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مقاله پژوهشی

ارزش تشخیصی سطح سرمی آنزیم‌های ADA، GGT و ALP در تشخیص و پیش‌آگهی سرطان سینه،یک مطاله سه مرکزی

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چکیده

مقدمه: *سرطان سینه شایع‌ترین نوع سرطان در زنان هندی و دومین نوع رایج در زنان روستایی هند است. سرطان را می‌توان با تشخیص زودهنگام هنگامی که اندازه تومور کوچک است درمان کرد تا به‌طور کامل با عمل جراحی برداشته شود. تعدادی از تومورمارکرها به‌منظور تشخیص زودهنگام سرطان مورد مطالعه قرار گرفته است. با این حال، این امکانات تنها در مراکز پیشرفته و مجهز به آخرین فناوری موجود هستند و آنها نیز گران هستند.*

مواد و روش‌ها: *این تحقیق یک مطالعه کنترل موردی بر روی ۲۶ بیمار است که در آنها سرطان پستان به‌طور کلینیکی و هیستوپاتواوژیکی تشخیص داده شده بود. نمونه‌ها ابتدا براساس TNM staging طبقه‌بندی گردید. سرم خون برای اندازه‌گیری آنزیم‌های ADA، GGT و آنزیم‌های کبدی (SGOT, SGPT, and ALP) جمع‌آوری گردید.*

نتایج: *این تحقیق یک مطالعه کنترل موردی بر روی ۲۶ بیمار است که در آنها سرطان پستان به‌طور کلینیکی و هیستوپاتواوژیکی تشخیص داده شده بود. نمونه‌ها ابتدا براساس TNM staging طبقه‌بندی گردید. سرم خون برای اندازه‌گیری آنزیم‌های ADA، GGT و آنزیم‌های کبدی (SGOT, SGPT, and ALP) جمع‌آوری گردید.*

نتیجه‌گیری: *میزان افزایش آنزیم‌ها را می‌توان به‌عنوان یکی از معیارهای تشخیصی مهم برای تعیین بیماران مبتلا به سرطان پستان در نظر گرفت. این ازمایشات در مناطق دور افتاده به‌راحتی در دسترس و مقرون به صرفه برای تشخیص زودهنگام سرطان سینه می‌باشد.*

واژه‌های کلیدی: ادنوزین دی امیناز،گاما گلوتامیل ترانس پپتیداز، الکالین فسفاتاز، مارکرهای انزیم، سرطان سینه.

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ارجاع: سودشنا بهارا، ایتشیری جنا، اسماراکا رانجان پاندا، سارتاک رانجاننایاک، سوبهاشری رای. ارزش تشخیصی سطح سرمی آنزیم‌های ADA، GGT و ALP در تشخیص و پیش‌آگهی سرطان سینه، یک مطاله سه مرکزی. مجله دانش و تندرستی در علوم پایه پزشکی ۱۴۰۰؛۱۶(۳):۵۴-۴۹.

Introduction

While cancer is the basis for high morbidity and high mortality rate worldwide, breast carcinoma is prevalent in women of developed countries and affects more than 40% of all cancer cases in developing countries (1). Its health care burden in India has been progressively mounting. Over 100,000 new breast cancer patients are estimated to be diagnosed annually in India (2,3). Due to the heterogeneous health care facility pattern, many regions of India are yet deprived of benefits of various awareness programmes, early diagnostic modalities, and many other multidisciplinary treatment programs. In recent years due to its growing incidence and increased attentiveness of people to various awareness and screening programmes, breast cancer accounts for the most common cancer in urban Indian females, and the second commonest in the rural Indian women (4).

Although breast cancer can be found out at an early stage by simple breast examinations, but most of the cases are being diagnosed in their advanced stages. Early detectable cancers can potentially be cured when the small size tumours can be completely removed. Unfortunately most of them are symptom less until the tumours becomes large enough to be surgically removed or metastasis has already taken place. Hence there is always a need of detecting cancers at an early stage so that curative measures can be taken.

In India, various Breast cancer awareness programs are going on but unfortunately these are more rigorous in cities rather than remote and rural areas5. Women often do not reach for medical care at an early stage due to a variety of factors like illiteracy, lack of awareness, and financial constrains. Therefore most of the carcinoma breast patients in India are still treated at locally advanced and metastatic stages (6). For the early detection of breast cancer patients, a number of biochemical tumour markers such as CA 15-3, CA 549, CA 27-29 and mucin like carcinoma-associated antigen (MCA) have been studied. However the analytical methods of many of these are unapproachable for general population as the facilities for these are only available at sophisticated and well equipped centres with latest technology and they are also expensive (7,8).

