ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 6, Issue, 2, pp.2625-2629, February, 2015

International Journal of Recent Scientific Research

RESEARCH ARTICLE

BIOMARKERS OF CARCINOMA BREAST IN FEMALES

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ARTICLE INFO

Article History:

Received 2nd, January, 2015
Received in revised form 10th, January, 2015
Accepted 4th, February, 2015
Published online 28th, February, 2015

Key words:

carcinoma breast, adenosine deaminase, lactate dehydrogenase, alkaline phosphatase.

ABSTRACT

Breast cancer a multifactorial disease with its uncertain etiology remains as one of the most dreaded of human diseases that claims the lives of thousands of women all over the globe every year. This may probably be due to the fact that it remains undiagnosed at an early stage perhaps because of the lack of awareness amongst the females and the fact that most cancers do not produce any symptoms until the tumours are too large to be removed surgically. Keeping in mind the fact that enzymatic pattern is significantly altered in malignancy; the present study was designed to evaluate the role of certain biomarkers in early detection of the disease in northwest Punjabi population. The study included 75 confirmed cases of carcinoma breast and 75 normal healthy females. Serum Adenosine Deaminase (ADA), Lactate dehydrogenase (LDH) and Alkaline Phosphatase (ALP) levels were evaluated. A significant increase was observed in serum ADA ($p<0.001$) and LDH ($p<0.001$) levels in carcinoma breast patients in comparison to normal control subjects. These levels increased significantly with increasing severity of carcinoma breast (stage I to IV). Postoperatively, serum ADA and LDH levels decreased significantly ($p<0.001$). In case of serum Alkaline Phosphatase (ALP), the trend was somewhat different showing significant increase at stage IV only which further increased after mastectomy showing the role of serum ALP estimations in metastatic conditions only? The postoperative increase in serum ALP levels could be attributed to the involvement of liver. There was observed a positive coefficient of correlation (r) between serum ADA and LDH levels suggesting that LDH estimation can serve as an adjunct to ADA estimations in diagnosis of various stages of breast cancer. Serum ADA levels > 30 IU/L and < 51.53±3.33 IU/L and LDH > 480 IU/L <557.23±25.75 IU/L could be helpful in early diagnosis of the disease.

INTRODUCTION

Breast cancer is one of the diseases most feared by women and perceived as fatal worldwide both in industrialized and developing countries. This disease has shown an increased prevalence worldwide with an estimated 75,000 new cases every year in India. Incidence of breast cancer is 23.2 per 100,000 in India. The increased prevalence may unfortunately be due to the fact that most cancers do not produce any symptoms until the tumours are either too large to be removed surgically or cancerous cells have already spread to other tissues i.e. metastasis has taken place. The most common symptoms of breast cancer include the appearance of a breast lump, spontaneous clear or bloody discharge from the nipple, change in the size or shape of breast, flattening or redness or pitting of the skin around the breast (Saslow *et al* 2004) These symptoms are common to benign conditions like mastitis and fibroadenoma of breast hence no clearcut demarcation can be achieved between benign conditions and the carcinoma breast. Moreover the TNM classification of The American Joint Committee on Cancer (AJCC) refers to classify the breast cancer after the tumour has been established. To increased prevalence can be checked if the disease is detected at an early stage and prior to the establishment of tumour in the soft tissues of breast.

The cytoplasm in the body cells contains most of the enzymes that catalyze all the biosynthetic and degradative metabolisms. Under normal conditions enzymes are retained within their cells of origin by the plasma membrane surrounding the cell. Each tissue maintains a steady and consistent enzymatic pattern which is significantly altered in malignancy. Various theories propounded are based on altered hormonal milieu, personal and demographic factors and certain agents such as radiant energy, oncogenic viruses, and chemical carcinogens which includes neoplastic transformation of the cell. But even today, inspite of the extensive clinical information concerning breast cancer and the undisputed value of radiological, histological procedure available, it is still difficult to define with certainity the extent of the progress of the malignant disease in any individual patient. Therefore, there is a need for noninvasive and sensitive methods to detect growth small in size which escape routine

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examination. This could be achieved to a certain extend by measuring products and metabolites derived from the tumours in the body. Hence, this study was undertaken to evaluate the importance of assessing serum ADA, LDH and ALP levels in carcinoma breast patients in early detection, assessing the prognosis and response to the treatment.

