Assessment and comparison of quality of life and sexual function after adjuvant therapy in post-mastectomy breast carcinoma: A prospective study



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ABSTRACT

Background: Quality of life (QoL) is an important measure in the evaluation of health, physical condition, and the effects of treatment and continues to gain importance. Health-related QoL gives information about the patient's experiences (physical and psychological) related to the disease and the applied treatments, as well as the potential prognosis. It is known that breast surgery and its combination with chemotherapy (CT) and radiation therapy causes negative changes in the patient's psychosocial well-being and QoL. Aims and Objectives: To study the QoL and sexual function in female breast carcinoma patients who received adjuvant CT and radiation therapy after mastectomy. Materials and Methods: This was a single institutional prospective study carried out in the Department of Radiotherapy, RG Kar Medical College and Hospital, Kolkata. From January 2017 to December 2019, according to inclusion and exclusion criteria, a total of 64 histologically proven breast carcinoma patients were included in this prospective study. Results: On symptom scale analysis, it has been noted that all the symptoms increased after receiving CT and reached their highest after the 6th cycle of CT, then improved after radiation and further improved at 6 months follow-up, except for the breast symptoms, which decreased after the 6th cycle of CT, increased immediately post-RT, and then again decreased at 6 months follow-up. Conclusion: Treatment with CT and radiation therapy significantly affects QoL, psychosexual function, and physical problems.

Key words: Breast cancer; Mastectomy; Adjuvant radiotherapy; Chemotherapy; Quality of life

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INTRODUCTION

Worldwide, breast cancer is the most common malignancy, constituting about 11.7% of all new cases and the 4th common cause of cancer-specific mortality at 6.9%.¹ The application of current treatment techniques (surgery, radiation therapy, chemotherapy [CT], hormone therapy, and biologic or targeted therapy) results in the cure of nearly two out of three patients diagnosed with breast cancer.^{2,3} Nevertheless, patients experience the diagnosis of any cancer as one of the most traumatic events ever.

Independent of prognosis, the diagnosis brings with it a change in a person's self-image and her role in the home and workplace.³

Quality of life (QoL) is an important measure in the evaluation of health, physical condition, and the effects of treatment and continues to gain importance.⁴ According to the definition of the World Health Organization, health not only means being free of any diseases but also a state of general well-being in terms of physical, mental, and social attributes. Health-related QoL (HRQL) gives information

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about the patient's experiences (physical and psychological) related to the disease and the applied treatments, as well as the potential prognosis.⁴ It is known that breast surgery and its combination with CT and radiation therapy cause negative changes in the patient's psychosocial well-being and QoL.4 It is important that patient-centered nursing care and optimized adaptation are ensured and that the state of well-being is continued during every stage after breast cancer diagnosis, until cure or death.⁵ Over 75 different determinants of HRQL were studied with either the EORTC or functional assessment of cancer therapy (FACT) questionnaires.⁶ Patients with comorbidities, treated with CT, with less social support, and with more unmet needs have poorer HRQL.^{6,7} HRQL improves over time. In Asia, patients with breast cancer, particularly those with other comorbidities and those treated with CT, with less social support and more unmet needs, have poorer HRQL. Appropriate social support and meeting the needs of patients may improve patients' HRQL.8

Aims and objectives

To study the QoL and sexual function in female breast carcinoma patients who received adjuvant CT and radiation therapy after mastectomy.

MATERIALS AND METHODS

Study design

This study was a single institutional, observational, questionnaire-based, single-arm longitudinal study carried out in the department of R. G. Kar Medical College and Hospital, Kolkata. Inclusion criteria were (a) diagnosed non-metastatic breast carcinoma patients who underwent upfront mastectomy; (b) age between 18 and 60 years; (c) patients who are candidates for adjuvant CT and radiation therapy after mastectomy; (d) no pre-existing uncontrolled co-morbidity or psychiatric illness; And (e) being literate and capable of comprehending and answering the questionnaires. The exclusion criteria were: (a) individuals who cannot comprehend the questionnaires or refuse to answer; (b) patients who are not currently active sexually; (c) those with a psychiatric illness; (d) post-hysterectomy with or without salpingo-oophorectomy patients; (e) patients who received neoadjuvant CT before surgery; and (f) patients with a history of diagnosis of another primary cancer (other than breast cancer) in some part of their life. The details of the study design and QoL assessment time points are illustrated in Figure 1:

