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A comparative study of the IMMULITE and Enzyme Linked Immuno Sorbent Assay for measuring Tumor Marker CA-125 in Ovarian Cancer Patients in Khyber Pakhtunkhwa

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Abstract

Different assays are used for screening and diagnosis of carcinoma worldwide. Here in Pakistan too, various techniques are used to screen cancers of different organs. Earlier the trend was to use ELISA assay for screening of ovarian cancer but nowadays special CHEMOLUMINESCENT assays are used to screen ovarian cancer. The objective of the study was to examine and compare the results of tumor marker CA-125, evaluated by CHEMOLUMINESCENT assays based (IMMULITE) and ELISA technique. Total of 80 patients having ovarian carcinoma and six healthy persons as control were included in this study. The study population included benign as well as malignant cases of ovarian carcinoma. The level of CA-125 was determined by ELISA and IMMULITE. Analysis of IMMULITE OM.MA assay showed 64 patients (80%) positive for cancer and in 16 patients (20%), the cancer marker was not detected. Analysis of the ELISA results showed 57 patients (72%) positive for cancer and in 23 patients (28%), the cancer antigen was not detected. Six healthy females as control group, showed very low concentration of CA-125. Pearson correlation coefficient (PCC) is 0.358, indicating a direct relation between the two assays values. Paired t-test data of 0.46 gave us an insignificant difference value between both assays. Although there is very small variation in sensitivity and specificity of both the assays but there is significant correlation between ELISA and IMMULITE assays. Hence ELISA can be used to determine CA-125 level in ovarian cancer patients where the IMMULITE assay is not available or is not affordable

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Introduction

Ovarian cancer (OC) is the major cause of gynecological cancer deaths worldwide (Hiss, 2011). In developing countries, OC is the 5th leading cause of death in females. Epidemiological studies reveal that the most common risk factor of OC is age, as with passage of time, the ovaries become smaller and crumpled in post-menopausal women, resulting in deep cleft formation along with smaller cyst in epithelial cells on ovarian surface. Null parity, family history, fertility drugs usage, and endocrinal disorders are other risk factors. Some other factors including pregnancy and lactation, multiparity and excessive use of oral contraceptives are also associated with lesser risk of OC because all of these cause decrease in ovulation cycles. Early diagnosis of OC is important for effective treatment, but unfortunately, as they are diagnosed mostly at their advanced stage (stage III) rather than early stages due to the unavailability of reliable protein biomarkers (Chuhan *et al.*, 2007).

In recent times, a lot of work has been done to discover OC biomarkers for initial detection, staging, grading of tumors, and clinical management as well as molecular therapeutic targeting (Hiss, 2011). The main reason of the poorly predicted ovarian carcinomas are the nonexistence of proper sign and symptoms of the disease at initial stages. At early stage, about 25 % of OC are diagnosed. To describe the incidence of cancer, highly sensitive strategies are required to detect the early stage disease (>75). Due to the absence of highly precise screening paradigm, it's important to discover the specific molecular biomarker for the detection of ovarian carcinoma at its primary stages (Robret, 2003).

As biomarkers aims to be careful in timely diagnosis and after therapy observation of cancer, so the best biological material is blood. Mostly, biomarker identifications are done with blood based strategies while some biomarkers for OC have also been identified in urine. Biomarkers discovery from glycoproteomic analysis of the body fluids has of great benefit in this regard. For OC identification, CA-125 is one of best biomarkers, which is secreted by abnormal / diseased Mullerian epithelial cells of the ovary (Markmen, 1997).

Bast *et al* in 1981 discovered the CA-125 by OC125, a monoclonal antibody from ovarian cancer cell line of mice (Yin *et al.*, 2001). Almost 50% of all the serum proteins are glycosylated, and glycosylation status of glycoprotein in diseased condition, e.g cancer may alter their degree of uniformity. The biomarker of OC is also a glycoprotein (Zhang *et al.*, 2010). About 83 % patients of OC reported with high concentration of CA-125 in their serum and only one healthy individual has high level of serum CA-125 level (Markman, 1997). Studies also show that CA-125 is much batter biomarker than HE4 and ROMA for prediction of OC. The diagnostic accuracy of other biomarkers e.g. HE4 and ROMA is still debatable (Pitta *et al.*, 2013).