A study showed that there was increase in the ADA activity and is maximum at stage III of breast cancer ($p<0.0001$). No significant difference between preoperative and post operative serum ADA activity was noticed (Chris Berrie *et al* 2004). In contrast to the observations of Chris Berrie et al, a significant decline was seen in the activity of ADA after mastectomy by some workers (Walia M *et al* 1995; Aghei M *et al* 2005; Mahajan M *et al* 2012). It was also reported by some workers that ADA activity of breast tumour cells was significantly elevated when compared to that of age matched normal healthy females. They observed a statistically significantly increased in ADA levels with advancing of stage of breast cancer, maximum value being seen as stage IV (Walia M *et al* 1995; Mahajan M *et al* 2012).

A significant higher value of LDH was observed as compared to non cancerous ones and a significant decline was observed after surgery(Kher A *et al* 1997).Warburg and Chrstian, suggested that high levels of glycolytic enzymes like lactate dehydrogenase might be found in the serum of patients with cancer because of the excessive glycolytic activity of the tumours (Maity C *et al* 1988).

Some workers did not find any significant difference in ALP levels in non-metastatic breast cancer (Mishra S *et al* 2004) while some other workers have revealed a significant rise in ALP in metastasis suggesting involvement of bone and liver(Stieber P *et al* 1992; Lamerz *et al* 1993; Ramaswamy G *et al* 2000; Vanhoof VO *et al* 1992; Seth RK *et al* 2003; Singh A *et al* 2013).

The latest analytical method are unapproachable for general population as the facilities are available only at sophisticated and well equipped centres with latest technology and are expensive. Therefore, there is need for simple relatively inexpensive biomarker investigations that can be easily assayed to diagnose the stage of breast cancer.

MATERIALS AND METHODS

A total number of 75 subjects participated in the present study which included 26, 25, 19 and 5 patients in stage I, stage II, stage III and stage IV respectively of carcinoma breast. 75 age matched normal healthy females were taken as controls from the general population. Informed consent was taken from all the subjects for drawing their blood sample. The patients suffering from carcinoma breast who were diagnosed by concerned surgeon on the basis of TNM classification were selected from the wards at Surgery Department, Guru Nanak Dev Hospital, Amritsar. Patients with myocardial infarction, jaundice or liver disease, pancreatic disease, diabetes mellitus and tuberculosis etc were excluded from the study. History of patients was collected and thorough clinical examination was conducted in all the cases according to the performa. Routine investigations were done in all cases. Blood samples were collected from healthy controls and carcinoma breast patients before mastectomy (preoperative) and one month after mastectomy (postoperative) and analyzed for serum ADA, LDH and ALP by kit methods.

METHODS

1. The estimation of serum Lactate Dehydrogenase (LDH) enzyme by DGKC Method, Kinetics (Kit Method) (1960).
2. The estimation of serum Adenosine Deaminase (ADA) enzyme by Guisti's Colorimeteric method (1974).
3. The estimation of serum Alkaline Phosphatase by method of Henry et.al (kit method).

Statistical analysis

The data for biochemical analysis are expressed as mean and standard deviation (SD). Statistical comparisons were performed by student's t-test. Unpaired t-test was used for finding significant difference between two groups. In carcinoma breast patients, changes in the serum levels of biochemical parameter after surgery was analyzed by paired t-test for each stage.

For all the tests p-value of <0.05 was considered as statistically significant.