Nowadays in order to investigate the diagnosis and characterisation of breast cancer, biochemical enzyme analysis has attracted great attention for many researchers (9,10,11) as enzymes were 1st identified as the tumour markers for carcinomas. According to reports of various recent researchers, there is a significant increase of serum levels of ADA (adenosine deaminase), GGT (gamma glutamyl transpeptidase) and ALP (alkaline phosphatase) and some other biochemical markers in patients with malignancy of breast. Assessment of serum levels of these biochemical parameters could substitute for the classical tumour markers in remote and rural areas where facility for these are not readily available.

In this study we focus on the activity of promising enzyme markers like ADA,GGT and ALP in breast cancer patients and to correlate their serum levels in different stages and finally to find out whether these parameters may have any diagnostic and prognostic significance in breast cancer patients.

Materials and Methods

The study was approved by the institutional ethical committee. An informed written consent was obtained prior to the study seeking permission to participate in the study.

The study was conducted in joint collaboration of Department of Biochemistry & Department of Oncology in IMS & SUM Hospital, Bhubaneswar under the aegis of Siksha 'O' Anusandhan University. This was a case-control comparative study conducted taking 26 clinically and histopathologically established female breast cancer patients attending the OPD or admitted to the department of surgical oncology. Same number of age matched breast cancer free females was taken as controls after exclusion of the disease by history and clinical examination.

Exclusion criteria includes Patients suffering from tuberculosis, rheumatic fever, jaundice and hepatobiliary disease, bone diseases, pancreatic disease, hemolytic anemia, congestive cardiac failure, myocardial infarction, ulcerative colitis, other malignancies and patients who have already received or were still under the treatment for malignancy.

The cases will first be categorized according to the TNM staging of Ca Breast basing on tumor size (T), involvement of lymph nodes(N) and metastasis(M) into various stages such as stage-I, stage –II, stage-III and stage-IV.

2 ml of blood was collected from both cases and controls from antecubital vein under aseptic precautions and was allowed to clot. After centrifugation, serum was collected for estimation of ADA, GGT and liver enzymes (SGOT, SGPT, ALP) and lipid profile.

The estimation of the parameters was carried out immediately. Serum ADA and GGT level was measured in semi autoanalyser (photometer 5010) by commercially available kits. Lipid profile, SGOT, SGPT & ALP levels were measured in autoanalyser (Roche Cobas Integra 401 plus) using ready to used reagents procured from Roche company.

The Demographic data collection related to the study was carried out by a previously prepared structured Questionnaire in local language. Test results of the above mentioned parameters were recorded and statistical analysis of the data were done using SPSS 20 software. Continuous data were expressed in terms of mean and standard deviation. Means were compared using students't-test and calculating P value. P- value of <0.05 was considered significant.

Results

Breast cancer is a female biased disease as the disease frequency is very low in males in comparison to females. In our study we also registered all female patients. Mean age of the study population is 50.9±7.76 yrs with maximum number of cases fall within the age group of 41-60 years.

Our study showed highest number of patients had stage II disease followed by stage III (Table-I). Breast lump was registered to be the most common presenting symptom of breast cancer and average duration of the symptoms to diagnosis was found to be 5 months(maximum number of cases fall with the duration of 4-6 months). Almost 76% of our study group had a delayed presentation (>60 days) which might have contributed to the large size of the tumor.

When we compared the delayed presentation with the late stage (stage 3, 4) of the disease, we found a significant correlation (Table -II).

Table I: Frequency of distribution of different stages of breast cancer among study population

Breast cancer staging	No of cases	Percentage (%)
Stage I	6	23.07
Stage II	11	42.30
Stage III	7	26.92
Stage IV	2	7.69

Table II: Association between delay presentation and severity of disease

Stage of Carcinoma	≤2 months No (%)	≥2 months No (%)	R.V	P.V
Stage I & II	10(58.82)	7(41.18)	0.811	<0.001
Stage III & IV	3(33.33)	6(66.67)		

We found significant increase in the mean serum ADA, GGT & ALP levels in carcinoma breast patients in comparison to healthy controls as shown in Table III.

