Observational Study of the association of GGT with Benign and Malignant Breast Tumors

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Abstract: Background: Gamma-glutamyltransferase (GGT) is a cell surface enzyme that uniquely enables glutathione catabolism by hydrolysing the gamma glutamyl bond between glutamate and cysteine. GGT is one of the enzymes which may rise in malignancies and since being economic and relatively easier to determine, remain as useful and practical marker in terms of prognosis and also early diagnosis. Breast cancer is a major public health issue in India. Most cases of Breast cancer in India are diagnosed at advanced stage, and outcome is not as good as earlier stages, most important reason being lack of screening and awareness. The objective of present study was to assess the clinical utility of serum levels of GGT in cases of breast lesions and to evaluate whether their levels are significantly higher in cases of breast malignancies as compared to cases of benign breast lesions. Methods: A total number of 160 subjects were studied, comprising of 80 cases of benign breast lesions and 80 Breast cancer subjects. Breast cancer subjects were further divided depending on stage of the cancer. Serum level of GGT was estimated in all subjects. Results: The present observational study has shown a significant elevation in serum levels of GGT in cases of carcinoma Breast as compared to benign breast lesions. These levels raised significantly with severity (stage) of carcinoma Breast. Conclusion: This study concludes that enzyme markers like serum GGT could serve as cost effective biomarkers for carcinoma breast and help in early detection of progression of the disease. [Pratibha M NJIRM 2017; 8(4):53-57]

Key Words: Carcinoma breast, Fibroadenoma, Gamma glutamyl transferase, Glutathione, Metastasis**.**

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Introduction: The enzyme gamma glutamyl transferase (GGT) uniquely enables glutathione (GSH) catabolism by hydrolysing the gamma glutamyl bond between glutamate and cysteine. The enzyme is widely present on the external surface of most cells [1]. GGT is constitutively expressed in several organs and is often significantly increased in malignant or premalignant lesions, where it is considered a factor conferring growth and survival advantages for the rapidly dividing neoplastic cells. GGT has also been shown to be inversely correlated with levels of several antioxidants, including β-carotene, α-carotene, β-cryptoxanthin and α-Tocopherol, which are known to lower incidence of several cancers [2]. Conditions that increase serum GGT, lead to increased free radical production and the threat of glutathione depletion [3]. GGT activity is able to promote iron-dependent DNA oxidative damage, thus potentially representing an important mechanism in initiation/progression of neoplastic transformation [4]. Functionally relevant polymorphisms in GGT may well affect the risk of developing cancer in tissues that express GGT [5]. GSH/GGT-dependent pro-oxidant reaction has been found to modulate the transduction of proliferative/apoptotic signals [1]. GGT-positive tumor cells have a selective growth advantage in vivo in comparison to GGT-negative tumor cells because they are able to use serum glutathione as a secondary source of cysteine thereby overcoming the growth restriction imposed by serum levels of cysteine [6].

Cancer is one of the major health issues worldwide. At global level it accounted for 11.4 million new cases in 2004. Cancer incidence in South East Asia region was 1.7 million in 2004[7]. Breast cancer is a major public health issue worldwide. In India, for the year 2012, 144,937 women were newly detected with breast cancer while 70,218 women died of breast cancer. Breast cancer is now the most common cancer in most cities in India, and 2nd most common in the rural areas. In India, the average age of developing a breast cancer has undergone a significant shift over last few decades with almost 48% patients are below 50 years of age. An increasing numbers of patients are in the 25 to 40 years of age. Most cases of Breast cancer in India are diagnosed at advanced stages, the most important reason being lack of screening and awareness[8].

Early detection of carcinoma is an important step towards treatment and a number of biochemical markers are being studied to evaluate malignancy and its impact on human survival rate. However, there is no ideal marker that has been proved to be a sensitive and specific indicator of early breast cancers. GGT is one of the enzymes which may rise in malignancies

and since being economic and relatively easier to determine, remain as useful and practical marker in terms of prognosis and also early diagnosis. In combination with other tumour markers, it shows higher specificity and sensitivity when compared with other liver enzymes especially when there is liver metastasis[9].

Hence, this study was undertaken to evaluate the clinical utility of assessing the serum levels of gamma-glutamyl transferase (GGT) in cases of breast lesions and also, to correlate the probable relationship between the serum levels of the enzyme and stages of breast cancer. The present observational study was carried out at the department of Pathology, Netaji Subhash Chandra Bose Medical College and Hospital, Jabalpur to study the serum levels of GGT in cases of benign and malignant breast lesions, also to evaluate whether the serum levels of GGT are significantly higher in cases of breast malignancies as compared to cases of benign breast lesions and to evaluate whether the serum levels of GGT are significantly correlated with stage of the disease in cases of malignant breast lesions.

