International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

A study on serum sirtuin 1 levels and Neutrophil-Lymphocyte ratio, Platelet-Lymphocyte ratio in breast cancer

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Abstract

This research aimed to compare serum Sirtuin 1 (SIRT1) levels between healthy females and those with breast cancer. It also sought to examine variations in SIRT1 levels across different cancer stages, explore correlations with inflammatory markers (NLR and PLR), and establish the potential of serum SIRT1 levels as a diagnostic marker for breast cancer. The study, conducted in a cross-sectional manner, involved recruiting 40 breast cancer patients and 36 healthy females. Demographic and laboratory data were collected, and blood samples underwent analysis for SIRT1 levels through ELISA and haematological parameters. Statistical analysis utilized GraphPad Prism. Significantly elevated serum SIRT1 levels were observed in breast cancer patients compared to controls ($p < 0.0001$). No substantial variation in SIRT1 levels was found across different breast cancer stages ($p = 0.212$). The research also unveiled positive correlations between SIRT1 and neutrophil count, as well as inflammatory markers NLR and PLR, along with a negative correlation with lymphocyte count in breast cancer patients. With an AUC of 0.933, SIRT1 demonstrated promise as a biomarker for breast cancer, and its association with the condition was highly significant ($p < 0.0001$). To summarize, the study identified higher serum SIRT1 levels in breast cancer patients, suggesting a potential role as a biomarker. However, SIRT1 levels did not significantly differ with tumor grade, lymph node involvement, or metastasis. The research also highlighted correlations between SIRT1, NLR, and PLR, indicating potential connections to the inflammatory and immune responses in breast cancer.

Keywords: Sirtuin 1, Neutrophil Lymphocyte Ratio, Platelet Lymphocyte Ratio, Breast cancer, Biomarker.

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1. Introduction

Breast cancer stands as the second leading cause of cancer-related deaths in women globally [1]. Various factors, including hormonal imbalances, genetic inheritance, ionizing radiation exposure, and unhealthy dietary habits, contribute to its development [2]. Treatment typically involves a combination of surgical procedures, radiotherapy, endocrine therapy, and/or chemotherapy. Despite advancements, the 5-year survival rate for breast cancer remains suboptimal, emphasizing the ongoing need to understand its progression mechanisms and identify new therapeutic targets [3].

Sirtuins, belonging to the class III histone deacetylases, exhibit varied expression levels in human cancers, with dual oncogenic and tumor-suppressive effects depending on cellular contexts. Integral to crucial biological processes, Sirtuins play a significant role in cancer initiation, promotion, and progression. SIRT1, a key player in breast cancer, functions as a deacetylase enzyme, altering gene expression and cellular behavior. It can act as a tumor suppressor by inhibiting certain oncogenes, preventing uncontrolled cell growth, or promote breast cancer progression by activating proteins involved in cell survival and proliferation. This dual nature highlights SIRT1's context-dependent role in breast cancer. Serum SIRT1 levels have drawn attention in cancer research for their potential implications in diagnosis, prognosis, and treatment. Elevated levels are associated with prostate, lung, colorectal, hepatocellular, ovarian, and gastric cancers, each with varying effects on prognosis [4-10]. In breast cancer, studies show mixed results. Some research has reported elevated serum SIRT1 levels in breast cancer

patients, indicating a potential role in disease progression [11]. However, further research is crucial to establish its precise diagnostic and prognostic value. SIRT1's complex role extends to inflammation regulation, exerting both pro-inflammatory and anti-inflammatory effects depending on the context. It impacts immune cell behavior, modulates metabolism, and influences cellular senescence, making it an intriguing target for studying inflammation-related diseases and potential therapeutic strategies. Inflammatory markers like the neutrophil-to-lymphocyte ratio (NLR) have gained attention as potential prognostic indicators in various cancers, including breast cancer. This study aims to assess the role of serum SIRT1 levels in breast cancer by comparing them between patients and healthy individuals, evaluating variations across cancer stages, exploring correlations with NLR and PLR, and investigating their association with the presence of breast cancer.