Study procedure

We have conducted this study on females between 18 and 60 years of age, post-modified radical mastectomy (MRM) breast carcinoma patients, and candidates for adjuvant

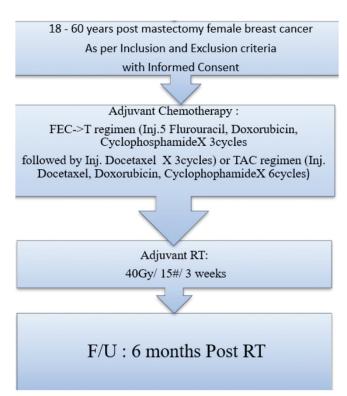


Figure 1: The study design and quality of life assessment time points

CT and radiotherapy, and to estimate changes in QoL in repeated measurements in this longitudinal study.

Data collection

Data was collected by self-reported questionnaires titled EORTC QLQ-C30 and EORTC QLQ-BR23 and their validated vernacular (Bengali/Hindi) translations. The EORTC QLQ-C30version 3.0 module contains 30 questions about functional scales (FSs) or symptoms for measuring a global health status or QoL scale, namely (1) FSs: (a) Physical functioning; (b) Role functioning; (c) Emotional functioning; (d) Cognitive functioning; (e) Social functioning; (2) Symptom scales or items: (a) fatigue; (b) nausea and vomiting (NV); (c) pain; (d) dyspnoea; (e) insomnia (IN); (f) loss of appetite (LOA); (g) constipation (CN); (h) diarrhoea (DR);, (i) financial difficulties.

The EORTC QLQ-BR23version 1.0 module contains 23 questions about symptoms for measuring the FSs, which include: (a) body image (BI), (b) sexual functioning (SF), (c) sexual enjoyment (EN), and (d) future perspective (FP). Similarly, the symptom scales and items include (a) side effects of systemic therapy, (b) breast symptoms (BS), (c) arm symptoms (AS), and (d) upset by hair loss (UHL).

Scoring system

All of the scales and single-item measures range in score from 0 to 100. A high scale score represents a higher response level. Thus, a high score on a FS represents a

high or healthy level of functioning. A high score for the global health status/QoL represents a high QoL. However, a high symptom scale or item score represents a high level of symptomatology or problems. The raw score (RS) was calculated as the mean of all the data for each scale. The range was calculated as the difference between the maximum possible and the minimum possible value of RS. Most items are scored 1–4, giving a range of 3. The exceptions are the items contributing to the global health status or QoL, which are 7-point questions with a range of 6. Using the RS and range, the values were linearly transformed to a scale of 0–100 to give a score of S by the given formulas:

Functional Scales: $S = \{1 - (RS-1)/range\} \times 100$.

Symptom Scales/Items: $S = \{(RS-1)/range\} \times 100$.

Global Health Status/QoL: $S = \{1-(RS-1)/range\} \times 100$.

Higher values in the function and QoL scales indicate a better level of functioning or higher QoL, whereas higher values in the symptom scales indicate increased symptoms or difficulties. Missing data will be accounted for according to the method prescribed in the EORTC manuals. Scores for individual patients will be analyzed, and the mean scores of all patients for each parameter will be calculated.

Data analysis

Data were analyzed using the Statistical Package for the Social Science Inc., Chicago, IL, software version 17.0. The Kolmogorov–Smirnov test was used to test for the "goodness of fit" of the normality of the sample distribution. With P<0.05, non-parametric tests were used. Categorical data were expressed as percentages and compared by the chi-square test. The continuous and repeated data were summarized as mean±SD. For repeated related *k* samples, the Friedman analysis of variance test was used. With P<0.05, subsequent *post hoc*, separate Wilcoxon signed-rank tests were used on the different combinations of related groups. All reported P-values will be two-tailed. All observations were considered statistically significant at P<0.05.

Ethical consideration

The purpose of the study was fully explained to all patients; written informed consent was obtained and they were assured of confidentiality and anonymity. This study was approved by the institutional ethics committee of RG Kar Medical College (RKC-5189, dated November 24, 2016).

