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Original Research Article

A PROSPECTIVE COMPARATIVE STUDY ON EFFICACY OF SELECTIVE ESTROGEN RECEPTOR MODULATOR AND ANTIOXIDANTS IN ANDI

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Abstract

Background: Breast cancer is a complex and heterogeneous disease with inherited genetic underpinnings that contribute to its pathology. The most common inherited breast cancer genes are BRCA1 and BRCA2, with mutations in high penetrance contributing to 5%-10% of all breast cancers. **Aim:** This study aimed to compare the efficacy of selective oestrogen receptor modulators and antioxidants in regression of ANDI. **Material and Methods**: This analytical prospective comparative study included 100 patients at the Govt Mohan Kumaramangalam Medical College and Hospital between October 2019 and September 2021. The patient group included 50 women with breast cancer and the control group consisted of 50 healthy women. Both groups were compared in terms of serum levels of antioxidant and oxidative stress biomarkers. **Results**: Regarding the antioxidant biomarkers, there was a significant difference between the patients and controls regarding the levels of serum ceruloplasmin and glutathione (P values .000), while vitamin C showed no significant correlation (P value .053). Regarding oxidative stress biomarkers, the correlation was significant for both peroxynitrite and MDA (P value .000 and .001) respectively,) but not for gamma-glutamyl transferase (P = 1.00). Reduced levels of both ceruloplasmin and glutathione are seen in patients with breast cancer, whereas vitamin C is not. Elevated levels of both peroxynitrite and MDA are observed in patients with breast cancer which may be used as serum markers for the early detection of breast cancer. **Conclusion**: Our results suggest that patients with breast cancer show reduced levels of ceruloplasmin and glutathione, while vitamin C is absent. Elevated levels of peroxynitrite and MDA may aid early detection.

INTRODUCTION

Breast cancer is an extremely complex and heterogeneous disease that is still not fully understood even after years of research. A meta-analysis of nine studies showed that hypermethylation of the BRCA1 gene is a high-risk factor for developing breast cancer which helps establish the foundations of its notoriety as a genetic disease.[1] However, while breast cancer is a genetic disease, its inherited genetic underpinnings are not the sole determinants of its pathology. Several GWAS studies have shown that susceptibility genes and genomic sequences account for less than one-third of all inherited breast cancers, with BRCA1 and BRCA2 accounting for 85% of all hereditary breast cancers.[2] Although some genetic elements of breast cancer are well-defined in their impact, such as BRCA1 and BRCA2, most of the women who develop breast cancer do not present a clear, concise risk profile, with only 5%–10% of all breast cancers considered due to mutations in inherited high penetrance.[3]

Breast cancer is heavily influenced by a combination of age, personal or family history, reproductive and hormonal factors, postmenopausal obesity, parity, and exogenous hormone use alongside genetic predispositions. The most common inherited breast cancer genes were BRCA1 and 2. These genes facilitate the repair of DNA strand breaks and certain founder mutations occur in specific ethnic populations. BRCA2-associated tumours are of a higher grade, ER-positive, and are unlikely to express HER2 receptor as opposed to BRCA1 which are mainly triple-negative genetic

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mutations that also play a role in both inherited and sporadic breast cancer.[4] P53 mutations are present in nearly 40% of human breast cancers acquired as a defect, with high penetrance genes being responsible for 5%–10% of all breast cancers.

Overall, the aetiology of breast cancer cannot be concisely defined owing to the heterogeneity of the disease process, and dominant gene mutations appear to be present in only a small number of breast cancers, with BRCA1 and BRCA2 being the most common. Instead, it is suggested that gene variations due to single nucleotide polymorphisms or SNPs likely explain the heterogeneous nature of breast cancer and the differences in tumour behaviour among individuals. The age at which women experience menarche, the first occurrence of menstruation, is also believed to affect their risk of developing breast cancer. Epidemiological investigations suggested that breast cancer risk associated with a young age at menarche was more pronounced among premenopausal women, and women who experienced menarche at a young age were more likely to develop ER/PR-positive tumours than ER/PR-negative ones.