RESULTS

There was statistically significant ($p < 0.001$) increase in serum ADA levels in females with carcinoma breast than in the age matched normal females (control). These patients were followed up after one month of surgery and it was observed there was statistically significant ($p<0.001$) decline in serum ADA levels after the surgery was done when compared with the preoperative patients. The levels of serum ADA were closer to the Mean±SD values observed for normal females.

Table1 Variation in Serum ADA levels at various stages of carcinoma breast and its comparison to normal individuals.

Parameter → / Individual		Serum ADA levels (Normal range: 0- 30 IU/L)			
Normal females (control) (n=75)	Range	13-25			
	Mean±SD (IU/L)	20.32±4.70			
		Preoperative		Postoperative	
		Range	Mean±SD (IU/L)	Range	Mean±SD (IU/L)
Females with CA breast (n=75)		46-78	60.58±8.89	15-29	21.86±1.46
Stages of CA breast					
Stage I (n=26)		46-57	51.53±3.33	15-23	21.15±1.15
Stage II (n=25)		47-68	59.84±4.57	16-22	21.88±1.58
Stage III (n=19)		58-72	70.10±2.68	15-27	22.47±1.26
Stage IV (n=05)		73-78	75.2±1.92	18-29	23.2±1.30

Table 2 p values of serum ADA levels in various stages of CA breast and normal healthy females

Comparison	P value	
Control vs preoperative	<0.001	
Control vs postoperative	<0.001	
Interstage	preoperative	Postoperative
StageI vs stageII	<0.001	0.06
StageII vs stageIII	<0.001	0.18
StageIII vs stageIV	<0.001	0.26
Preoperative stage I vs postoperative stage I	<0.001	
Preoperative stage II vs postoperative stage II	<0.001	
Preoperative stage III vs postoperative stage III	<0.001	
Preoperative stage IV vs postoperative stage IV	<0.001	

Table 6 p values of serum ALP levels in various stages of CA breast and normal healthy females

Comparison	P value	
Control vs preoperative	<0.001	
Control vs postoperative	<0.001	
Interstage	preoperative	Postoperative
StageI vs stageII	<0.001	<0.001
StageII vs stageIII	0.35	0.006
StageIII vs stageIV	<0.001	0.76
Preoperative stage I vs postoperative stage I	0.50	
Preoperative stage II vs postoperative stage II	0.32	
Preoperative stage III vs postoperative stage III	0.82	
Preoperative stage IV vs postoperative stage IV	<0.001	

There was statistically significant ($p < 0.001$) increase in serum ALP levels in females with carcinoma breast than in age matched normal females (control). There was statistically significant increase in serum ALP levels with advancement of stage of breast cancer (stage I to IV) & maximum being observed at stage IV Postoperatively, there was no significant decline in serum ALP levels at stage I,II and III. A statistically significant decline in serum ALP levels was observed at stage IV when compared to preoperative state and the values never turned back to normal (41-71 IU/L in case of normal control females).

Table3 Variation in Serum LDH levels at various stages of carcinoma breast and its comparison to normal individuals.

Parameter → / Individual		Serum LDH levels (Normal range: 280-480 IU/L)			
Normal females (control) (n=75)	Range	157-293			
	Mean±SD (IU/L)	277.97±63.36			
		Preoperative		Postoperative	
		Range	Mean±SD (IU/L)	Range	Mean±SD (IU/L)
Females with CA breast (n=75)		509-1305	731.76±190.42	259-486	357.21±62.21
Stages of CA breast					
Stage I (n=26)		509-595	557.23±25.75	259-426	324.92±49.25
Stage II (n=25)		604-794	694.92±50.14	339-431	370.48±67.33
Stage III (n=19)		781-1052	891±73.31	345-469	366.05±53.78
Stage IV (n=05)		1198-1305	1218.4±67.07	364-486	425.2±46.97

Table 4 p values of serum LDH levels in various stages of CA breast and normal healthy females