RESULTS

Between January 2017 and July 2018, we enrolled 65 consecutive patients who met the inclusion criteria. The

baseline patient profile is listed in Table 1. Fifty-four patients were eligible for statistical analysis in the initial 65 patients recruited in the study. 5 patients were left outside the study due to reluctance to answer SF questionnaires, incomplete questionnaires, and missing data on 2 or more scheduled visits; 3 patients did not complete the treatment due to various reasons; and the rest 3 patients developed uncontrolled comorbidities after 2 or 3 cycles of CT; hence, the regimen had been changed.

EORTC QLQ-C30 subscale scores at each assessment

The mean score for global QoL at baseline was 41.20; after the end of the 3rd cycle of CT, it was 30.25; after the end of the 6th cycle of CT, it was 25.30; immediately, after EBRT, it increased to 39.81; and 6 months after EBRT, the mean score increased to 67.28, which even surpassed the baseline QoL. Hence, the GHS or QoL in general has progressively decreased after the administration of different cycles of CT, but immediately after EBRT, it improved a little, and 6 months after EBRT, it improved significantly (Figure 2).

Table 1: Baseline patients' profile of patients	
Baseline profiles of patients	n (%)
EDUCATION	
Graduate	20 (37%)
HS	22 (40.7%)
Primary school	12 (22.2%)
Residential Status	
Rural	24 (44.4%)
Urban	30 (55.6%)
Menopausal status	
Postmenopausal	21(38.9%)
Premenopausal	33(61.1%)
Hormone receptor profile	
ER, PR +ve; HER2/neu	15(28%)
negative15(28%) (Luminal A)	
ER, PR +ve;	14(26%)
HER2/neu +ve (Luminal B)	
ER, PR -ve;	25 (46%)
HER2/neu +ve (Luminal C)	

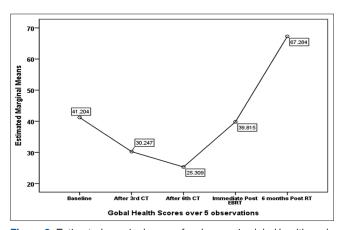


Figure 2: Estimated marginal means for changes in global health scale scores over the study period

FS

FSs consist of physical functioning (contains 5 questions about the patient's daily physical activities). Role functioning (contains 2 questions about the patient's limitations in professional life, daily life, or leisure time activities). Emotional functioning (contains 4 questions regarding the patient's anxiety, worries, irritable mood, and depression) Cognitive functioning (contains 2 questions about the patient's state of attention and memory) Social functioning (contains 2 questions about the patient's family life and social activities) From the above 15 answers out of 15 questions, we can state that a score of 4 is "very much" and 1 is "not at all'. With a linear transformation score, RS 4 corresponded to 0 on the FS, and score 1 corresponded to 1–100. We had considered that a high RS meant patients had more complaints with the corresponding low FS of 0 and a less healthy life. Our study revealed that patients' physical, role, emotional, cognitive, and social functioning got worse from baseline to the assessment after the 3rd cycle of CT and got worse after the 6th cycle of CT (mean linear transformation scores are decreasing). However, immediately after EBRT, the mean linear transformation score increased, and 6 months after EBRT, it reached its highest, as summarized below. Hence, we can conclude that all 5 functions. as stated above, were better after radiation in comparison to CT and significantly improved 6 months after the completion of EBRT (Figure 3).

Fatigue

Fatigue is a symptom that remained more or less constant throughout the different phases of observation in our study. It reached a maximum after the 6th cycle of CT (mean score: 68.93) from the baseline and through the successive cycles of CT; thereafter, it came down immediately post-radiation, and 6 months after the completion of radiation, it became significantly reduced

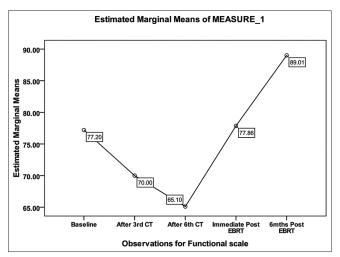


Figure 3: Estimated marginal means for changes in functional scale scores over the study period

(mean score: 32.10), even less than that of the baseline, but did not disappear completely.

NV

NV increased significantly after the 3rd cycle of CT in comparison to baseline (mean score: Baseline: 8.33, after the 3rd cycle CT: 65.12) and reached its highest after the 6th cycle of CT (mean score: 79.63). Immediate post-RT, it decreased substantially (mean score: 44.13), and 6 months after the completion of RT, it decreased further (mean score: 16.05).

