

Assessment of the Variation in Benign and Malignant Breast Lumps in Relation to Serum Uric Acid, Serum Bilirubin and Serum Creatinine Values: A Comparative Study

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Abstract

Aim: The aim of the present study was to compare variation in benign and malignant breast lumps in relation to serum uric acid, serum bilirubin and serum creatinine values

Methods: An observational and prospective study was conducted on all the outpatient and inpatient diagnosed to have a breast lump, admitted in the Department of General Surgery for the period of 2 years. 200 patients were included in the study and 100 patients were having benign breast lumps and other 100 patients malignant. Approval of institutional ethics committee was provided. All the patients presented to surgery outpatient clinic with the features suggestive of breast lump were subjected to detailed history and clinical examination. Standard protocol for triple assessment was followed.

Results: Majority of the patients belonged to 16-25 years with benign breast lump and in the malignant breast lump most of the women belonged to 46-55 years. On comparing, positive correlation was found between BIRADS score and serum uric acid and serum bilirubin. P value was found to be highly significant statistically. No significant correlations were found after comparing HER 2/neu positive and HER 2/neu negative, ER/PR positive and ER/PR negative and various stages of breast cancer. Although, no significant correlation was found between biochemical parameters used in this study (serum uric acid, serum bilirubin and serum creatinine) and age, BIRADS score, stage of tumor, tumor grade.

Conclusion: The evaluation of serum uric acid and serum bilirubin may be useful for predicting prognosis in females with malignant breast lump and can provide help in making diagnosis during the initial assessment of the patient presenting with breast lump.

Keywords: Biochemical parameters, Breast cancer, Prognosis of carcinoma breast, Anti-oxidant property

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Introduction

Breast carcinoma is the most common malignancy and leading cause of death in women worldwide. [1] Breast cancer (BC) begins in breast tissue which is made up of glands called lobules, and the ducts which connect lobules to the nipple. [2-4] It is heterogeneous in its clinical, genetic and biochemical profile. The incidence rate of breast cancer is much lower in Asian countries as compare to western countries. Its incidence is increasing in all regions of the world with majority of rise seen in developing countries. [5] The age-standardized incidence rate for breast cancer in India is one-third that of Western countries. [6] It is the leading cause of cancer death among females worldwide, with an estimated 2.3 million cases and 690,000 deaths in 2020. It accounts for 11.7% of all cancer cases and 6.9% of all cancer deaths among females. [7] The biochemical changes in carcinogenesis is mandatory to understand accurately. Determination of enzymes

activities and analysis of concentrations of various cellular components can be useful in the treatment of cancers. [8]

The two main categories of investigations in cancer biochemistry- cancer cell metabolism and how the cancer can affect the host cell metabolism.[9] Quantitatively, the biochemical distortions may be detected at two levels. At first, similar molecules are found in both normal cells and cancer cells, but with different quantities. Then, molecular constituents existing in normal cells are depleted in the cancer cells. The biochemical changes in the cancer cell may be in a serious condition, which result in a total deletion of an important molecule, leading to an error in metabolism, [10] Measuring serum uric acid (SUA), urea and glucose can determine health and proper function of various organs like kidneys, liver, and other organs of BC patients. [11]

High level of serum uric acid is important in diagnosis, cancer risk, recurrence and medical management of breast carcinoma. Serum uric acid provide a primary defense against human cancer based upon its capacity to scavenge singlet oxygen and its capacity to inhibit lipid peroxidation. [12] Uric acid is the end product of purine metabolism. Catabolism of purines by enzymatic hydrolytic deamination to form xanthine and hypoxanthine. These are than oxidized to Uric acid.¹³ Bilirubin reacts with diazotized sulfanilic acid in acidic medium to form pink coloured azobilirubin with absorbance directly proportional to bilirubin concentration. [13] Bilirubin formed from hemoglobin breakdown and its role is an antioxidant and to correlate bilirubin with uric acid to examine oxidant-antioxidant status of these parameters in breast cancer women.¹⁴ Serum uric acid, serum bilirubin, serum albumin, serum creatinine however, all of these are having relatively less specificity. However, elevated level of serum uric acid is postulated to be associated with poor prognosis of the breast cancer patient coinciding with ER and PR negative status.

Hence, this study was directed to assess prognostic value of above-mentioned parameters in the patients of breast lump. And since the present study includes all breast lumps whether it is benign or malignant, it will also perform major role in comparing the variation between benign and malignant breast lumps concerning with aforementioned parameters.