In recent times, for the screening trials of ovarian carcinoma, the conc. of serum CA-125 has been tested, which clearly shows that the tumor marker CA-125 level in serum is a powerful index with the help of which OC is detected among post-menopausal and asymptomatic females. Hefler in 2000 studied tumor marker CA- 125 level in the serum of patients as an independent prognostic biomarker of the disease (Hefler *et al.*, 2000).

In Khyber Pakhtunkhwa, the assessment of the CA-125 level in serum, correlation with female patient's age, histological tumor grade and stage of tumor in correlation with tumor marker CA-125 level in blood serum has been investigated in our previous study (Ahmad *et al.*, 2015). Elevated tumor marker CA-125 level can be detected in the serum of only 40-50% of ovarian tumor stage 1 patients, but when this biomarker is used in combination with some other markers, the sensitivity increases to 80% (Zou *et al.*, 2010). Evolution of disease can be expected in many females by rise in CA-125 concentration (Rustin *et al.*, 2013). The CA125 antigen is detected by the OC125 monoclonal antibody, first defined by Bast in 1981 (Moss *et al.*, 2013). Transvaginal color doppler studies combined with serum CA-125 test is the

powerful tool to detect OC in females of known family history. Biomarkers of OC, particularly, CA125, have led the way for further studies to promise the early detection (Eagle, 1997).

This study was aimed to find out the concentration of serum CA-125 in the patients of ovarian cancer who were under treatment at Institute of Radiotherapy and Nuclear Medicine (IRNUM), Peshawar through ELISA and IMMULITE assay and then compared both of the essays to find out the compatibility and reliability of the assays for clinical diagnosis.

Materials and methods

Ethical approval

The Ethical Committee of the Centre of Biotechnology & Microbiology, University of Peshawar, has approved this study.

Clinical presentation

From IRNUM, Peshawar we selected patients of ovarian cancer diagnosed by biopsy examination. A total 20 benign cases (n=20) and 60 malignant cases (n=60) were included in study. The included six healthy females (n=6) in this study served as control group, which were free of any gynecological abnormality.

Blood collection

The blood was collected from the diagnosed patients of ovarian cancer as well as from six healthy females as control after written informed consent. Serum was isolated by centrifugation of blood at 8000 revolution per minute (rmp) for 5 minutes and stored at-4 ^{o}C for further use.

CA-125 Concentration determination

By using commercial ELISA based tumor marker CA-125 kit (Monobind, USA) and IMMULITE 1000 OM-MA kit (USA) the concentration of CA-125 tumor marker level in the blood serum of patients and healthy individuals were determined. Before proceeding with the assays, all the reagents, references serum, and controls were taken to ambient temperature (22-30℃). CA-125 level was determined by both the assays.

ELISA for CA125

CA-125 level was measured using CA-125 based commercial ELISA kit. The assay principal based on the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-CA-125 antibody. For each of the 80 patients and healthy control the same procedure was adopted.

IMMULITE for CA-125

The principal of IMMULITE assay based on capture by a murine monoclonal antibody and detection by a rabbit polyclonal antibody for the CA-125 antigen. The test was carried out according to the directions of the manufacturer. Assays were performed on 80 samples along with six controls.

Statistical analysis

Analysis is done through the PCC (Pearson Correlation Coefficient), which is a measure of precision and BCF (the bias correction factor), a measure of accuracy. We also applied sample independent t-test on our data which gives the significant difference between both assay values.

Results

A total 80 patients with the age of 30-70 years, who had ovarian carcinoma formed the basis of this study (Table 1). Out of eighty individuals, twenty individuals were of benign ovarian cancer (Table 2) (n=20). 72% of benign patients show high concentration of CA-125 level (≥ 35U/ml) and placed in positive patient's category while rest 25 % patients show low concentration of CA-125 and categorized as negative patients (Table 2, and Figure. 2). Six healthy females (n=6, age ranges 20-30) serves as control group.

The patients were distributed into 4 groups (A, B, C, D) on the basis of age. Group A of 20-40 years, group B of 40-50, group C of 50-60, and group D of 60-70 years of patients. Group A with 16 patients, group B

with 34 patients, group C had 24 and 6 patients were in group D. High number of patients were of group B and C (Figure. 3). Analysis of the 80 ovarian cancer patients by IMMULITE OM.MA assay showed 64 patients (80%) positive for cancer and in 16 patients (20%) the cancer marker was not detected. Analysis of the ELISA results showed 57 patients (72%) to be positive for cancer and in 23 patients (28%), the cancer antigen was not detected (Figure. 3). We used six (6) healthy females as control and their blood showed very low concentration of CA-125 equals to nil (Fig. 1).