Table III: comparison of serum level of ADA, GGT & ALP in both cases & controls

Parameters	Controls (n=26)	Cases(n=26)	P.V
Serum ADA(U/L)	20.33 ± 3.65	44.54 ± 8.36	<0.001
Serum GGT(U/L)	15 ± 4.04	28.43 ± 5.65	<0.01
Serum ALP(IU/L)	65.13 ± 5.41	106.82 ± 7.62	<0.001

Table- IV shows the inter-stage comparison of serum ADA, GGT & ALP level along with its comparison with the control group. It shows significant increase in serum ADA & GGT levels in various stages of breast carcinoma in comparison to controls (P<0.01). But on comparing these values of stage II with stage I, we documented a non-significant increase in stage II.But stage III & IV had highly significant increase of serum ADA & GGT levels (P<0.001) in comparison to both stage I & II.

Analysis of serum ALP level revealed significant increase in stage III & IV in comparison to stage I & II. Comparing stage I & II, we did not document any significantly prominent rise though there was high serum level of ALP in stage II. When the different stages were compared with control, significantly marked rise was observed in stage III & IV. Stage I & II though showed increased level of serum ALP level in comparison to controls, but it didn't possess any significant rise.

Table IV: comparison of serum levels of ADA, GGT & ALP between controls and different stages of Ca Breast patients

parameters	Controls (n=26)	Stage I (n=6)	Stage II (n=11)	Stage III (n=7)	Stage IV (n=2)
S. ADA	20.33 ± 3.65	34.61±5.72*	39.21±7.14*	45.67±3.22**	51.82±8.74**
S. GGT	15 ± 4.04	20.32±4.38*	23.45±5.34*	31.12±6.43**	38.42±3.67**
S. ALP	65.13 ± 5.41	78.88±2.98	87.12±6.31	108.13±5.21*	141.67±6.87*

The values are expressed as their Mean ± SD, *P<0.05 compared with controls : significant,**P<0.001 compared with controls: highly significant

Biochemical analysis of the study group revealed significant increase in total cholesterol level than the control group (Table-V). HDL-Cholesterol and LDL-Cholesterol also registered marked changes in the study group in comparison to the control group. Liver enzymes like SGOT and SGPT documented a prominent rise in breast cancer patients than controls.

Table V: comparison of lipid profile and liver enzymes in control and study groups

parameters	Controls (n=26)	Cases (n=26)	P.V
Total Cholesterol	135.67±24.47	186.20±23.70	<0.01
Triglyceride	110.21±16.71	146.61±27.21	NS
HDL-Cholesterol	46.51±3.05	39.81± 2.20	<0.01
LDL-Cholesterol	66.67±22.90	113.81±23.51	<0.01
VLDL-Cholesterol	22.24±3.51	28.78±5.50	NS
SGOT	35.20±20.12	63.17±13.01	<0.001
SGPT	32.76±17.89	52.34±12.45	<0.001

Discussion

In India, carcinoma breast is one of the most growing and most feared health problems in women and regarded as fatal worldwide. It has revealed an increased prevalence worldwide, both in industrialized and developing countries. In India, incidence of breast cancer has been recorded to be 23.2 per 1, 00,000 population (12).

Each tissue of our body always maintains a steady and consistent pattern of enzyme activity which significantly alters in malignant conditions where membranes become leaky enough to shed out its constituents into the surrounding milieu at an increased rate with the rapid replication of cells (13). Ultimately when the cell destroys all the contents of the cells along with the mitochondrial and membrane bound enzymes discharged leading to prominent rise in enzyme activity in case of malignancy. Therefore extensive researches have been done to evaluate the association of various biochemical and enzyme markers with the diagnosis and prognosis of carcinoma breast.

The present study involved 26 histopathologically confirmed breast cancer patients with majority of patients (61.54%) included in the age group of 41-60 yrs. Mean age of the study population was recorded to be 50.9 ± 7.76 yrs.

According to SEER Cancer Statistics Review, the median age at the time of breast cancer diagnosis was 61.24 yrs during the period from 2008-2012. This means, half of the women who developed breast cancer were 61 years of age or younger at the time of diagnosis. The median age of diagnosis is younger for black women (58 yrs) than white women (62 yrs). However, breast cancer rate increases 0.7% per year for women 50 years of age and older (14). Our study is in consistent with the above said statistical review.

Observing Table-1 of our study, we conclude that stage II tumour is the most common variety followed by stage III. In contrast to our study, various researchers reported stage III tumour as the most common type followed by stage IV15,16,17. Colleoni et al also observed approximately 48% of patients having higher stage of the disease18. Furthermore we found significant association between delayed presentation and late stage of the disease which is in agreement with various other researchers (15,19).