Comparison	P value	
Control vs preoperative	<0.001	
Control vs postoperative	<0.001	
Interstage	preoperative	Postoperative
StageI vs stageII	<0.001	0.007
StageII vs stageIII	<0.001	0.81
StageIII vs stageIV	<0.001	0.03
Preoperative stage I vs postoperative stage I	<0.001	
Preoperative stage II vs postoperative stage II	<0.001	
Preoperative stage III vs postoperative stage III	<0.001	
Preoperative stage IV vs postoperative stage IV	<0.001	

There was statistically significant ($p < 0.001$) increase in serum LDH levels in females with carcinoma breast than in the age matched normal females (control). These patients were followed up after one month of surgery and it was observed there was statistically significant ($p<0.001$) decline in serum LDH levels after the surgery was done when compared with the preoperative patients.

Table 7 Coefficient of correlation (r) between LDH and ADA in various stages of CA breast.

Stage	Preoperative		Postoperative	
	r	p	r	p
Stage I (n=26)	+0.38	<0.01	+0.49	<0.001
Stage II (n=25)	+0.35	<0.01	+0.76	<0.001
Stage III (n=19)	+0.37	<0.01	+0.87	<0.001
Stage IV (n=05)	+0.97	<0.001	+0.93	<0.001

A positive correlation was established between serum ADA and LDH levels in carcinoma breast patients. Preoperatively, a significant correlation was seen in different stages of carcinoma while postoperatively, a highly significant positive correlation was observed.

Table5 Variation in Serum ALP levels at various stages of carcinoma breast and its comparison to normal individuals.

Parameter → / Individual		Serum ALP levels (Normal range: 40-111(IU/L)			
Normal females (control) (n=75)	Range	41-71			
	Mean±SD (IU/L)	47.10±11.74			
		Preoperative		Postoperative	
		Range	Mean±SD (IU/L)	Range	Mean±SD (IU/L)
Females with CA breast (n=75)		38-197	77.05±39.09	39-102	76.48±18.95
Stages of CA breast					
Stage I (n=26)		38-53	48.03±11.57	39-55	46.15±6.30
Stage II (n=25)		51-83	79.12±21.85	48-79	74.08±10.91
Stage III (n=19)		62-89	84.52±14.20	67-88	84.89±13.67
Stage IV (n=05)		59-197	189.2±46.64	82-102	98.2±53.20

DISCUSSION

Breast cancer is the leading malignancy and also the leading cause of death from cancer in women. The commonest cause being late marriage, birth of first child at a later stage, shorter period of breast feeding, industrialization etc. So far the criteria followed for diagnosis is based on histopathological technique measuring tumour size, lymph nodes involvement and metastasis. A number of workers have tried evaluating the role of biomarkers in diagnosis of carcinoma breast but till date biochemical markers have not given promising results about diagnostic criteria. In view of this, our study was undertaken for the categorization of the patients according to stages of cancer by evaluating serum ADA, LDH and ALP.

It was observed that level of serum Adenosine Deaminase (ADA) was significantly ($p<0.001$) high in patients of carcinoma breast as compared to equal number of normal healthy females (Table 2). When these patients were classified into different stages on the basis of TNM criteria of classification, it was observed that ADA levels keep on increasing with advancing stage of breast cancer.(stage I to IV,Table1). Serum ADA is sensitive to stimulation by growth factors and cytokines during rapid tissue proliferation (Borzenko BG *et al* 1997). The increase in the serum ADA levels may be a result of the lymphoid proliferation in the metastatic lymph nodes or leakage of the enzyme from the primary tumour cells.

After the mastectomy, serum ADA levels tend to decline in all the patients and a significant ($p < 0.001$) decline in ADA levels was seen in patients at each stage of breast cancer (Table 7). Moreover the levels of ADA after mastectomy were found to be closer to Mean±SD value observed in normal females and the values were within the normal range of the method (0-30 IU/L,Table 1).