Table 1. Blood CA-125 concentration of patients (benign and malignant Ovarian Carcinoma) Cut off value for CA125=25ng/ml.

No. of patients	IMMULITA CA-125 value (ng/ml)	ELISA CA-125 value (ng/ml)
1	59.0	477
2	230	25.8
3	160	144
4	4.70	13.7
5	36.8	20.1
6	34.1	12.6
7	23.5	0.61
8	223	227
9	67.3	41.0
10	66.2	67.6
11	59.3	482
12	11.0	10.9
13	39.0	30.0
14	17.2	8.09
15	531	77.2
16	8.00	9.41
17	14.5	6.95
18	6.96	4.50
19	249	111
20	22.9	294
21	476	377
22	15.3	6.54
23	30.5	27.0
24	60.0	70.0
25	182	74.9
26	10.0	19.8
27	2029	461
28	120	453
29	76.2	66.8
30	120	81.2
31	0.21	4.59
32	2500	241
33	34.0	14.6
34	25.1	26.8
35	117	9.39
36	60	450
37	13890	473
38	150	101
39	82.3	12.0
40	115	135
41	561.9	487

42	35.0	26.1
43	476	11.5
44	485	31.6
45	59.40	25.1
46	122	78.2
47	3.55	11.6
48	2320	441
49	7.23	16.0
50	18.2	34.8
51	168	57.0
52	1.50	1.59
53	105	98.7
54	590	448
55	559	11.7
56	268	116
57	2260	102
58	33.6	53.3
59	593	475
60	1.00	15.0
61	180	15.3
62	63.7	100.5
63	146	7.80
64	29.0	19.0
65	50.0	60.0
66	90.0	115
67	250	190
68	25.0	42.4
69	55.0	35.0
70	65.0	30.0
71	445	300
72	112	100
73	310	50.0
74	300	180
75	118	33.7
76	3.20	227
77	1.00	415
78	2.11	98.7
79	45.0	27.9
80	400	180

The cut off value for ELISA and for IMMULITE is 25 u/ml. we find out the mean and standard deviation for assay as well as PCC (Pearson Correlation Coefficient) and BCF (the bias correction factor).

PCC is significant at the level of 0.385 which shows that there is direct relation between the two assay values (Table 4). Sample independent t-test on our data gives the significant difference between both assay values as 0.046 (Table 5). It clarify that there is significant difference between IMMULITE and ELISA, both values are different in each test but are positively correlated, and have direct relationship. ELISA values of CA-125 concentration measurement are more consistent while IMMULITE showing high variations among their values which is confirmed by their standard deviation difference (Table 3). Pair t-test applied to results and concluded that both assay results were insignificant with positive correlation of .0385 Pearson correlation coefficient. Standard deviation of two means (SD of Elisa = 155.5, SD of IMMULITE = 1626.2).

Table 2. Benign ovarian carcinoma patients.

No of benign patients	IMMULITE CA-125 conc. u/ml	ELISA CA-125 conc. u/ml
1	53.1	77.2
2	63.7	10.5
3	146	98.7
4	29.0	19.0
5	50.0	60.0
6	90.0	115
7	25.1	16.8
8	25.0	12.0
9	55.0	35.0
10	65.0	30.0
11	15.3	6.54
12	112	100
13	310	50.0
14	300	180
15	118	70.0
16	3.20	11.7
17	1.00	15.1
18	2.11	7.80
19	45.0	27.9
20	400	180

The Table 1 demonstrates ELISA and IMMULITE CA-125 readings from the ovarian cancer patients of the study. To test the difference between two mean, pair t-test is carried out (Table 5). There was insignificant difference observed between the mean concentrations measured by ELISA compared to IMMULITE measurements by (Paired samples t-test: P 0.046, and the standard deviation of ELISA is much smaller (SD =155.1), as compared to IMMULITE (SD = 1626.2). The results of ELISA assay show more consistence as compared to IMMULITE assay.

Table 3. Descriptive Statistics Comparing of CA-125 concentration (ng/ml of Blood) Measured using Immulite and Elisa method. Group 1 indicating Immulite and group 2 is for Elisa values, N is our sample size and that is 80.

