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Evaluation of CA125 and CA19-9 during different Stages of Urothelial Carcinoma In Pre and Post-therapeutic level

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Abstract: *The aim of the study was to evaluate the role of CA125 and CA19-9 tumour markers in different stages of urothelial carcinoma and monitoring the response to therapy. Several levels of CA125 and CA19-9 (each purchased from Fujirebio Diagnostics, Sweden) was measured by ELISA before and 7 days after treatment of different stages of 182 urothelial carcinoma patients and compared with the 200 normal subjects as control. The results showed that among 182 urothelial cases, the median serum level of CA125 and CA19-9 were 107.7U/ml, 118.6U/ml, 120.4U/ml and 73.1U/ml, 81.1U/ml, 196.4U/ml in case of superficial transitional cell carcinoma, muscle invasion and metastatic variety respectively. The fall of the titre of CA125 were 90.6%, 91.4%, 81.7% and CA19.9 were 87.1%, 85.8%, 85.1% in case of transitional cell carcinoma, muscle invasion and metastatic variety respectively. The study revealed that CA125 and CA19-9 could be prognostic markers of urothelial carcinoma.*

Key words: *CA19-9, CA125, ELISA, urothelial carcinoma*

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I. Introduction

Bladder cancer is a major health problem in the world with more than 72,570 new cases and about 15,210 deaths predicted in the US yearly. It is the second most common cancer of the genitourinary tract. Bladder cancer is more common among men than women and more common among whites than blacks, but the reason behind this gender biasness is unknown [1]. Smoking is the greatest risk factor for urothelial carcinoma and increases risk of developing the disease four-fold compared to non-smokers[2]. The risk of bladder cancer goes up with age about 9 out of 10 people with bladder cancer are over the age of 55. Diet, occupational chemical exposure to benzidine dye, rubber, leather, textiles and painting industry are other risk factors[1,3]. Genetic predisposition like activation of some oncogenes such as ras and cerb B2 has been reported in bladder cancer, as has the inactivation of tumor suppressor genes such as p53, p16 and retinoblastoma. There are three types of bladder cancer- transitional, squamous and adenocarcinoma. Over 90 percent are transitional cell carcinoma (TCC), pure squamous cell carcinoma (SCC) is uncommon approximately 5% and primary adenocarcinoma accounts for 1-2%. TCC again subdivided into superficial, muscle invasive and metastatic variety [4]. Painless hematuria is one of the most common presenting symptoms. Frequency and painful urination are late symptoms that result from sloughing, infiltration and secondary infection[5]. Tumor markers are the substances produced by tumor cells or by other cells in response to cancer or certain benign non-cancerous conditions[6]. These substances are found in blood, urine, tumor tissues or in other tissues[6,7]. Different tumor markers are found in different types of cancer[8-10]. In addition, tumor markers level may not be altered in all patients having cancer especially the cancer is in early stage[11,12]. Some rumor markers level may also be raised in patients with non-cancerous conditions[6,13]. Tumor markers are substances usually protein in nature, produced by cancer tissue itself. Some tumor markers are specific for particular cancer while non-specific markers are also found in different cancerous conditions [14,15]. On the other hand many of the well-known markers are also raised in several non-cancerous conditions like endometriosis, cardiac failure, pleuropulmonary disease, chronic liver disease, connective tissue disease, peritoneal dialysis and recent surgery[6,9,16]. Consequently these are not diagnostic for cancer[17-21]. Carbohydrate antigen CA 125 is a high molecular mass glycoprotein produced both by ovarian cancer cells as also by normal cells of tissue derived from coelomic epithelium[6,15]. It is generally found to be higher in malignant conditions compared to benign conditions[6]. The usual malignant conditions associated with raised levels of this tumor marker include lung, bladder, gastric, hepatic, breast and pancreatic cancers[6,8,21]. CA125 is a monoclonal antibody associated mainly with colorectal carcinoma but it can also be associated with other