Methods: The present Observational Study was conducted from November 2014 to November 2015 at Department of Pathology, N.S.C.B. Medical College and Hospital, Jabalpur. Sample size was calculated by using power and sample size command (sampsi) in STATA 12 (StatCorp LP, Texas USA) statistical software. The assumptions for comparing the mean difference of GGT between benign and malignant group was considered 20 – 60. Minimum required samples size was drawn 71 cases in each group with 5% alpha and 80% power. To adjust the non-response error which assumed maximum up to 10%, the sample size was further increased and finally decided to enroll 80 sample in each group. A total number of 160 subjects participated in the present study which included 80 cases each of benign and malignant lesions of the breast. All were in the age group of 18 to 80 years. Study was conducted on inpatients and out patients of N.S.C.B. Medical College And Hospital, Jabalpur. Cases were histopathologically or cytologically diagnosed as benign and malignant lesions of the breast. A careful history was taken and thorough clinical examination was conducted in all the cases. Serum GGT levels were measured and complete blood count (including hemoglobin, platelets, total and differential leucocyte count) was done in all the cases.

GGT was determined using GGT test system is a device intended for the quantitative in vitro determination of L-Gamma-Glutamyltransferase (GGT) activity in serum and plasma. The GGT estimation was done by the colorimetric method. The substrate L-γ-glutamyl-3-carboxy-4-nitroanilide, in the presence of glycylglycine is converted by GGT in the sample, to 5-amino-2-nitro-benzoate which absorbs at 405 nm.

Inclusion criteria: Patients with Breast lesions from inpatient and outpatient department of N.S.C.B. Medical College and Hospital, Jabalpur, in the age group of 18 to 80 years .Study subjects were divided into two subgroups:

- Group 1: Patients having Benign breast lesion.
- Group 2: Patients having malignant Breast lesion.

The cases were proven as benign and malignant cytologically or histopathologically. Breast malignancy patients were further divided according to stage grouping. Stage grouping was based on TNM staging system.

Exclusion criteria: Patients with Liver disease, history of alcoholism, pancreatic disease, bone diseases other than metastasis, diabetes mellitus, heart disease, other malignancies and those taking anti-epileptic or hepatotoxic drugs during the last three months were excluded from the study.

Statistical analysis: The data was compiled and entered in the Microsoft excel sheet. It was analyzed using statistical software SPSS IBM (Chicago) version 21. The data was represented in tables and charts. The frequency was displayed of all variables and mean and standard deviation was calculated for quantitative variables. Unpaired student t test was applied for comparing means [quantitative data] and chi square test was applied for qualitative data. The test was considered significant if $p<0.05$, at 95% confidence level.

Ethical consideration: This study was approved by the Research and Ethical Committee of NSCB Medical College and Hospital, Jabalpur.

Results: As shown in table I, in present study maximum numbers of cases of both benign and malignant lesions were in 40 – 49 years of age group (33.8%) followed by 30 – 39 years of age group (23.1%). Minimum number of cases were in >60 years of age group.

As shown in table II maximum numbers of cases belonged to stage III followed by stage II in the present study comprising of 46.4% and 38.7% case respectively. While stage IV and stage I consisted 11.3% and 3.6% cases respectively. In the present study the mean serum levels of GGT was raised in cases of Malignant lesions when compared to cases of Benign lesions, and the rise was highly significant (p value < 0.001) (table III). The mean levels of serum GGT increased serially from stage I to stage IV in the present study. Highest level of enzyme was observed in stage IV of the disease (table IV).

As shown in table V when serum GGT levels were compared in different stages of breast cancer the enzyme levels raised significantly (from stage I to stage IV) with increase in severity (stage) of the disease. However *no* significant difference was observed in the enzyme level when stage I was compared with benign disease. In present study it was observed that serum levels of GGT were higher in malignant lesions when compared to benign lesions with comparable size of the tumor (table VI).

Table I: Age wise distribution of patients

Age group	No of patients	Percentage
<20	13	8.1
20—29	30	18.8
30—39	37	23.1
40—49	54	33.8
50—59	12	7.5
60—69	7	4.4
>70	7	4.4
Total	160	100.0

Table II: No. of patients in various stages of Breast cancer

Stage	No of patients	Percentage
I	3	3.6%
II	31	38.7%
III	37	46.4%
IV	9	11.3%
Total	80	100%

Table III: Comparison of serum GGT levels in malignant and benign breast tumors

Serum GGT level	Malignant		Benign		p value
	Mean	SD	Mean	SD	
	33.7	23.4	15.96	8.91	<0.001

Table IV: Mean Serum GGT Level according to the clinical stage

Stage	Mean Serum GGT	Std. Deviation
I	13.67	4.93
II	24.32	10.09
III	31.49	17.23
IV	82.44	22.67

Table V: Comparison of Mean Serum GGT Level in different clinical stages of breast cancer patients

GGT mean	P value
Benign Vs stage I	0.51
Stage I Vs Stage II	0.035 [sig]
Stage I Vs Stage III	0.024 [sig]
Stage I Vs Stage IV	0.0006 [sig]
Stage II Vs Stage III	0.036 [sig]
Stage II Vs Stage IV	0.0019 [sig]
Stage III Vs Stage IV	0.0039 [sig]

Table VI: Correlation of serum GGT with size of tumor in Malignant and benign lesions.