Group statistics				
Group	N	Mean	Std. deviation	Std. error mean
1	80	4.8318E2	1626.29057	181.82481
2	80	1.2490E2	155.10354	17.34110

Discussion

The concentration of serum CA-125 was detected high in both of the assays in 70% of the patients. This is almost an agreement with study conducted in 2001in which 80% of study population had high concentration of CA-125 (Yin *et al.*, 2001). Another study conducted by Xing *et al.* in 2011, in which 80% of the patients with non-mucinous epithelia OC, measured through Electro-chemiluminescent immunoassay, had high level of CA-125 (Xing *et al.*, 2011).

The elevated level is correlated with high grade and stage of cancer as shown by our earlier study (Ahmad *et al.*, 2015) and both assays result were agreed with elevated stage and grade of carcinoma.

Table 4. Correlation between CA-125 conc. u/ml IMMULITE and ELISA value.** . Correlation is significant at the 0.01 level (2-tailed).

Pearson correlation coefficient		IMMULITE	ELISA
IMMULITE	Pearson Correlation	1	.358**
	Sig. (2-tailed)		.001
	N	80	80
ELISA	Pearson Correlation	.358**	1
	Sig. (2-tailed)	.001	
	N	80	80

The Ca-125 concentration obtained with ELISA and IMMULITE assay in the serum samples of Pre-menopausal healthy females were ≤ 25 U/ml and same results were obtained by Thomas *et al.*, 1995 while investigating CA-125 concentration through Abbott lMx CA 125 and Abbott CA 125 RIA assays (Thomas *et al.*, 1995).

Table 5. Paired samples T-Test.

		Paired Differences					T	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	IMMULITE - ELISA	3.58279E2	1577.48827	176.36855	7.22646	709.33209	2.031	79	.046

For patient care, the laboratory results alone are only one aspect and should not be sole bases for treatment, particularly if these outcomes conflict with some other determinants (Pitta *et al.*, 2013). Same as in our study, 30-40 % of individuals had low CA-125 marker concentration but their clinical reports conform the presence of OC which clarify that only the CA-125 concentration determination is not enough to confirm the diagnosis.

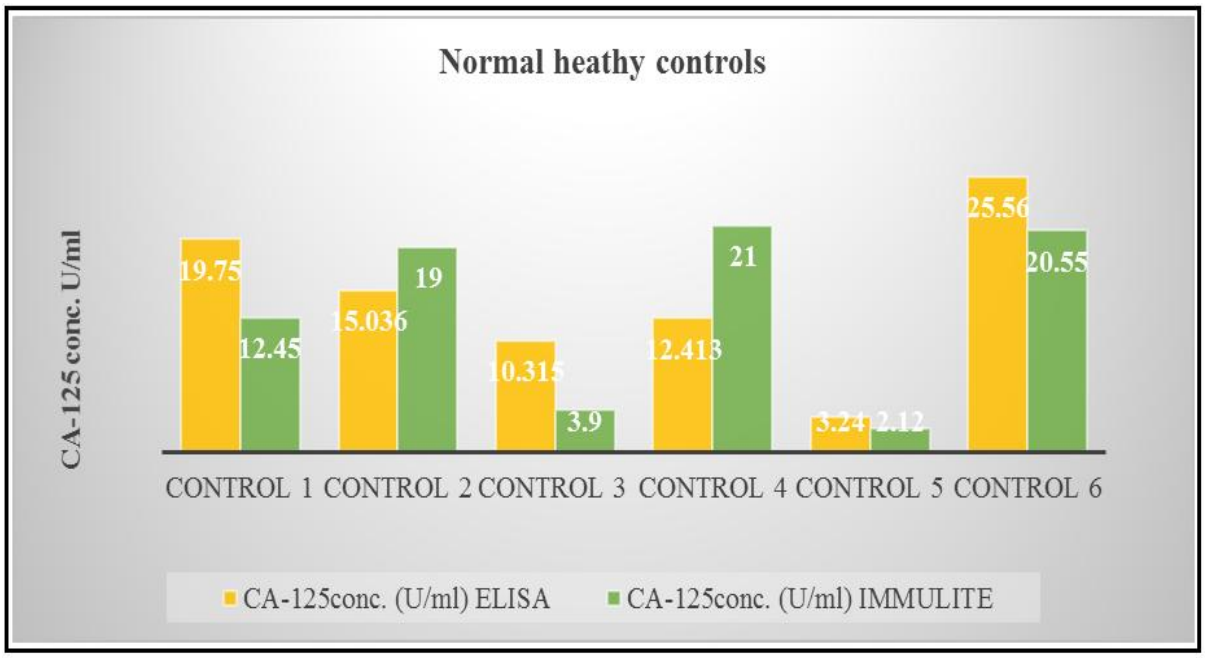

Fig. 1. Healthy females (control group).