Material & Methods

An observational and prospective study was conducted on all the outpatient and inpatient diagnosed to have a breast lump, admitted in the Department of General Surgery, JNKTMCH, Madhepura, Bihar, India for the period of 2 years. 200 patients were included in the study and 100 patients were having benign breast lumps and other 100 patients malignant. Approval of institutional ethics committee was provided. All the patients presented to surgery outpatient clinic with the features suggestive of breast lump were subjected to detailed history and clinical examination. Standard protocol for triple assessment was followed.

Proper consent was also taken. Around 5 ml of venous blood was collected from antecubital vein under aseptic precautionary measures using sterile disposable syringe. Blood was then allowed to clot and serum was separated by centrifugation and stored for testing. Total 100 patients were included in this study (sample size-100), out of which 50 patients were having benign breast lumps and other 50 patients were having malignant breast lumps. Categorization of the patients was done on the basis of their histopathological examination report. Radiological findings were also recorded in all the patients taking part in this study. In the patients with

surgical intervention and in whom surgery was contemplated underwent excisional biopsy all were subjected to histopathological examination of the lump. Breast lumps proved to be benign by fine needle aspiration cytology (FNAC)/biopsy was not subjected to further histopathological analysis (ER/PR and HER2/neu status). Breast lumps proved to be malignant by histopathological examination then further subjected to analysis of ER/PR status and HER2/neu status.

Inclusion Criteria

The study included only females with 16 years of age and onwards.

Exclusion Criteria

Female patients who have not attained menarche with history of any trauma to the breast and patients not giving consent for any sort of surgical intervention were excluded from the study.

Methodology

Blood samples (venous blood, 5 ml) of the patients were taken and processed under all aseptic precautions. Autoanalyzer was used for estimation of abovementioned biochemical parameters by biochemistry technician in central pathology laboratory (CPL) of JNKTMCH, Madhepura, Bihar, The autoanalyzer machine was based on the principle of spectrophotometry. The automated clinical chemistry analyzer used for this study was Biosystems BA 400. It incorporates optical LEDs based system with 8 working wavelengths (340, 405, 505, 535, 560, 600, 635, 670 nm) for spectrophotometric readings, having photometric range of -0.2 to 3.5 A. For present study, normal range for serum uric acid is 3 mg/dl to 5 mg/dl (standardized as per our laboratory report).

Statistical Analysis

Data collected were transformed into variables, coded and entered in Microsoft excel. Data was analyzed and statistically evaluated using statistical process control calculator run on a personal computer (SPCC-PC-21 version). Quantitative data was expressed in mean±standard deviation and depends on normality distribution difference between two comparable groups were tested by student's t-test (unpaired) or Mann Whitney 'U' test while for more than two groups analysis of variance (ANOVA) test or Kruskal Wallis H test followed by posthoc test was used. Qualitative data were expressed in percentage. Statistical differences between the proportions were tested by chi square test or Fisher's exact test. Spearman correlation coefficient was used to see the correlation between two quantitative variables. 'P' value less than 0.05 was considered statistically significant. Statistical differences between the proportions were tested by chi square test or Fisher's exact test.

Results

Table 1: Age wise distribution of breast lumps

Age groups in years	Benign	Malignant	Total
16-25	60	0	60
26-35	34	0	34
36-45	6	10	16
46-55	0	40	40
56-65	0	35	35
66-75	0	15	15
Total	100	100	200

Majority of the patients belonged to 16-25 years with benign breast lump and in the malignant breast lump most of the women belonged to 46-55 years.

Table 2: Association of BIRADS score with biochemical parameters in breast tumours

BIRADS score	N	Mean	P value
Serum uric acid (mg/dl)			
1	32	4.26±0.54	<0.001
2/3	70	4.34±0.86	
4a	15	6.74±0.22	
4b	36	6.90±0.32	
4c	29	6.76±0.46	
5	18	6.88±0.42	
Serum bilirubin (mg/dl)			
1	32	0.92±0.18	<0.001
2/3	70	0.96±0.24	
4a	15	1.26±0.16	
4b	36	1.36±0.24	
4c	29	1.48±0.32	
5	18	1.52±0.34	
Direct bilirubin (mg/dl)			
1	32	0.28±0.12	<0.001
2/3	70	0.30±0.15	
4a	15	0.52±0.08	
4b	36	0.58±0.08	
4c	29	0.59±0.18	
5	18	0.64±0.16	
Indirect bilirubin (mg/dl)			
1	32	0.62±0.16	<0.001
2/3	70	0.68±0.12	
4a	15	0.76±0.16	
4b	36	0.84±0.18	
4c	29	0.94±0.24	
5	18	0.88±0.22	
Serum creatinine (mg/dl)			
1	32	0.96±0.14	0.18
2/3	70	0.84±0.16	
4a	15	0.88±0.18	
4b	36	0.92±0.14	
4c	29	0.96±0.18	
5	18	0.86±0.14	