While older age at menarche was not differentially associated with increased breast cancer risk when defined by ER or PR status, late menarche was not associated with a reduced risk of developing ER-negative/ER tumours in five of the studies. The associated risk for later menarche was similar for ER-positive/PR-positive and ER-negative/PR-negative tumours. In addition to age at menarche, women who experienced postmenopausal obesity were at increased risk of developing breast cancer. A consistent association between postmenopausal obesity and ER-positive/PR-positive tumours was identified in three of the four studies (one cohort and two case-control) that assessed this relationship (reviewed in Althuis et al. 2004). Furthermore, risk estimates increased incrementally with increasing BMI and reached statistical significance in two of the studies: the Iowa Women's Health Study also showed that postmenopausal obesity was associated with an increased risk for hormone receptor-positive breast cancer, regardless of ER, PR, or ER/PR joint status (Althuis 2004).[5] Parity, or the number of times a woman carries a pregnancy to gestational age, is another non-genetic factor that affects a woman's likelihood of developing breast cancer. The reduction in breast cancer risk associated with parity was more consistently observed for ER-positive than ER-negative tumours and risk estimates ranged from 0.5-0.8, with the greatest reductions noted for multiparous women.[6]

Aim
This study aimed to compare the efficacy of selective oestrogen receptor modulators and antioxidants in the regression of ANDI.

MATERIALS AND METHODS

This analytical prospective comparative study was conducted with 100 patients in the Department of General Surgery, Govt. Mohan Kumaramangalam Medical College and Hospital, Salem, between October 2019 and September 2021. The study was approved by the institutional ethics committee before initiation, and informed consent was obtained from all patients.

Inclusion Criteria
The study included female patients aged between 18 and 55 years who had breast lumps (benign size, < 5 cm), mastalgia, cysts, and nodularity.

Exclusion Criteria
Patients with associated gynaecological disease of age less than 18 years and more than 55 years with malignant breast disease, breast lump of size more than 5 cm with other comorbid systemic diseases such as diabetes, malignancies, long-term steroids, etc., with haemodynamic instability, and those not willing to give consent were excluded.

This study included 100 women divided into equal groups. The patient group included 50 women with breast cancer and the control group consisted of 50 healthy women. Both groups were compared regarding the serum levels of antioxidant biomarkers (vitamin C, ceruloplasmin, glutathione) and oxidative stress biomarkers, malondialdehyde (MDA), peroxynitrite, and gamma-glutamyl transferase.

Ormeloxifen 60 mg (DS) was administered twice weekly for three months to all patients enrolled in our study. A second cycle of ormeloxifene (60 mg once weekly) was administered to the benign lumps, which required further regression. Mastalgia, nodularity, and lump size were assessed serially during the initial and monthly visits. The intake of tablets and self-reporting were documented as compliance. Pain at the initial visit and subsequent visits was documented using a visual analogue scale using a VAS ruler. It is a linear analogue 10-point scale with markings from 0 to 10, where '0' denotes the absence of pain and' 10' denotes extreme pain.

The nodularity of the breast was documented on the Lucknow Cardiff scale, given the extent of the breast involved with nodularity and extent of nodularity (consistency). This scale was documented by the same clinician for each patient at each visit, to prevent observer bias. This scale is a five-point ordinal scale with grades 0 to 4, in which grade 0 denotes no nodularity with soft consistent breasts and grade 4 denotes the maximum nodularity. Results were documented as per the clinical examination, visual analogue scale for pain, Lucknow Cardiff scale for nodularity, and ultrasonography for the size of lumps. Bi-monthly follow-up was performed in all patients for 12 weeks, followed by monthly follow-ups for the next three months for all cases.

Statistical Analysis.

RESULTS

The mean age was 51 years, and 70% of the patients were between the age group 50-60 years. More than 80% of the patients were 40 years old; the maximum age of the patients was 75 years, and the minimum age was 32 years.