Our study reported a significant increase in serum ADA value in carcinoma breast patients in comparison to controls. Inter-stage comparison also yielded a progressive rise from stage I-IV with significant change in stage III & IV in comparison to stage I & II. Similar results were reported by

various researchers like Aghaei M11, Choudhari A(20), Majoomdar M (21) and Borzenko BG (22,23).

In case of solid cancers, various factors like local tissue hypoxia, increased turnover of malignant cells and overall increased nucleotide metabolism lead to prominent rise of purine nucleoside adenosine. Consequently serum ADA activity in malignant tissues also increases to detoxify the increased amount of adenosine and deoxyadenosine substrate (24,25). It has also been proposed that increased ADA activity might be one type of physiologic attempt of the malignant cells to provide sufficient substrates to the cancer cells for accelerating the salvage pathway activity (26).

Similarly serum GGT is significantly increased in Carcinoma breast patients in comparison to controls. Correspondingly Serum GGT levels in Stage III & IV have significantly prominent rise than stage I & II. Similar results were also suggested by Mishra S1, Seth LR (27) and Guddanti R (28). It may be due to an adoptive response of increased reactive oxygen species production in the blood which ultimately causes induction of GGT mRNA by multiple signalling pathways (29). In metastatic breast carcinoma, hepatocellular damage and biliary obstruction also lead to increased activity of serum GGT.

Along with various isoenzymes of ALP distributed in various organs like liver, bones and intestines, another isoenzyme named Regan isoenzyme has also been recognized in various malignancies (30). This isoenzyme may be one of the contributing factors of increased ALP in breast carcinoma. Again in stage III & IV of breast cancer , neoplastic invasion of the liver results in localized intra hepatic cholestasis which ultimately leads to increased ALP level because of the increased synthesis of enzymes by proliferating endothelial cells and also due to the pressure necrosis of liver cells.

In agreement with the above evidence, Present study documented a significant increase in the serum ALP level compared to controls. Our findings correlate well with various other studies conducted within various parts of the globe (1, 31, 32, 33).

The present study shows significant elevation of serum ADA, GGT, ALP in carcinoma breast cases than controls. The extent of rise in these parameters can be taken as one of the important criteria to establish its diagnostic role in carcinoma Breast patients. These might prove to be easily accessible and affordable biomarkers in remote areas for the early detection of the disease. Serum ALP level can act as a potential marker for the early detection of cancer with or without metastasis.

Overall these parameters are simple, reliable, accurate, cost effective and can be easily assayed in smaller laboratories of rural & remote areas. Unlike other cancers, as breast cancer is eminently treatable at an early stage, our study suggests that estimation of less specific biomarkers like ADA, GGT, & ALP can be used as routine screening tests in all suspected breast cancer patients which help in its early detection and subsequent early and effective management.

However further studies are required in this zone of India on larger population with longer follow up to substantiate our findings so that strong guidelines can be set up for the utility of these enzymes for the diagnosis and assessment of progression of carcinoma breast.

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BRIF REPORT

Diagnostic and Prognostic Efficacy of Serum ADA, GGT & ALP Levels in Carcinoma Breast Patients - A Tertiary Center Study

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Abstract:

Introduction: *Breast cancer accounts for the most common cancer in urban Indian females, and the second commonest in the rural Indian women. Early detectable cancers can potentially be cured when the tumour is small enough to be completely removed surgically. A number of biochemical tumour markers have been studied for the early detection of cancer. However these facilities are only available at sophisticated and well equipped centers with latest technology and they are also expensive.*

Methods: *This was a case-control comparative study conducted taking 26 clinically and histopathologically established female breast cancer patients. The cases will first be categorized according to the TNM staging of Ca Breast. Serum was collected for estimation of ADA, GGT and liver enzymes (SGOT, SGPT, and ALP) and lipid profile. Results: There was significant increase in the mean serum ADA, GGT & ALP levels in carcinoma breast patients in comparison to healthy controls. Inter-stage comparison of serum ADA, GGT & ALP level in various stages also showed significant hike.*

Results: *There was significant increase in the mean serum ADA, GGT & ALP levels in carcinoma breast patients in comparison to healthy controls. Inter-stage comparison of serum ADA, GGT & ALP level in various stages also showed significant hike.*

Conclusion: *The extent of rise in these parameters can be taken as one of the important criteria to establish its diagnostic role in carcinoma Breast patients. These might prove to be easily accessible and affordable biomarkers in remote areas for the early detection of the disease.*

Keywords: Adenosine deaminase, Gamma glutamyl transpeptidase, Alkaline phosphatase, Enzyme markers, Breast cancer.

Conflict of Interest: No

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