On comparing, positive correlation was found between BIRADS score and serum uric acid and serum bilirubin. P value was found to be highly significant statistically.

Table 3: Association of HER2neu status with biochemical parameters in malignant tumours

	HER2 neu -ve (n=80)	HER2 neu +ve (n=20)
Serum uric acid (mg/dl)	6.84	6.81
Serum bilirubin (mg/dl)	1.44	1.38
Direct bilirubin (mg/dl)	0.55	0.52
Indirect bilirubin (mg/dl)	0.84	0.83
Serum creatinine (mg/dl)	0.91	0.82

No significant correlations were found after comparing HER 2/neu positive and HER 2/neu negative.

Table 4: Association of ER/PR status with biochemical parameters in malignant tumours

	ER/PR -ve (n=175)	ER/PR +ve (n=25)
Serum uric acid (mg/dl)	6.82	6.92
Serum bilirubin (mg/dl)	1.45	1.37
Direct bilirubin (mg/dl)	0.54	0.58
Indirect bilirubin (mg/dl)	0.86	0.78
Serum creatinine (mg/dl)	0.86	0.98

No significant correlations were found ER/PR positive and ER/PR negative.

Table 5: Association of stage of tumour with biochemical parameters in malignant tumours

	Stage 2A (N=45)	Stage 2B (N=25)	Stage 3A/4 (N=30)
Serum uric acid (mg/dl)	6.92	6.85	6.78
Serum bilirubin (mg/dl)	1.42	1.45	1.43
Direct bilirubin (mg/dl)	0.56	0.57	0.60
Indirect bilirubin (mg/dl)	0.88	0.84	0.86
Serum creatinine (mg/dl)	0.91	0.94	0.90

No significant correlations were found various stages of breast cancer.

Table 6: Correlation of biochemical parameters with age, BIRADS score, stage and grading

Biochemical parameters	Age in years	BIRADS score	Stage	Tumour grade
Serum uric acid (mg/dl)				
R value	0.722	0.773	-0.146	0.032
P value	0.000	0.000	0.308	0.820
N	200	200	100	100
Serum bilirubin (mg/dl)				
R value	0.690	0.732	0.078	-0.029
P value	0.000	0.000	0.612	0.858
N	200	200	100	100
Direct bilirubin (mg/dl)				
R value	0.752	0.730	0.236	0.007
P value	0.000	0.000	0.102	0.966
N	200	200	100	100
Indirect bilirubin (mg/dl)				
R value	0.426	0.518	-0.059	-0.085
P value	0.000	0.000	0.686	0.562
N	200	200	100	100
Serum creatinine (mg/dl)				
R value	0.045	0.001	-0.098	-0.002
P value	0.675	0.990	0.505	0.992
N	200	200	100	100

Although, no significant correlation was found between biochemical parameters used in this study (serum uric acid, serum bilirubin and serum

creatinine) and age, BIRADS score, stage of tumor, tumor grade.

Discussion

Research findings concerning the typical bioenergetics and biosynthetic pathways in tumors show changes in metabolic regulation, for instance, increase in anaerobic glycolysis, the earliest biochemical pathway that successfully detected in cancer cells. [15] Chemotherapy and radiotherapy generally destruct the cancerous cells in the tissues. Normal cells are sometimes destroyed by these treatments. [16] Chemotherapy represents blocking or reversing the process of carcinogenesis by using chemotherapeutic agents. Clinical and experimental researches have revealed that various chemotherapeutic drugs have cytotoxicity which is mainly mediated by the formation of free radicals. Reactive oxygen species play a mandatory role in the pathogenesis of different diseases and cancers, affecting different organs like stomach, colon, ovaries and breast. [17] Biochemical tests are determined to measure the levels of enzymes, reactive oxygen species, chemicals and organic waste products found in body fluids. These tests can find out the functions of a variety of organs whether these organs work properly during chemotherapy or not. For an instance, liver function tests can detect the extent of liver damage due to chemotherapy. Abnormal findings of biochemical profile can detect the possible drawbacks of chemotherapy and the progression of the cancer. [18]