2. Materials and methods

The cross-sectional study was conducted at the Department of Biochemistry in collaboration with Medical Oncology at Justice KS Hegde Charitable Hospital (Mangalore, Karnataka) and the Central Research Laboratory, KSHEMA, Mangalore. Approval for the study was obtained from the Central Ethics Committee, NITTE Deemed to be University (NU/CEC/2021/212), and informed consent was acquired from all participants prior to their inclusion. Forty women diagnosed with breast cancer and thirty-six healthy females were enrolled in the study. Inclusion criteria comprised female patients with primary breast carcinoma diagnosed by histopathology and receiving anticancer drugs. This group was further categorized into three subgroups: 11 patients with hormone-positive tumors (ER+ve, PR+ve), 13 with Triple Negative Breast Cancer (TNBC), and 16 with HER2-positive cases. Exclusion criteria for both cases and controls included patients with Diabetes Mellitus, congestive heart disease, inflammatory and autoimmune disorders, and renal stones. Patient data, including demographic characteristics, co-morbidities, tumor characteristics (grade and stage), and laboratory parameters, were obtained from the Medical Records Department. Blood samples (3 ml) were collected in plain tubes to evaluate Sirtuin 1 levels in serum using Enzyme Linked Immune-Sorbent Assay (ELISA), and 2 ml of EDTA samples were collected for the assessment of hematological parameters, which were analyzed using an automated cell counter. Tumor estrogen receptor (ER) and progesterone receptor (PR) statuses were determined immunohistochemically [12].

Statistical analysis, performed using GraphPad Prism 8. Included the Mann-Whitney U test for comparing data between cases and controls, Kruskal-Wallis test for subgroup comparisons, Spearman's correlation test for assessing correlations, chi-square test for associations, and ROC analysis to determine the diagnostic utility of sirtuin 1 as a biomarker in breast cancer.

3. Results

The study comprises two groups: cases (consisting of 40 individuals) and controls (comprising 36 individuals). The average age of the cases is 49.90 years with a standard deviation of 9.231, while the control group has an average age of 40.06 years with a standard deviation of 7.223 ($p<0.0001$).

The data presents the distribution of tumor types, TNM classification (including tumor size, lymph node invasion, and metastasis status), and the status of estrogen receptor (ER), progesterone receptor (PR), and HER2 (human epidermal growth factor receptor 2) in a group of breast cancer patients. Specifically, there were 7 cases (17.5%) of ductal carcinoma, 5 cases (12.5%) of lobular carcinoma, and 28 cases (70%) of other tumor types. Regarding TNM classification, tumor sizes ranged from T1 to T4, lymph node invasion varied from N0 to N3, and 6 cases had metastasis while 34 did not. The status of hormone receptors showed that 27 cases were ER-positive and 13 were ER-negative, while 13 cases were PR-positive and 27 were PR-negative. Additionally, 16 cases were HER2-positive, and 24 were HER2-negative (Table 1).

In the study, serum SIRT1 levels in breast cancer patients were exceptionally significantly higher than those in the control group ($p < 0.0001$). No notable difference was observed in serum SIRT1 levels among different tumor grades ($p = 0.228$), graded according to the modified Bloom–Richardson system [13]. The levels exhibited a descending order from T3 to T1, with T1 being the lowest. Sirtuin 1 levels did not show significant variations in groups based on lymph node involvement ($p = 0.193$), categorized as N1, N2, N0, and N3 in descending order, as well as in relation to metastasis ($p = 0.134$). There was also no significant difference in sirtuin 1 levels among different groups based on hormone response ($p = 0.067$) (Table 2).

There was no significant difference in the serum sirtuin 1 levels in different stages of breast cancer ($p=0.212$) (table 3). A significant positive correlation was observed between serum SIRT1 levels and neutrophil count ($p=0.007$) in breast cancer patients. Additionally, there was a negative correlation with lymphocyte count ($p=0.015$) and positive correlations with both NLR ($p=0.012$) and PLR ($p=0.008$) as indicated in table 4.

The effectiveness of Sirtuin 1 as a biomarker for breast cancer was evaluated through ROC analysis (fig 1), revealing an area under the curve of 0.933. The biomarker demonstrated a sensitivity of 80% and a specificity of 80.6%, with a determined cutoff value for Sirtuin 1 level at 1.0121 ng/ml. The association of sirtuin 1 levels with breast cancer was found to be highly significant with a chi-square value of 27.81($p<0.0001$). (Table 5).

4. Discussion

Our study delves into the intricate landscape of serum sirtuin1 (SIRT1) levels in breast cancer patients, comparing them to a control group. The findings highlight a significant elevation in serum SIRT1 levels among breast cancer patients compared to controls, indicating a potential association or the prospect of SIRT1 serving as a biomarker for breast cancer. This aligns with previous research, reinforcing the relevance of SIRT1 in the context of breast cancer. The examination of SIRT1 levels across different tumor grades revealed no statistically significant differences, suggesting that SIRT1 levels may not correlate with the aggressiveness or grade of breast tumors. Similarly, there were no notable variations in SIRT1 levels concerning lymph node involvement or metastasis, implying that serum SIRT1 levels might not strongly associate with these aspects of breast cancer. A study conducted by Ibrahem *et al.* and our findings are similar [14]. The study supporting the higher

levels of SIRT1 in breast cancer patients and its association with tumor staging and lymph node infiltration [14]. Chung *et al*. discovered SIRT1 was found to have a part in breast tumor invasiveness progression and adverse clinical outcomes [15]. In contrast, Sung *et al.* lower-grade breast cancer had been associated with elevated SIRT1 levels in breast tumor tissue [16]. Furthermore, the study found that, when compared to the control group, breast cancer patients blood levels of SIRT1 were considerably greater. These elevated levels positively correlated with tumor size, histological grade of tumor, and involvement of lymph node in breast cancer patients. Abdelmawgoud and El Awady documented increased SIRT1 activity in breast cancer tissues as compared to adjacent healthy tissues and benign breast tumors, which aligns with the findings of Jin *et al*, who reported up-regulation of SIRT1 in human breast cancer tissues [17-18].