malignant conditions like leukaemia, non-Hodgkin's lymphoma, mediastinal teratoma and pleural effusion associated with Meig's syndrome [6,8,22,23,24]. Carbohydrate antigen CA19-9 is 210kD tumor-associated glycoprotein antigen as carbohydrate determinant on glycoprotein. CA19-9 is characterized by monoclonal antibody 1116-NS19-9 by immunizing BALB/c mice with human colorectal cancer line. This antibody reacts with CA19-9, which has been identified as a sialylated lacto-N-fucopentosell, an oligosaccharide sharing structural features with Lewis A blood group antigen. It is well- known marker for pancreatic carcinoma and is being investigated for other malignancies like carcinoma bladder, cholangiocarcinoma and gallbladder carcinomas. The CA19-9 concentration correlated well with the clinical response to treatment. It may be used as a prognostic marker but not as a screening tool due to its low sensitivity[25,26,27]. The isolated paper shows that CA125 and CA19-9 both may act as tumor markers in advanced stages of urothelial carcinoma[12,24,25,28,29]. But the role of CA125 and CA19-9 as prognostic markers in different stages of urothelial carcinoma is still controversial[8,30,31]. So the aim of this study are i) To evaluate 'the role of CA125 and CA19-9 as prognostic markers in different stages of urothelial carcinoma and ii) To compare the pre and post-operative serum marker levels.

II. Material And Methods

This study was conducted in the Biochemistry, Urology and Pathology department of IPGME&R over a period of 18 months and was approved by the ethics committee of IPGME & R. One hundred eighty two (182) patients were selected from those with histologically confirmed urothelial carcinoma. Two hundred (200) normal age and gender matched control subjects were selected from those who had no previous history of any urological disorders.

Study Design: Prospective observational analytical study.

Study Location*:* This study was conducted in the Biochemistry, Urology and Pathology department of IPGME&R and was approved by the ethics committee of IPGME&R.

Study Duration: March 2013 to September 2014.

Sample size: 382 Subjects.

Sample size calculation: The sample size was estimated on the basis of a single proportion design. The target population from which we randomly selected our sample was considered 20,000. We assumed that the confidence interval of 5% and confidence level of 95%. The sample size actually obtained for this study was 378 patients for both groups. We planned to include 382 patients (control 200 normal subjects and 182 bladder cancer patients for study group) with 1% drop out rate.

Subjects & selection method: The study population was drawn from consecutive urothelial carcinoma patients who presented to Urology department of IPGME&R over a period of 18 months and was approved by the ethics committee of IPGME&R were admitted and operated in Urology department of IPGME&R. Subjects were divided into two groups (control 200 normal subjects and 182 bladder cancer patients for study group).

Inclusion criteria:

1. Different stages of urothelial carcinoma.
2. Either sex.
3. Aged ≥ 18 years.

Exclusion criteria:

1. Pregnant women.
2. Patients with genetic disorders.
3. Patients with other causes of increased CA125 and CA 19-9.
4. Patients who are physically inactive.
5. Moribund patients.

***Procedure methodology*:**

After written informed consent was obtained, a well-designed questionnaire was used to collect the data of the recruited patients prospectively. The questionnaire included socio-demographic characteristics such as age, gender, nationality, height, weight, and consanguineous marriage, physical activity, occupation and lifestyle habits like smoking and alcohol. Enzyme - linked immunosorbent assay (ELISA) kit of CA 125 CanAg CA125 EIA from Fujirebio Diagnostics, (Sweden) and CA19-9 CanAg CA19-9 EIA from Fujirebio Diagnostics, (Sweden).

Specimen collection and handling: Blood samples were obtained by venipuncture and the serum was separated according to common procedures. The samples were stored at -20°C for 24 hours. For longer period, samples were stored at -70°C or below. Samples were brought to room temperature before analysis.