Pain (P)

The questions about pain consist of two numbers: one is about having (generalized) pain, and the other is about whether there is any limitation in daily life activity due to pain. The mean pain score at baseline was 56.17, whereas after the 3rd cycle of CT, it increased to 62.96. We found that patients often felt body aches or muscle pain after receiving CT. It further increased after the 6th cycle of CT (mean score: 69.44). However, decreased after the completion of RT (53.70) and reached a minimum value (44.13) 6 months after the completion of RT. The changes in pain scores were statistically significant at each assessment.

Dyspnea

This result showed that there was a significant change in dyspnea after the 3rd cycle of CT (mean 66.05) in comparison to the baseline level (mean 39.50) and increased a bit after the 6th cycle of CT (mean 75.92). Immediate post-RT decreased significantly (mean 49.38) and 6 months after the completion of RT, it reached its minimum value (mean 22.84).

IN

Most of the patients were having some problems regarding sleep at baseline, as they came after MRM with a diagnosis of breast cancer. The mean score was 30.24 at baseline. However, after receiving successive cycles of CT, IN worsened. It reached its highest at the assessment after the 6th cycle of CT (mean 59.88). Immediate after radiation, IN was improved to some extent (mean 40.12) and further improved 6 months after the completion of RT (mean 23.45).

LOA

The result showed that the mean score of LOA sharply increased from baseline (mean 30.24) to the assessment after the 3rd cycle of CT (mean 70.99). It further increased after the 6th cycle of CT (mean 87.04) and then decreased significantly immediately post-RT (mean 52.47). Six months after the completion of EBRT, it reached its minimum value (mean 25.92).

CN

CN was not a very common side effect of CT or radiotherapy, though post-menopausal patients aged over 50 years complained more of CN than younger patients. The baseline mean score of CN was 16.05; after the 3rd cycle of CT, it remained almost the same (mean 16.67); it increased a bit after the 6th cycle of CT (mean 22.22); and thereafter, it came down to the same as the baseline.

DR

At baseline, none of the patients complained of DR, but after the 3rd cycle of CT (mean 30.25), many patients experienced DR. The mean score further increased after the 6th cycle of CT (37.65), but it reduced significantly immediately post-RT (mean 9.87). Six months after the completion of EBRT, it reached baseline again (Figure 4).

ΒI

The BI 4 questionnaires were "Have you felt physically less attractive as a result of your disease or treatment?", "Have you been feeling less feminine as a result of your disease or treatment?" and "Did you find it difficult to look at yourself naked?" and "Have you been dissatisfied with your body?", with score 4 being "very much" and 1 meaning "not at all'. With a linear transformation score, RS 4 corresponded to 0 on the FS, and score 1 corresponded to 1–100. We had considered that a high RS meant patients had more complaints with the corresponding low FS of 0 and a less healthy life. Our study revealed that patients were very much concerned at baseline after surgery with low linear transformation BI scores. However, as postsurgery adjuvant treatment continued, their mean linear transformation scores for BI improved, i.e., they started accepting the body deformity and had more positive wellbeing, which was clearly reflected in their SF and FP scores.

SF

We have used two questions: "To what extent were you interested in sex?" and "To what extent were you sexually active? (with or without intercourse)" for the sexual function score. For both questions, RS of 4 meant "very much" and 1 meant "not at all'. As these were positive questions, linear transformation scores of 100 meant healthier individuals with high sexual activities. These questionnaire scores were, however, differently interpreted from the BI and FP scores. We have chosen sexually active patients in our study, but the mean score for the above 2 answers was 40.74, which sharply came down to 6.80 after the 2nd assessment (3rd cycle of post-CT) and thereafter reached 0 after the 6th cycle of CT. Immediate post-RT, it has been seen that very few of the patients became sexually active (mean 1.54), though with a loss of interest. At post-RT 6 months follow-up, the mean score became 24.07, which was significantly less than the baseline mean score (Figure 5).

ΕN

This score used 1 question: "If you have been sexually active, to what extent was sex enjoyable for you?" and >90% of patients were reluctant or avoidant to this question not only after starting adjuvant therapy but also at baseline. Hence, no assessment could be done on this parameter.