Conversely, Cao *et al.* reported significantly lower SIRT1 expression levels in breast cancer tissues compared to normal breast tissues [19]. Notably, our data demonstrated that SIRT1 serum levels were significantly higher in breast cancer patients with estrogen receptor-negative (ER–ve) and progesterone receptor-negative (PR -ve) tumors compared to those with estrogen receptor-positive (ER +ve) and progesterone receptor-positive (PR +ve) tumors, which is consistent with Jin *et al*.'s report indicating contribution of SIRT1 to the estrogen and estrogen receptor carcinogenic pathway in carcinoma of breast [18]. The intricate interplay between SIRT1 and factors like estrogen receptors, gene polymorphisms, and tumor characteristics warrants further exploration to unravel the complexity of SIRT1's involvement in breast cancer. The study's correlation analysis unveils significant associations between serum SIRT1 levels and various blood parameters in breast cancer patients. The positive correlation with neutrophil count and negative correlation with lymphocyte count provide insights into the potential interplay between SIRT1 and the immune response in breast cancer. Additionally, the positive correlations with NLR and PLR further highlight the link between elevated SIRT1 levels and inflammatory and immune responses in breast cancer patients. Neutrophils have been a subject of extensive investigation, and there is now compelling evidence suggesting their role in promoting tumor growth and contributing to metastasis development [19-20]. They have the ability to secrete matrix metalloproteases, in particular, proteases which facilitates tumor invasion. Neutrophils also actively participate in tumor progression by activating signal transducers and activators of transcription 3 (STAT3) and promoting neo-angiogenesis [21]. Recently, researchers have turned their attention to tumor infiltrating lymphocytes (TILs), particularly in BC26. TILs represent a specific subset of T cells with a robust immune response directed against tumor cells. These lymphocytes, belonging to the innate immune system, are adept at detecting cancerous cells and signaling the immune system to eliminate them. Consequently, a low TIL level may potentially predict a weaker response to neo-adjuvant chemotherapy and might be linked to an unfavorable prognosis [22-23]. The ROC curve analysis underscores the potential of SIRT1 as a robust biomarker for breast cancer, with a high level of accuracy in distinguishing between cases and non-cases. The determined sensitivity, specificity, and cutoff value further emphasize the diagnostic utility of SIRT1 in breast cancer. Your comprehensive exploration of SIRT1 in the context of breast cancer sheds light on its multifaceted roles, from its potential as a biomarker to its intricate involvement in immune responses and inflammatory processes. The study lays a foundation for further research to unravel the complex dynamics of SIRT1 in breast cancer and its implications for diagnosis, treatment, and prognosis.

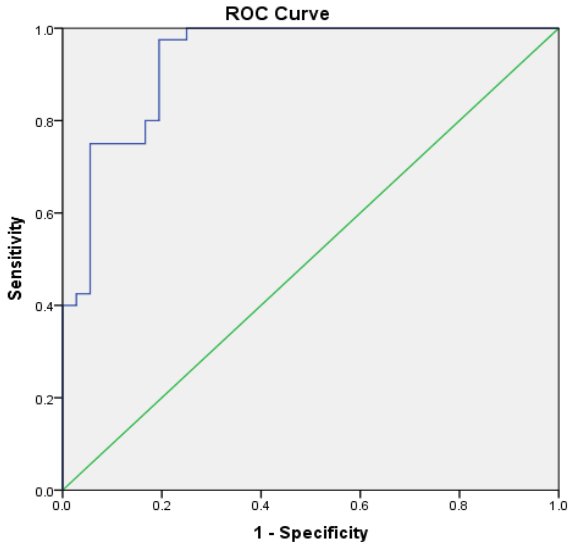

Fig 1: ROC for Sirtuin 1 for predicting breast cancer

Table 1: Clinical data of the breast cancer subjects

Characteristics		Cases (n=40)
Tumor type	Ductal carcinoma	7(17.5)
	Lobular carcinoma	5(12.5)
	Others	28(70%)
TNM Classification		
Tumor size	T0	0
	T1	5 (12.5%)
	T2	5 (12.5%)
	T3	14 (35%)
	T4	16 (40%)
Lymph node invasion	N0	7 (17.5%)
	N1	22 (55%)
	N2	8 (20%)
	N3	3 (7.5%)
Metastasis	Present	6 (15%)
	Absent	34 (85%)
ER Status	Positive	27 (67.5%)
	Negative	13 (32.5%)
PR status	Positive	13 (32.5%)
	Negative	27 (67.5%)
HER status	Positive	16 (40%)
	Negative	24 (60%)