Procedure for measurement of CA125 & CA19-9: The quantitative determination CA125 & CA19-9 concentration in human sample was done by a microplate immunoenzymometric assay. In this method, CA125 & CA19-9 calibrators, patients' specimen or controls were first added to a streptavidin coated well. Biotinylated

monoclonal and enzyme-labeled antibodies (directed against distinct and different epitopes of CA125 and CA19-9) were added and the reactants were mixed. Reaction between the various CA125, CA19-9 antibodies and native CA125, CA19-9 formed a sandwich complex that bound with the streptavidin coated well. After the completion of the required incubation period, the enzyme-CA125 and CA19-9 antibodies bound conjugates were separated from the unbound enzyme-CA125 and CA19-9 conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well was quantitated by reaction with a suitable substrate to produce colour. The value CA125 and CA19-9 expressed in U/ml and 37U/ml was taken as cut-off upper value of normal[6,25.]

Statistical analysis:

The statistical evaluation was done by Wilcoxon's matched pairs signed rank test. Software used: - Statistical version 6 (Tulsa, Oklahoma: Stat Soft Inc., 2001) p Value: significant in all cases (<0.001).

III. Result

A total of 382 (male - 366, female - 16) subjects were studied. Out of 382, 200 normal subjects were taken as control and 182 subjects having urothelial carcinoma of varying stages were taken as cases. The age (Fig.1, Table 1) and sex (Fig.2, Table 2) distribution of study population is given in table 1 & 2.

Table no 1: Age distribution of study population

Age (in year)	No. of patients in control group	Percentage%	No. of patients in study group	Percentage%
Blow 40	10	5%	8	4.39%
40 – 50	42	21%	40	21.97%
50 – 60	84	42%	76	41.75%
60 – 70	56	28%	50	27.47%
70 – 80	8	4%	8	4.39%
Above 80	0	0%	0	0%
Total	200	100%	182	100%

Figure 1: Age distribution of study & control population

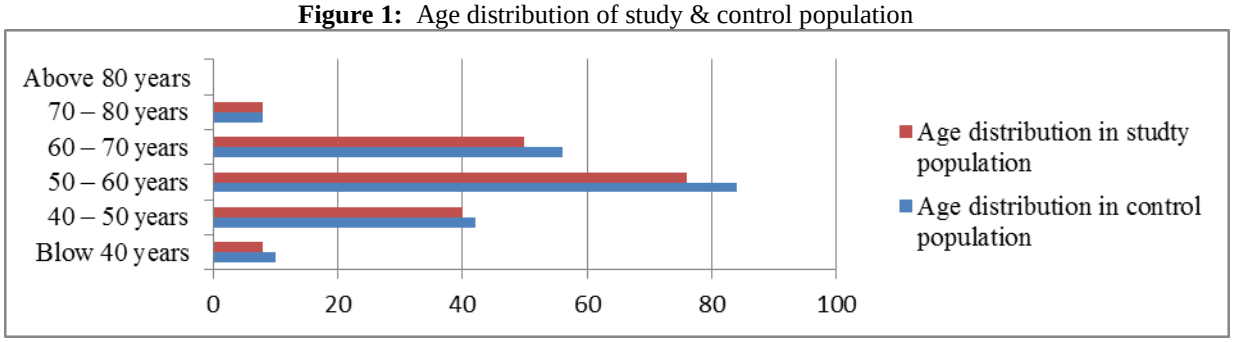


Fig. 2: Incidence of carcinoma of bladder is significantly much more in male patients than in female patients. Carcinoma of urinary bladder is a male predominant disease. We got male-female ratio 23:1. Incidence of the disease was found to be maximum in 50-60 years age group.

Figure 2: Sex distribution of both groups.