The studies also recorded CA-125 expression in some normal physiological conditions like during embryonic development, CA125-related antigen has been confined in the mesonephric duct, epidermis, periderm, muscle cells, umbilical cord and in amnion. Normal adults also expressed CA-125 associated antigen in endo-cervix, active secreting mammary gland, and some time in lungs and kidneys. Moreover, CA-125 has been found in sera, breast milk, ascites and uterine secretion also. CA-125 concentration change in condition effecting endometrium e.g pregnancy, menstruation and endometriosis (Jankovic, 2005).

In our study three of our normal healthy controls show enough concentration of cancer antigen CA-125 in their blood stream in non-cancerous condition as stated in previous study of Jankovic, 2005.

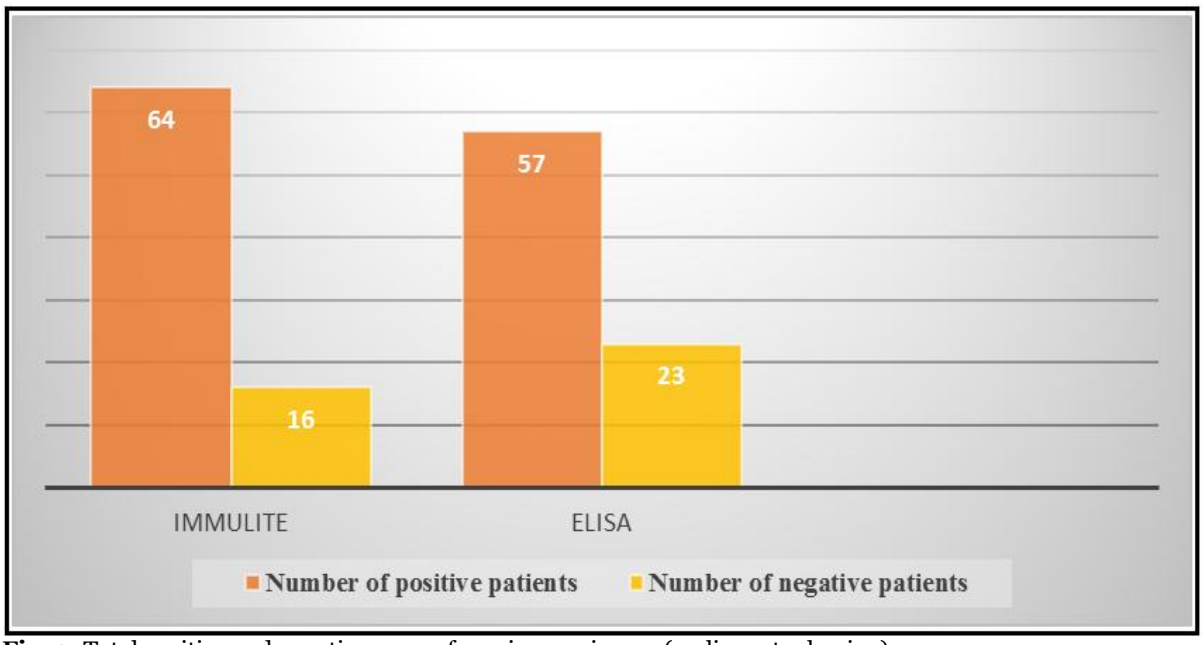

Fig. 2. Total positive and negative cases of ovarian carcinoma (malignant + benign).

Some studies revealed the low clinical sensitivity and specificity of CA-125 as a tumor marker and suggested it as the second line investigation to determine the nature of ovarian lesion (Moss *et al.*, 2013).

There are some other malignant conditions which report high concentration of CA-125 including breast cancer, Non Hodgkin lymphoma and Non Hodgkin myoma of gastro-intestinal origin. In benign condition such as endometriosis, pregnancy and ovulatory cycle's concentration of Ca-125 level also have been found elevated (Scholler & Urban, 2007).

In our study 72% benign population show elevated CA-125 level (Table 2).These finding were similar as found by Vuento *et al* in 1997, that 14 women had an elevated tumor marker CA-125 conc. ranging between 30.3 and 1410 U/ml but in primary transvaginal sonography none of these had suspicion of any uterine or ovarian malignant tumor (Vuento *et al.,* 1997).