Table 2: Comparison of serum sirtuin 1 levels

Breast cancer group	Groups	Sirtuin 1 level (ng/ml)	p value
	Breast cancer (n=40)	1.344(1.09-2.18)	<0.0001****
	Healthy females (n=36)	0.69(0.62-0.84)	
Tumor size	T1 (5)	0.94 (0.93-3.12)	0.228
	T2 (5)	1.46 (1.11-4.80)	
	T3(14)	1.65 (1.26-4.96)	
	T4(16)	1.31 (1.08-1.65)	
Lymph node	N0 (7)	1.29 (0.94-1.46)	0.193
	N1 (22)	1.52 (1.21-3.98)	
	N2 (8)	1.52 (1.05-1.92)	
	N3 (3)	1.20 (0.84-1.28)	
Metastasis	Present (6)	1.41(1.22-2.18)	0.134
	Absent (34)	1.12(0.93-3.75)	
Hormone status	HER positive (16)	1.69(1.30-4.47)	0.067
	Hormone positive (11)	1.34(1.33-2.80)	
	Hormone negative (13)	1.22(0.96-1.45)	

*p value <0.05 is significant, Test used is Mann Whitney U test, Kruskal Wallis test followed by Dunns test

Table 3: Comparison of serum sirtuin1 levels in different stages of breast cancer

Stages	Sirtuin 1 level	P value
Stage I (3)	0.946	0.212
Stage II (6)	1.360 (0.95-4.27)	
Stage III (25)	1.43(1.25-2.12)	
Stage IV (6)	1.12(0.93-3.75)	

Table 4: Correlation of serum sirtuin1 levels with Haematological Parameters, NLR and PLR in breast cancer group

Parameter	Breast cancer (n=40)	r value	p value
Haemoglobin (gm/dl)	11.35 (10.70-12.40)	0.087	0.591
Neutrophil count (%)	69.45 (59.25-76.75)	0.421	0.007**
Lymphocytes count (%)	25.15 (17.25-32.65)	-0.38	0.015*
Total leukocyte count (cu.mm)	6150 (4225-7885)	0.296	0.063
Eosinophil count (%)	3 (1.10-5.95)	-0.036	0.823
Platelet count (c/cumm)	255500 (202500-312250)	0.165	0.307
NLR	2.66 (1.80-4.48)	0.392	0.012*
PLR	9749 (7440-15794)	0.411	0.008**

*p value<0.05 considered to be significant, test used is spearman correlation

Table 5: Association of sirtuin 1 level with breast cancer

Serum level	Breast cancer group (n=40)	Healthy group (n=36)	Chi- square	P value
<1.012 ng/ml	8	29	27.81	<0.0001****
>1.012 ng/ml	32	7		

*p value<0.05 considered to be significant, test used is chi square test.

5. Conclusions

The study revealed significantly elevated serum SIRT1 levels in breast cancer patients compared to controls, indicating a potential association between SIRT1 and breast cancer. However, no significant differences in SIRT1 levels were found based on tumor grade, lymph node involvement, or metastasis in the studied breast cancer patients, suggesting that SIRT1 may not be strongly influenced by these specific factors in this particular study. Furthermore, the study unveiled associations between serum SIRT1 levels and changes in several blood parameters. Positive correlations with neutrophil count, NLR, and PLR, along with a negative correlation with lymphocyte count, suggest a potential link between SIRT1 and inflammatory and immune responses in breast cancer patients. These findings contribute to our understanding of the disease and its progression, emphasizing the potential of SIRT1 as a biomarker for predicting breast cancer. The robust association of sirtuin 1 with breast cancer underscores its significance in the context of this prevalent and impactful disease.

Acknowledgement

Sincere thanks to Dr Suchetha Kumari N, Principal Scientific Investigator, Central Research Laboratory, KS Hegde Medical Academy, Mangalore for the generous support in carrying out the work.

Conflict of interest

Authors declare no conflict of interest.

Authors contribution

Desy TM., software, validation, analysis and interpretation of data, writing review and editing of manuscript; Usha Adiga., Study Conceptualization, design, validation and funding acquisition; Sachidananda Adiga., Project administration and resources.; Vijith Shetty, acquisition of patients and data curation. All authors read and approved the final manuscript.

Informed consent

Informed consent was obtained for experimentation and that it conforms to the standards currently applied in the country of origin**.**

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