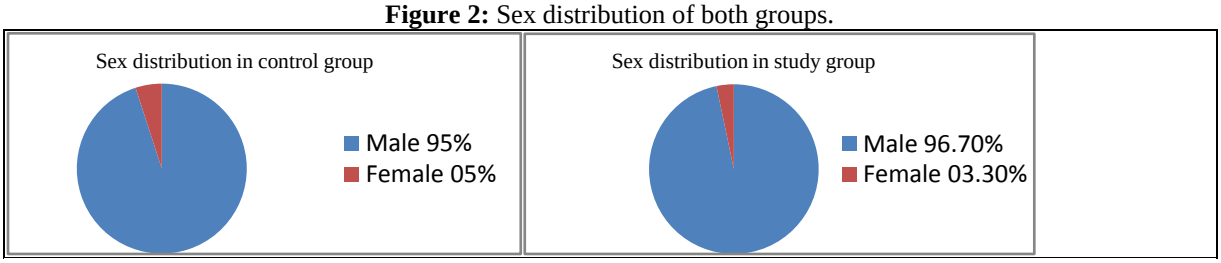


Table no 2: Sex distribution of study population

Sex	No. of patients in control group	%	No. of patients in study group	%
Male	190	95%	176	96.70%
Female	10	5%	6	03.30%
Total	200	100%	182	100%

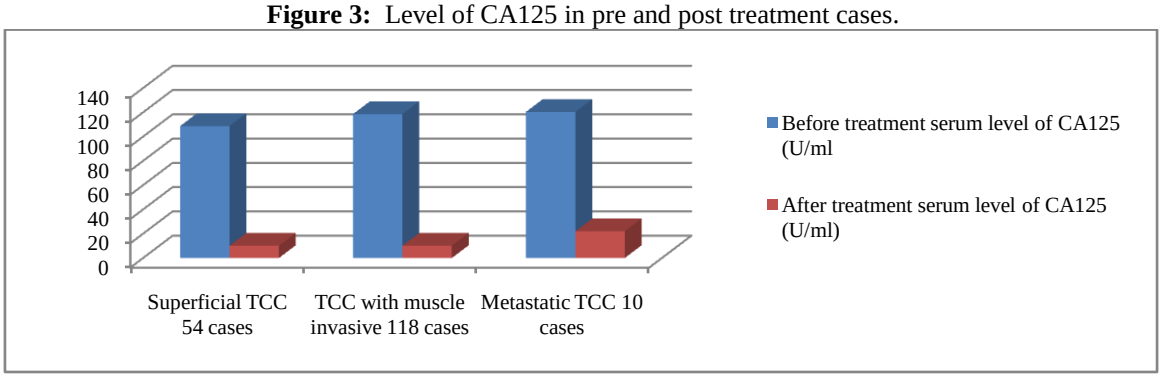
Out of 182 subjects 54 cases had superficial TCC bladder, 118 had with muscle invasion but no distant metastasis, and 10 patients had TCC with metastasis. Among 54 cases of TCC bladder patients 40 cases had high serum concentration of CA125 and 20 cases had high serum concentration of CA19-9. Among 118 cases

having TCC with muscle invasion 80 cases had high concentration of CA125 and 40 cases had high concentration of CA19-9. The level of CA125 and CA19-9 in pre and post treatment period is given in table 3.

Table 3: Level of CA125 & CA19-9 in pre and post treatment cases.