Our results showed that both of the assays had high CA-125 level in post-menopausal women of age above than 45. This is also in consistent with the results of Moss *et al*, 2013 as they have also stated the age limits above 50. Measurement of CA125 by IMMULITE OM-MA is a simple and sensitive procedure (Gabauer, 1999).

In the report by MOR *Et Al.*, in 2005, showed perfect correlation between RCA microarray immunoassay and ELISA assays and were able to investigate group of four protein of OC i.e. Leptin, Prolactin, OPN and IGF-II. Similarly, for the confirmation of the array data through multiplex detection, Jiang *et al*, in 2013

quantitatively measured the expression levels of cytokines individually by performing single –targeted ELISA, and compared the results with the array data and found similar for the relative expression levels of proteins. In his study, he had confirmed the effectiveness of screening through ELISA and detected OC with high specificity and high sensitivity (Jiang *et al.,* 2013).

Similar to the study of Jiang *et al*; 2003, this study resulted in the significant correlation between IMMULITE assay and ELISA assay while finding out the evaluation of OC serum marker CA-125 concentration in female patients of Khyber Pakhtunkhwa. Our results clarify that like IMMULITE assay ELISA is as sensitive as specific and by comparing the values of both of assays we came to know that there is no significance difference. So it is suggested that ELISA assay may be used in our clinics to test the tumor marker level. Clinically, just a high level of CA-125 alone in the serum of effected patients is not a diagnostic value for the cancer test and confirmation and should be used in conjugation with some other clinical manifestation and other diagnostic parameters.

As the variation in sensitivity and specificity of both the assays are very small with a significant correlation between the two assays. Hence ELISA can be used to determine CA-125 level in ovarian cancer patients where the IMMULITE assay is not available or is not affordable.

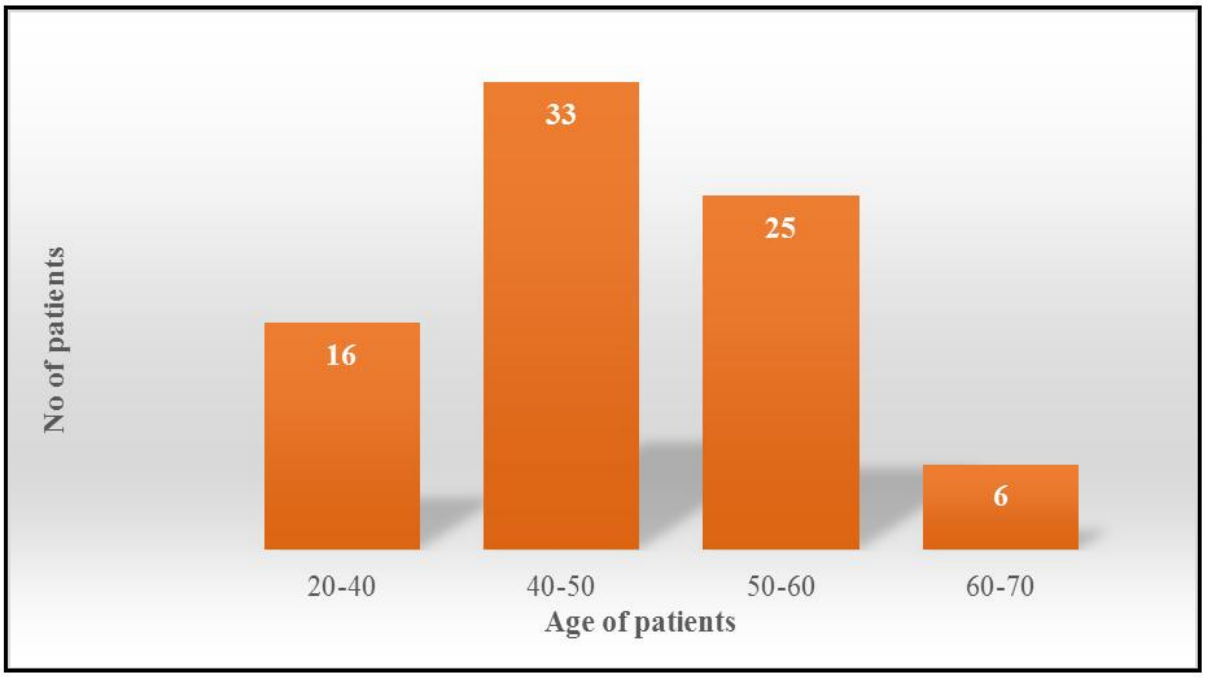

Fig. 3. Age wise distribution of total malignant and benign patients.

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