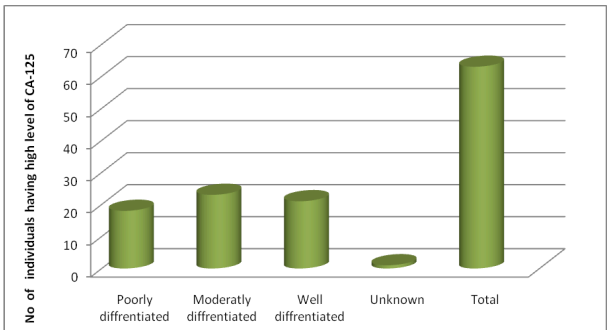

Figure 2. Histological Tumor Grade of Ovarian Cancers

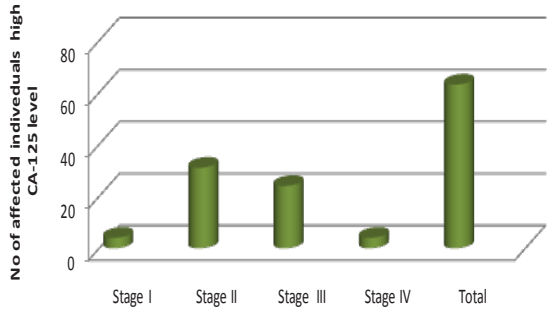

Figure 3. Cancer Stages of Ovarian Cancers

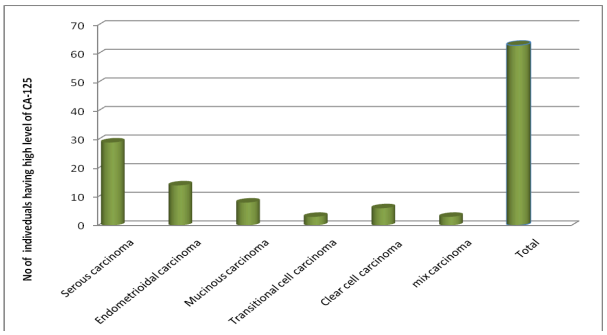

Figure 4. Histological Subtypes of Ovarian Carcinomas

Discussion

Worldwide, cancer is the major cause of morbidity and mortality. As cancer is a progressive disease, can be converted to serious stages and hence finally death of the person can occur. Different etiological factors contribute in causing and progression of cancer. Beside this, there is an important role of historical background, genetics, environment, lifestyle and socioeconomic culture in incidence of cancer. In developing countries, cancer rushes

out 53%, while the mortality rate due to cancer is 56%. While by 2020, the total expected number of cancer cases may exceed up to 29% in developed countries and 77% in developing countries as a result of urbanization and change in nutritional values (Hanif et al., 2009).

Ovarian cancer is one of most common gynecological malignancy and the 4th most vital cause of death in U.S and Europe (Jacobs & Menon, 2004). Some studies show that the ovarian cancer is up to 13.6% in Pakistani population, but the exact number of population affected by ovarian cancer in Pakistan is still unknown (Soslow, 2008).

This study, based on determining the level of tumor marker CA-125 in ovarian cancer patients, admitted in Irnum, has shown that out of 63 patients, CA-125 level was high in 52% of the patients. Earlier in KPK, such studies have not been done and hence we for the first time reported level of CA-125 in ovarian cancerous patient. Elevated serum level in about 50% of the patients in this study is almost in accordance with other studies (Bian et al., 2013; Devan et al., 2013; Lawiciki et al., 2013; Arun-Muthuvel and Jaya, 2014; Karadag et al., 2014).

CA-125 level based cancer proportion is different in different age groups. In this study, the incidence rate of ovarian cancer has been shown to be more in age group more than 40 years (Figure 1). Results of other studies are also in similar fashion, regarding age (Soslow, 2008). As it has been noted that the chances of ovarian cancer are more with the increase in age, usually after menopause, as ovaries become functionless so more and more disturbances can occur (Muccluggagee, 2011).

In this study, the level of serum CA-125 was high in moderately differentiated carcinoma (Figure 2). While its proportion was low in well and poorly differentiated carcinoma respectively. Other studies have also shown similar relationship of histological tumor grade with elevation in tumor marker value (Soslow, 2008).

Since ovarian tumors are histologically divided into Serous, Endometrial, and Mucinious, Clear cell, Transition cell carcinoma and mix carcinoma (malignant Brenner's tumor). In this study most of the cases based on level of CA-125, were serous types of carcinoma (Figure 4). This is an agreement with other study that has shown that amongst the carcinmoma about 83% of the carcinoma are are serous (Yancik, 1993). Mucinous carcinoma was about 5% in our study and it has been shown by different studies that it is uncommon and contributes only about 3% of the total ovarian carcinomas (Junejo et al., 2010). Studies regarding proportion of endometrioid in western countries have shown that it is about 10%, while in the present study its proportion was 23% (Table 1). Only 5% of the carcinomas were clear cell according to other studies while in the current study it is only about 3% (Figure 4). hence our results are almost with an agreement with all other studies regarding subtypes of ovarian carcinoma.

The true prevalence of transition cell carcinoma is impossible, but carcinoma's with a transitional cell pattern are arisen into Brenner Tumor are exceeding rare (Soslow, 2008).

Determination of different stages in ovarian cancer has an important prognostic value. The current study reflected different stages of ovarian cancer and among these the most prevalent stage was III, followed by stage I,II and IV (Figure 3) . Simir results were presented by one Western study (Priya et al, 2008), according to that, bout 20% women had ovarian cancer stage I, 19% population had stage II, 36% had stage III and up to 12% had stage IV (Soslow, 2008).

In conclusion CA-125 level was high in more than 50% of the carcinoma patients, hence it can be considered as screening marker for the diagnosis of ovarian cancer. But still some molecular study should be in practice that can confirm the presence of cancer.

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