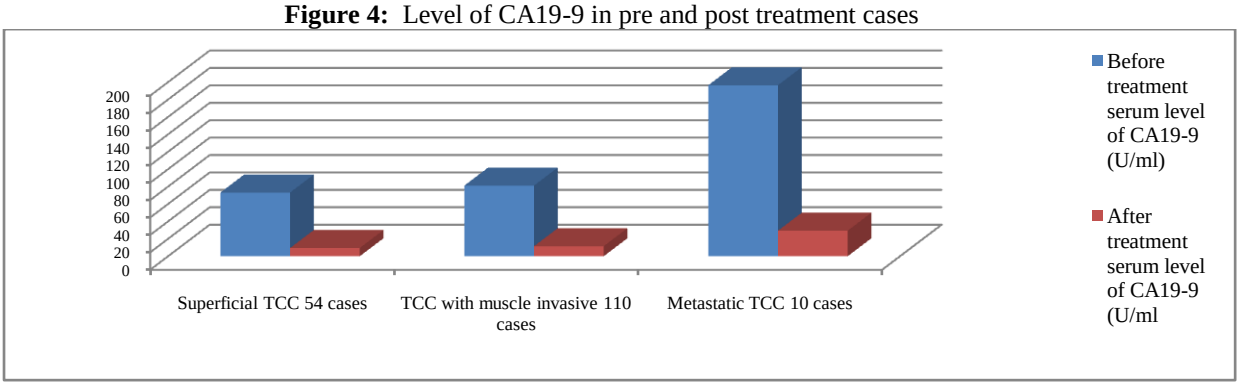
	No of cases	Before treatment serum level of CA125 (U/ml)	After treatment serum level of CA125 (U/ml)	% Fall of titer	Before treatment serum level of CA19-9 (U/ml)	After treatment serum level of CA19-9 (U/ml)	% Fall of titer
Superficial TCC	54	108.7	10.1	90.6%	73.1	9.4	87.1%
TCC with muscle invasive	118	118.6	10.2	91.4%	81.1	11.5	85.8%
Metastatic TCC	10	120.4	22.0	81.7%	196.4	29.3	85.1%

Figure 3: Level of CA125 in pre and post treatment cases.



But 7 days after onset of treatment either surgical or non-surgical intervention among 54 cases having superficial TCC bladder only 4cases had raised concentration of CA125 and 4 cases had raised concentration of CA19-9. In the group of patients having TCC with muscle invasion, out of 118 cases 10 cases had high concentration of CA125 and 4 cases had high concentration of CA19-9. The patients having metastatic TCC bladder, out of 10 patients only 4 patients had raised concentration of CA125 and 4 patients had raised concentration of CA19-9 (table 5). Also we know that CA19-9 and CA125 levels in serum of normal patients are 5-37 U/ml[25]. In 99.6% of healthy adults, serum CA19-9 levels are lower than 37 U/ml. Value less than 100 U/ml considered as grey zone values in which malignant and benign diseases may overlap.