FP of life

The FP questionnaire was "Were you worried about your health in the future?" with score 4 being "very much" and 1 meaning "not at all'. With a linear transformation score, score 4 corresponded to 0 on the FS, and score 1 corresponded to 1–100. We had considered that a high RS meant patients had more complaints with the corresponding low FS of 0 and a less healthy life. Our study revealed that

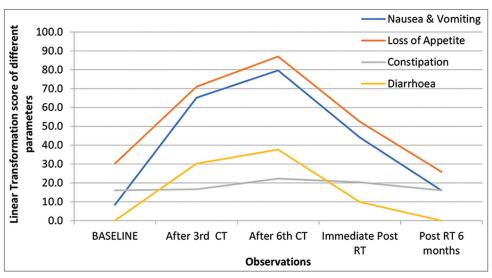


Figure 4: Graphical representations of symptom scale about gastrointestinal symptoms of QLQ C30

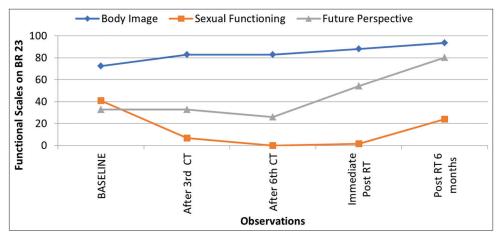


Figure 5: Graphical representations of functional Scales of BR 23

patients were very much concerned at baseline after surgery with low linear transformation functional scores. However, as post-surgery treatment went on, after the 6th cycle of CT, scores dipped further as patients were apprehensive of radiation and its toxicities, despite adequate counseling. They had the conception that radiation was more "toxic" than CT, and many wanted to avoid the radiation treatment. At 6 months, however, their mean linear transformation score for FP improved with a sense of positive well-being in patients as treatment-related adverse events reduced, except arm complaints in a few individuals, and none of the patients had disease recurrence.

Systemic therapy side effect

A total of 7 questions are in the QLQ-BR23 Questionnaire to assess the symptoms caused by systemic therapy side effects, i.e., dry mouth, altered taste for food or drink, Eye symptoms like painful, irritated, or watery eyes; hair loss; ill feeling; hot flushes; and headache. Our study revealed that the mean score increased sharply from the baseline (10.14) to the assessment after the 3rd cycle of CT (50.41). It further increased after the 6th cycle of CT (mean 61.46). However, the score decreased significantly immediately after radiotherapy (mean 28.04) and reached near baseline 6 months after the completion of EBRT (mean 12.79).

BS

The BS (pain, swelling, sensitivity to touch, itching, dryness, and desquamation) remained the same at baseline (mean 48) and after the 3rd cycle of CT (mean 48). After the 6th cycle of CT, it decreased a bit (mean 42.28). Immediate post-RT, it decreased a little (mean 45.83), and 6 months after the completion of CT, the symptoms decreased significantly (mean 16.36).

AS

The results showed that the AS (pain over the arm or shoulder, arm swelling, elevation, or movements of the

arm) increased a little from baseline (mean 55.76) to the assessment after the 3rd cycle of CT (mean 57). It further increased a little at the assessment after the 6th cycle of CT (mean 61.32). Immediate post-RT, it increased significantly (mean 73.25) and thereafter started decreasing and reached just below the baseline (mean 54.52).

UHI

Females are usually very sensitive and concerned about the hair loss issue. As both the regimens used in our study induce alopecia, all the patients reacted to this issue and were mentally upset a lot due to hair loss. The mean score increased from 6.79 at baseline to 57.41 after the 3rd cycle of CT. It reached a maximum after the 6th cycle of CT (mean score: 67.90). The score came down to 26.54 immediately after RT and further came down to 3.09, which was less than the baseline.

Comparison of two different CT regimens according to alopecia complaints

On comparing both CT regimens, there was no difference with Greenhouse-Geisser analysis (F (2.115, 109.963)=1.202, P=0.306).

Comparison of NV in two different CT regimens

There are two arms of CT regimens: TAC and FEC-T. The comparison was made for CT's choice of CT according to emetogenic complaints. By repeated measures analysis, it was evident that the TAC regimen was more emetogenic than the FEC-T regimen; however, the difference was not statistically significant (Greenhouse-Geisser analysis, F (2.494, 129.695=2.508, P=0.073).