Carcinoma breast, especially locally advanced breast cancer, was more common in the lower socioeconomic group in 35 of 50 cases (70%). The incidence is higher in the lower economic class due to ignorance or negligence and illiteracy, and lower in the higher socioeconomic class as they are educated, aware, and more concerned. Of the 50 patients, 25 (50%) had locally advanced breast cancer, mostly belonging to the lower socioeconomic strata, because they presented at a late stage of the disease. [Table 1]

The mean serum glutathione level was 6.247 ± 1.35688, which was normal in all patients. The mean serum vitamin C level was 5.4788 ± 2.8798 mg/dL, which was normal in 21 patients (70%). The mean serum ceruloplasmin was 27.03 ± 6.531, which was normal in 28 patients (93.3%).

The mean serum MDA level was 5.3129 ± 1.15844, which was above $4\mu mol/L$ in 15 patients (96.7%), and the serum peroxy nitrite level was elevated in 10 patients (96.7%), with a mean level of $5.212\pm.15844$ $\mu mol/L$. The serum GGT level was normal in 29 patients (96.7%), with a mean level of 30.43 ± 10.792 $\mu mol/L$. Serum MDA levels were elevated in 96.7% of patients with breast cancer and 60% of the control group, and the correlation was very significant for breast cancer (0.001).

Regarding the antioxidant biomarkers, there was a significant difference between the patients and controls regarding the levels of serum ceruloplasmin and glutathione (P values .000), while vitamin C showed no significant correlation (P value .053).

Regarding oxidative stress biomarkers, the correlation was significant for both peroxynitrite and MDA (P value .000 and .001) respectively,) but not for gamma-glutamyl transferase (P = 1.00). Reduced levels of both ceruloplasmin and glutathione are seen in patients with breast cancer, whereas vitamin C is not. Elevated levels of both peroxynitrite and MDA are observed in patients with breast cancer which may be used as serum markers for the early detection of breast cancer. [Table 2]

In the Fibroadenoma group (at the end of 12 weeks), the lumps completely disappeared in 60% of the patients, with a 75% regression in 30% of the patients and a 25 to 40% regression in 10% of the patients. These patients were followed up with ormeloxifene therapy 60 mg once weekly for the next 12 weeks, with complete disappearance of lumps. There was no response in Isolated Breast cyst cases (macrocysts), but complete regression occurred in all cases if cysts were associated with nodularity (fibrocystic disease). No allergies to the drug were reported. There were no other complications, except prolonged menstrual cycles, in 10% of the patients. [Figures 1 and 2]
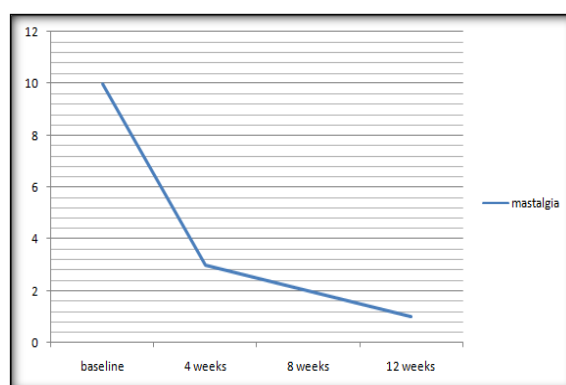

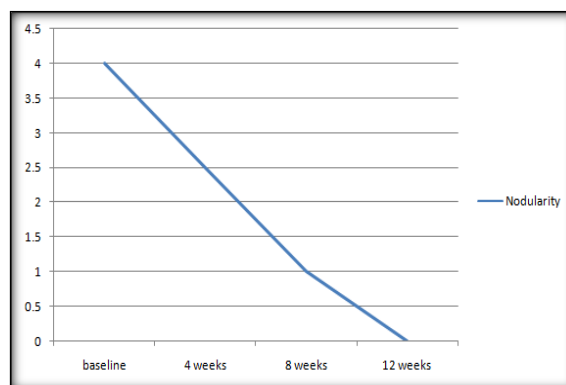**Figure 1: Assessment of mastalgia in ormeloxifene therapy**