Figure 4: Level of CA19-9 in pre and post treatment cases

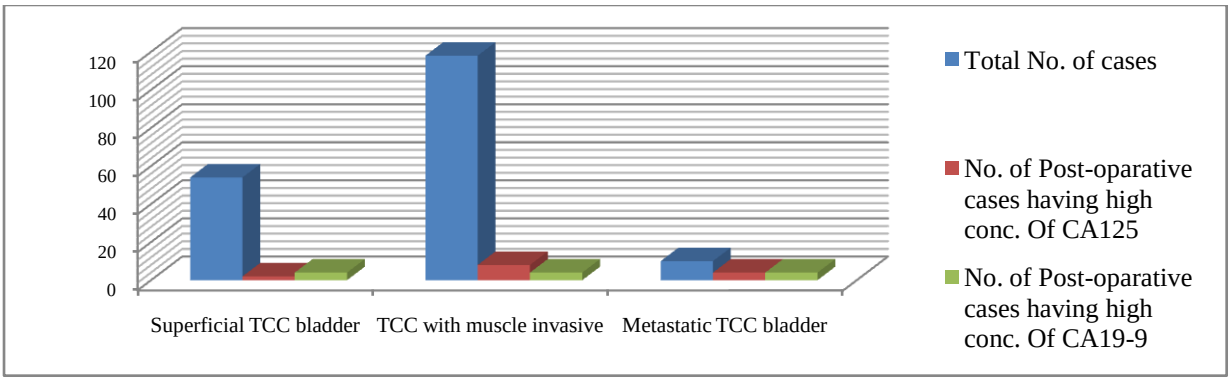


Among 182 urothelial cases the median serum level of CA125 were 107.7 (77.1417.2), 118.6 (75.8-134.7), 120.4 (108.2-389.8) in case of TCC, muscle invasive and metastatic variety respectively and serum level of CA19-9 were 73.1 (68.3-126.2), 81.1 (67.4-170.3), 196.4 (165.8-396.4) in case of TCC, muscle invasive and metastatic variety respectively. But 7 days after onset of treatment (surgical / non-surgical) the titer of CA125 were 10.1 (7.3-10.9), 10.2 (8.3-11.2), 22.0 (21.2-419.8) in case of TCC, muscle invasive and metastatic variety respectively & CA19-9 were 9.4 (8.0-10.3), 11.5 (10.0-14.0), 29.3 (24.3-479.2) in case of TCC, muscle invasive and metastatic variety respectively. The fall of titer of CA125 were 90.6%, 91.4%, 81.7% and CA19-9 were 87.1%, 85.8%, 85.1% in case of TCC, muscle invasive and metastatic variety respectively (Table - 3).Among 10 cases having metastatic TCC bladder 4 patients had raised levels of both markers (table 4).

Table 4: Distribution of cases according to high concentration of CA125 and CA19-9 in post-treatment period.

	No. of cases	No. of cases having conc. Of CA125	No. of cases having high conc. Of CA19-9
Superficial TCC bladder	54	2	4
TCC with muscle invasive	118	8	4
Metastatic TCC bladder	10	4	4

Figure 5: Number of Postoperative cases having high conc. of CA125 &CA19-9 in comparison to total no of cases



IV. Discussion

Bladder cancer is one of the commonest malignancies there are about 50,000 new cases of bladder cancer diagnosed each year in the United States and more than 10,000 cancer deaths are attributed to this disease.

In India, according to the recent reports of the National Cancer Registry Programme, the overall incidence rate of the urinary bladder cancer is 2.25% (per 100,000 annually), 3.67% among males and 0.83% for females [32].The most common symptom is painless hematuria. The next most common symptoms are dysuria and frequency. Twenty-six percent of the patients' male preponderance is much more frequent in Indians than in other races. Younger patients present with low-grade disease [32]. Urinary bladder cancer ranks ninth in worldwide cancer incidence, it is the seventh most common malignancy in men and seventeenth in women [34,35].

Nearly 90% of patients with muscle invasive tumors present with de novo invasive tumor, no prior history of bladder malignancy.

Dyer and associates evaluated that CA125 appears to be a useful marker of patients with advanced urothelial cancer[12]. In this study the author showed that a major group had features of urothelial cancer as well as elevated levels of CA125. But a few patients manifested a biochemical rise of CA125 prior to clinical progression.

Kazuhiko et al concluded that serum CA19-9 may serve as a significant marker for advanced cancer and is useful for predicting prognosis of disease [11]. But in early stage the role of CA125 as tumor marker is still controversial [8,30,31].

In this study the levels of both CA125 and CA19-9 were raised in three groups of urothelial carcinoma. But the maximum rise of CA125 and CA19-9 occurred in metastatic variety (Table 3). Moreover, after 7 days of onset of therapy (surgical / non-surgical) the titer of CA125 and CA19-9 both fall in three group of cases. The declining rate of CA125 is 90.6%, 91.4% & 81.7% in group of TCC muscle invasive and metastatic variety respectively. The declining rate of CA19-9 is 87.1%, 85.8% & 85.1% in group of TCC, muscle invasive and metastatic variety respectively, (Table 3).The post therapeutic fall of CA125 titer is minimum in metastatic variety and more or less same in other two groups. But no significant different results among three groups have been found in case of CA19-9. In our study, we found that the CA125 and CA19-9 levels are higher than normal levels of the patients having urothelial carcinoma. But when the patients are treated with any kind of therapy (surgical / non-surgical) the levels of these markers fall.

So we can conclude that when the cancer cells are in active state, the titre of CA125 and CA19-9 increase but after treatment the cells become in inactive state and the titre declines[29].

V. Conclusion

Here CA125 and CA19-9 both are important for detection of bladder cancer and provide significant prognostic importance for maintaining the disease in response to successful therapy. Furthermore the decline in CA125 was more in compare to CA19-9.

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