These findings are consistent with those of other workers(Walia M *et al* 1995; Cabolt O et al, 1998; Aghei M *et al* 2005; Mahajan M *et al* 2012; Maksoud NAE *et al* 2009). In their study it was stated that serum ADA levels are significantly increased in carcinoma breast and the levels keep on increasing with increasing size of tumour (stage I to IV) and that it could be useful parameter for the diagnosis of breast cancer and for monitoring its progression and remission after surgical resection or chemotherapy.

Serum LDH levels were significantly ($p <0.001$) high in patients of carcinoma breast as compared to normal healthy females (Table 4). When the levels of serum LDH in these patients at various stages of breast cancer were estimated, it was observed that LDH levels increased significantly ($p <0.001$) with increasing size of tumour, number of lymph nodes and metastases etc i.e. from stage I to IV (Table 3). This increase in serum LDH levels could be responsible for excretion of large amount of lactic acid by the tumour cells consequent upon the anaerobic glycolytic pathway operating in tumour cells when compared to normal cells. Serum LDH is the consequence of the disruption of the cell membrane of a large fraction of dividing malignant cells whose metabolic hallmark is anaerobic glycolysis because of increased LDH enzyme activity (Koukourakis *et al* 2009), hence increased levels of lactic acid.

After the mastectomy, serum LDH levels tend to decline in all the patients and the decline in LDH levels was seen in patients at each stage of breast cancer(Table 3). Moreover, the values were within the normal range of the method (240-480 IU/L,Table 8) suggesting there by serum LDH levels are good biomarker for diagnosis of breast cancer and for identification of stage of cancer and its remission consequent upon mastectomy. The present findings are consistent with those of some workers (Chandrakanth KH *et al* 2011; Shrivastavan A *et al* 1999) who stated that enzymatic pattern is significantly altered in malignancy with release of large amount of these enzymes in the plasma where the cells are replicating rapidly in comparison to the normal conditions.

The levels of serum ALP were significantly ($p <0.001$) high in patients of carcinoma breast as compared to normal healthy females. When the levels of serum ALP in these patients at various stages of breast cancer were estimated. It was observed that serum ALP levels increased significantly with advancing stage of cancer (stage I to IV) , maximum increase being observed at stage IV(Table 5).

After the mastectomy, the decline in serum ALP levels was relatively insignificant at stage I, stage II and stage III of breast cancer but significant decline was observed in stage IV ($p<0.001$,Table 6). The levels of serum ALP never turned back to the figure observed in case of age matched normal females that served as control.

These findings are suggestive of the fact that ALP can be used to detect occurrence and progression of carcinoma breast (stage I to stage IV). A significant increase in levels of serum ALP at each stage when compared to that of normal healthy females and no significant decline in the values of ALP even after surgical removal of the tumour are suggestive of the fact that serum ALP levels increase with increase in number of lymph node involved and the occurrence of metastasis in bone and liver. Moreover all these patients after mastectomy were subjected to chemotherapy and radiotherapy and hence increased serum ALP levels could be also due to hepato-toxic effects of various chemotherapeutic agents.

To conclude serum ADA, LDH, and ALP estimations together can be used as diagnostic biomarker for early detection of breast cancer, progression of disease , occurrence of metastasis.

CONCLUSION

The present study suggests that estimation of serum Adenosine deaminase, Lactate Dehydrogenase and Alkaline Phosphatase together can serve as important biomarkers for diagnosis and development of various stages of carcinoma in females. The classification of breast cancer into various stages can be achieved early and in a better way with the knowledge of these biomarkers rather than knowing only the size of tumour and involvement of lymph nodes etc.

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How to cite this article:

Amritpal Kaur *et al*. Biomarkers of carcinoma breast in females. *International Journal of Recent Scientific Research, Vol. 6, Issue, 2, pp.2625-2629, February, 2015*

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