Figure 2: Assessment of nodularity in ormeloxifene therapy

Table 1: Age and ANDI classification

Age	Normal process	Aberration
	Breast development	
<25 years	Lobular and ductal	Fibroadenoma
	Stromal	Juvenile hypertrophy
	Cyclical activity	
25-40 years	Lobular	Cyclical mastalgia
	Stromal	Cyclical nodularity
	Involution	
35-55 years	Lobular	Macrocysts
	Stromal	Sclerosing lesions
	Ductal	Duct ectasia

Table 2: The levels of the antioxidant biomarkers and oxidative stress biomarkers

		Levels	Frequency (%)	P value
Antioxidant biomarkers	Serum glutathione	Range	0.88–8.45	.000
		Mean ± SD	6.247±1.35688	
		Normal	30(100%)	
	Serum vitamin C	Range	0.92–8.24	0.053
		Mean ± SD	5.4788±2.87980	
		Normal	21(70%)	
		Low	9(30%)	
	Serum ceruloplasmin	Range	14–46	.000
		Mean ± SD	27.03±6.531	
		Normal	28(93.3%)	
		Low	2(6.7%)	
Oxidative stress biomarkers	Serum malondialdehyde	Range	3.24–8.33	.000
		Mean ± SD	5.3129±1.158	
		Normal	1(3.3%)	
		Elevated	29(96.7)	
	Serum peroxynitrite	Range	4.44–93.18	.000
		Mean ± SD	5.2129±1.158	
		Normal	1(3.3%)	
		Elevated	29(96.7)	
	Serum gamma-glutamyl transferase	Range	6–46	1
		Mean ± SD	30.43±10.792	
		Normal	29(96.7)	
		Low	1(3.3%)	

DISCUSSION

Prognosis of breast cancer is related to a variety of clinical, pathological, and molecular features, including the stage of the carcinoma, histological type, grade, and lymph node metastasis. ER and PR are of increasing importance and influence in the management of this malignancy. The mean age was 51 years, and 70% of the patients were between the age group 50-60 years. More than 80% of the patients were 40 years old; the maximum age of the patients was 75 years, and the minimum age was 32 years. This finding is like several other studies, in which it was found that more than 75% of the cases were above 50 years.[4] A similar study conducted in 2009 reported that the mean age was 52.5 years and 85.7% of the patients were more than 40 years. 70% of cases were postmenopausal women.[7] It is in concurrence with some recent studies conducted, in which, the postmenopausal age group was more common, some showing up to 70% while some others showing up to 59% case prevalence.[6,8]

In our study, carcinoma breast, especially locally advanced breast cancer, was more common in the lower socioeconomic group (35 of 50 cases, 70%). The incidence is higher in the lower economic class due to ignorance or negligence and illiteracy, and lower in the higher socioeconomic class as they are educated, aware, and more concerned. Of the 50 patients, 25 (50%) had locally advanced breast cancer, mostly belonging to the lower socioeconomic strata, as they presented at a late stage of the disease. This is in concurrence with a recent study in which 65% of the affected patients were from the low socio-economic class.[9]

In our study, from a total of 50 cases, 50% of cases were ER-positive and 44% of cases were PR-positive. Among the patients, 63.5% had locally advanced breast cancer with ER and PR positivity.

A study reported that ER and PR were positive in 70%-80% of tumours, and HER-2/neu expression was present in 15%-20% of tumours.[10] A population-based study conducted in 2003 documented the incidence of invasive carcinoma-based hormone receptor status between 1992 and 1998. Hormone receptor positivity has been found to increase from 75.4% to 77.5% in the United States, with an increase in prevalence over the years.[11]

In a study conducted in 2005 in New York with 3655 breast carcinomas, 71.6% were ER positive and 47.4% were PR positive. Some of these studies were conducted in Western populations.12 An Indian study conducted in 2000 documented the ER and PR status of breast carcinoma in 798 cases. The procedure was performed using immunohistochemistry.[13] Of the 798 tumours, 32.6% were ER-positive and 46.1% were PR-positive which was also in concordance with a study in Karachi.[14]

Ceruloplasmin which is formed mainly in the liver and to a lesser extent extra-hepatically, is normally present in the serum at concentrations between 80 and 120 IU and plays a major role in the binding of free radicals and enhancing their scavenging and metabolism. Serum vitamin C level was normal in 90% of the control group and 70% of the breast cancer group, although the difference between both groups showed no significant correlation, the patient group showed that 30% of them showed a low level of vitamin C compared with 10% from the control group.