Size cm	Type	Mean GGT	SD	No of patients	P value
< 2	Malignant	16.0		1	0.89
	Benign	17.6	11.1	29	
2-3	Malignant	38.5	24.5	22	<0.001
	Benign	15.4	7.9	42	
4-5.	Malignant	33.0	27.1	27	0.16
	Benign	13.0	4.2	4	
>5	Malignant	31.6	19.2	30	0.052
	Benign	14.0	4.2	5	

Discussion: The enzyme γ-glutamyl transferase uniquely enables glutathione (GSH) catabolism by hydrolysing the γ-glutamyl bond between glutamate and cysteine [1]. GGT is located on the outer aspect of plasma membrane of most cell types,and is often expressed at high levels in malignant tumors and their metastases [10]. Several experimental models have elucidated the ability of cellular GGT to modulate crucial redoxsensitive functions, such as antioxidant/antitoxic defences and cellular proliferative/apoptotic balance, and its role in tumor progression, invasion and drug resistance [2]. In a study

there was found positive association between GGT and overall cancer risk of prostate, breast, liver and pancreatic cancer [11].

A number of biomarkers have been studied to evaluate malignancies. However, no specific markers for breast cancer have been discovered and those that are currently available lack the sensitivity and specificity for early detection of cancer and timely treatment. This study involving serum GGT levels aims to find the correlation of breast cancer with the enzyme levels in determining its prognostic significance if not for diagnostic role. This may improve the patient survival rate with a timely treatment. Serum levels of GGT along with other biomarkers may differentiate benign and malignant lesions and help in early detection of disease. It may serve as better marker in terms of prognosis [9].

Breast carcinoma is one of the commonest malignancies in females with its increasing incidence. Despite the extensive research for many years throughout the world, the etiopathogenesis of cancer still remains obscure. Oxidative stress plays an important role in the pathogenesis of chronic diseases, such as cancer and atherosclerosis. For the early detection of carcinoma of various origins, a number of biochemical markers have been studied to evaluate the malignancy. Tumor associated markers reflect behavioural changes from tissue to blood, resulting in changes in levels of enzymes, proteins and hormones both in cancerous tissue and blood because of unchecked proliferation of cells. Therefore, alteration in particular enzyme contents in serum could be a good index of malignancy in its early and best manageable stage [12].

In view of this, present study was undertaken to study the serum levels of GGT in cases of benign and malignant breast lesions and to evaluate whether serum levels of GGT are significantly higher in cases of breast malignancies as compared to cases of benign breast lesions.

In present study serum GGT levels were significantly ($p<0.001$) elevated in carcinoma breast cases (33.7 ± 23.4 U/L) when compared with benign lesions (15.96 ± 8.91U/L) (table no. III) in present study .
Our findings are in accordance with Shashi Seth et al [13] and Chanrakanth et al [12], found significant increase in serum GGT levels in patients with breast cancer as compared to benign breast disease.

In present study the mean levels of serum GGT increased serially from stage I to stage IV (table IV). Highest enzyme level was observed in stage IV of the disease.

When serum GGT levels were compared in different stages of breast cancer the enzyme levels raised significantly (from stage I to stage IV) with increase in severity (stage) of the disease (table V) .Similar findings were observed by Choudhari et al [14], Chandrakanth et al [12], Seth L R et al [15], in patients with breast cancer there was a steady and progressive increase in serum GGT levels from stage I to stage IV .

Although serum GGT level is non-specific parameter and it is difficult to ascertain its diagnostic importance in breast cancer patients, yet its prognostic importance cannot be undermined. A combination of GGT along with other tumor markers could be used as important biochemical parameter in patients with breast cancer, which are cost effective, and can be easily assayed in smaller laboratories not yet exposed to any sophisticated technology for more reliable cancer marker [16].

Conclusion: Breast cancer is a leading cause of cancer death in the less developed countries of the world. This is partly because a shift in lifestyles is causing an increase in incidence, and partly because clinical advances to combat the disease are not reaching women living in these regions. An urgent need in cancer control today is to develop effective and affordable approaches to the early detection, diagnosis, and treatment of breast cancer among women living in less developed countries of the world. The present observational study has shown a significant elevation in serum levels of GGT in cases of carcinoma Breast and these estimations together with clinical and histopathological findings may serve as potential markers in assessing the prognosis in patients of breast cancer. Serum GGT level could serve as an important biochemical aid for early detection and monitoring the progression of carcinoma breast. Serial levels of GGT in patients of proliferative breast lesions can help in early detection of progression of malignancy, if levels go on increasing. Although being non-specific it is difficult to ascertain its diagnostic importance in cancer patients,

its role as prognostic markers seems to be more significant. Serum GGT estimation is cost effective, and can be easily assayed in smaller laboratories not yet exposed to any sophisticated technology for more reliable cancer marker. Further studies on a larger sample with follow up are needed to substantiate our findings which can establish strong guidelines for the utility of serum GGT levels for the diagnosis and assessment of progression of carcinoma breast.

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