Majority of the patients belonged to 16-25 years with benign breast lump and in the malignant breast lump most of the women belonged to 46-55 years. On comparing, positive correlation was found between BIRADS score and serum uric acid and serum bilirubin which was close to the result reported by several other studies, and manifested that serum uric acid level may be a protective agent and its role as antioxidant and raised serum uric acid levels as risk factor for incidence and cancer mortality in breast cancer of females. [19-22] Complete blood picture is a prerequisite investigation for breast lump patients before use of any sort of treatment guideline. Blood chemistry panel is common tests used to evaluate a variety of chemical components excreted from body tissues or synthesized during the breakdown or metabolism of various substances. The blood chemistry panel measures the levels of chemicals, enzymes, and organic waste products detected in the blood stream. It evaluates the healthiness and proper functioning of various organs during the course of disease. The abnormal blood chemistry report also suggests the spread of breast cancer to the bone, kidney or liver.

No significant correlations were found after comparing HER 2/neu positive and HER 2/neu negative. No significant correlations were found ER/PR positive and ER/PR negative. No significant correlations were found various stages of breast cancer. Although, no significant correlation was

found between biochemical parameters used in this study (serum uric acid, serum bilirubin and serum creatinine) and age, BIRADS score, stage of tumor, tumor grade. The risk of cancer incidence can be reduced by antioxidants because antioxidants may help the body's defense mechanism and combat free radicals that can be a cause of cancer development. Antioxidants may also reduce the risk of breast cancer. [23] Uric acid which is the final product of purine nucleotide metabolism is considered as a crucial antioxidant found in the plasma. [24] In this study, breast cancer patients had significant lower mean serum uric acid concentration than normal subjects. Moreover, mean serum uric acid level that determined after the third cycle of chemotherapy of breast cancer patients was significantly lower than that measured as the baseline level in breast cancer patients ($p < 0.02$). In the study of Abdel-Salam et al, significant decrease in uric acid level has been seen in breast cancer patients after chemotherapy; and uric acid levels were lower in cancer with metastasis than that of cancer patients with no metastasis. [24]

Antioxidant like uric acid and bilirubin may be important in determining the oxidant and antioxidant status in female patients with breast cancer. In the etiology of cancer, involvement of oxidant such as hydrogen peroxide (H_2O_2), singlet oxygen ($1/2 O_2$) and superoxide anion (O_2^-) and hydroxyl radicals (OH) are significant and well recorded in previous studies. [25] It has been postulated previously that oxidative stress contributes to carcinogenesis. [26,27] Bilirubin is a potent antioxidant and has been shown to protect against cancer development. Lower serum bilirubin level has been associated to raised risk of cardiovascular disease and cancer. [28] The association of serum bilirubin level with survival has been evaluated in metastatic breast cancer patients and the results showed that hyperbilirubinemia was related with worse survival. [30] The level of bilirubin in blood stream may have different predictive effect in metastatic and non-metastatic breast cancer patients. In our study, serum bilirubin level in breast cancer patients were found to be slightly more than normal range. This may be due to bilirubin scavenge during oxidative load or oxidative stress in breast cancer.

Conclusion

We concluded that raised level of serum uric acid may be due to its protective role in response to increased oxidative stress and high serum uric acid level may suggest poor prognosis in patients with breast cancer. And thus, it may play a major role in providing adequate management for patients with breast cancer. The level of bilirubin was slightly higher than normal range or within normal range, it could be due to bilirubin scavenge during oxidative load or oxidative stress in female patients with breast cancer. The level of serum creatinine was within normal range for both benign and malignant breast

lump patients in absence of any renal impairment by other etiology. The evaluation of patient's biochemical profile along with abovementioned parameters, may be used as a credible predictive mean in the monitoring of disease, metastasis and for different treatment modalities for breast cancer. These results suggest that evaluation of serum uric acid and serum bilirubin may be useful for predicting prognosis in females with malignant breast lump (breast cancer) and can provide help in making diagnosis during the initial assessment of the patient presenting with breast lump.

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