Many epidemiological studies have confirmed the protective effect of vitamin C in lowering the risk of cancer development and its association with better survival rates among cancer survivors. Vitamin C regulates tumour necrosis factor-related apoptosis-inducing ligands, and low levels of vitamin C are

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associated with the reduction of this pathway and an increased risk of cancer, suggesting the potential role of normal serum vitamin C in the prevention of breast cancer. Ceruloplasmin is an acute-phase protein that is synthesised mainly in the liver and is found to play a role in the development of cancers, including breast cancer, which is attributed to its effects on angiogenesis and neovascularization. This has been shown in breast cancer cell lines in some studies.

In our study, the levels of ceruloplasmin were within normal levels in 93.3% of patients with breast cancer, while it was low in all individuals in the control group; although it was not elevated in the patient group, the difference was very significant between both groups. This signifies that the measurement of serum ceruloplasmin is valuable in the diagnosis of breast cancer, and in some studies, its level has been correlated with the clinical stage of the tumour; thus, it may be used in other clinical and imaging studies for the diagnosis of breast cancer. Glutathione is an important marker in patients with breast malignancy that is independent of hormone receptor status and the clinical stage of the tumour, which may indicate disseminated disease.

In our study, serum glutathione was normal in all patients with breast cancer and elevated in 83.3% of the control group. This finding is contrary to most published papers which show the reverse, which may be attributed to the small sample size. Long-term follow-up and estimation of glutathione levels may be performed to predict prognosis, recurrence, and responsiveness to chemotherapy, although this is still under investigation. GGT levels are elevated in most malignant and non-malignant liver disorders; however, GGT levels have been linked to other types of cancer, such as breast cancer. In a Swedish cohort study involving 545,460 individuals, they found an association between GGT levels and different types of cancer and a particularly high association with breast cancer.[15]

Our results suggest no significant correlation between breast cancer and GGT; its level was normal in 100% of the control group and 96.7% of the breast cancer group, and the P-value for this correlation was 1:00. The level of serum peroxynitrite was elevated in 96.7% of the patient group and 6.7% of the control group, and the association was very significant, with a very clear difference between both groups. Many studies have also concluded that increased levels of peroxynitrite are observed in patients with breast cancer. Some theories have now been presented about the possible use of antibodies against peroxynitrite which may help in the diagnosis of early breast cancer. Lipid peroxidation is associated with a high risk for cancer development and is formed by the peroxidative metabolism of unsaturated fats.

In our study, the level of serum MDA was elevated in 96.7% of patients with breast cancer and 60% of the control group, and the correlation was

significant for breast cancer. This provides evidence for a relationship between lipid peroxidation and breast cancer development.

CONCLUSION

In conclusion, several different compounds were assessed for their antioxidant effects. Although most compounds did not produce significant results, vitamin E surprisingly exhibited an oxidant and not an antioxidant capacity. This result is interesting because vitamin E is traditionally considered to have antioxidant qualities; however, the findings presented in this study present a different picture. Although the results of these experiments are not definitive, they provide a different perspective on the compounds used and their ability to reduce oxidative stress and lower protein carbonylation levels. Reduced levels of both ceruloplasmin and glutathione are seen in patients with breast cancer, whereas vitamin C is not. Elevated levels of both peroxynitrite and MDA are observed in patients with breast cancer which may be used as serum markers for the early detection of breast cancer.

Ormeloxifene was significantly effective in treating the Aberrations of development and cyclical changes in breasts. Its efficacy has not been documented in the treatment of breast involution aberrations. It has a targeted efficacy profile, prevents adverse effects, and has a low economic burden.

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