Sexual function activity according to menopausal status

By repeated measures analysis, it was evident that post-menopausal women had worse SF scores of BR-23, which persisted even after treatment, Greenhouse-Geisser analysis (F (1.958, 101.822=3.232, P=0.045).

Systemic side effects more in urban women

According to residents, systemic effects were more profound in urban women during the CT cycles.

DISCUSSION

With the increased survival of breast cancer patients and the younger age at diagnosis, the focus of caregivers has shifted to the QoL of the survivors. Previous evidence has indicated that breast cancer survivors suffer from several chronic problems, such as physical (pain and fatigue), psychological (fear of recurrence and inability to cope with the disease), and psychosocial (family worries and sexual problems).9-11 Therefore, there is a great need to provide education, information, and support to the patients and their caregivers over time. In our study, the mean global QoL was impaired significantly after receiving CT but improved immediately post-radiotherapy. Six months post-radiation it was improved and even surpassed in comparison to the baseline global QoL. On subset analysis, it has been found that all the FSs deteriorated after receiving CT, especially the physical, emotional, and social functioning, which was hampered more than cognitive and role functioning, but all returned to the baseline level immediately post-radiation and further improved significantly 6 months after radiation. The concept of QoL has been widely recognized for its prognostic role in treatment and disease outcomes.¹² Although, the EORTC QLQ-BR23 and the FACT-Breast (FACT-B) differ in their focal points, both questionnaires have been proven to be valid, reliable, and prominent tools in breast cancer-specific QoL assessment.

A study by Holzner et al. on breast cancer survivors raised the question of whether enough attention to the QoL of long-term survivors of breast cancer patients had been paid or not. The authors used the EORTC QLQ-C30 and QLQ-BR23 questionnaires and the FACT-B for the assessment of QoL. The main focus of this study was to look at the effect of time elapsed since initial treatment on the patient's QoL. Reduced QoL, especially in the areas of emotional, social, and SF, was found not only after initial treatment (1-2 years) but also after long post-treatment survival (>5 years). From these findings, the need for specific psycho-oncological interventions is derived. The findings imply that, besides recovering from the acute consequences of cancer therapy, long-term survivors of breast cancer (>5 years after initial treatment) still may have a special need for psycho-oncological support¹⁰.

In our study, on symptom scale analysis, it has been noted that all the symptoms increased after receiving CT and reached their highest after the 6th cycle of CT, then improved after radiation and further improved on the

6-month follow-up, except for the BS, which decreased after the 6th cycle of CT, increased immediately post-RT, and then again decreased at the 6-month follow-up. AS reached its highest peak immediately post-radiation. The most predominant symptom after receiving adjuvant CT was LOA, followed by NV, fatigue, and alopecia. Regarding FP, all patients were anxious, worried, and apprehensive from the baseline interview to adjuvant CT and EBRT; however, at 6 months post-RT follow-up, they showed a positive attitude and felt a sense of well-being as none of the patients had disease recurrence during our study period.

Our study revealed that patients were very much concerned at baseline after surgery with BI. However, as post-surgery adjuvant treatment continued, their mean linear transformation scores for BI improved, i.e., they started accepting the body deformity and had more positive well-being. Though all patients were sexually active at baseline, the study showed that interest and activity were greatly diminished after the 3rd CT, and all became sexually inactive after the 6th CT. However, at 6 months post-RT, most of the pre-menopausal women regained their sexual activity, although not as much as before the diagnosis of breast cancer. Post-menopausal women lost interest and remained sexually inactive. Regarding EN, >90% of patients were reluctant or avoidant to this question; hence, no assessment could be done on this parameter.

Parmar et al. assessed the QoL about surgery, adjuvant CT, radiation therapy, and hormone therapy in 299 Indian women with operable breast cancer using QLQ-C-30 and BR-23 questionnaires.¹³ The global QoL scale, FSs, and symptom scales showed no significant change from visits 1 to 3 between BCT and mastectomy. Women who underwent BCT maintained a better BI through visits 1 (P<0.001) and 2 (P=0.055) compared with women who underwent mastectomy. The differences disappeared by visit 3 (average 9 months after surgery). Women who had BCT had significantly more local symptoms than after mastectomy on visits 2 and 3 (P<0.01). CT significantly affected global QoL with poor scores during treatment (P=0.016). The results also showed lower FSs, especially concerning sexual function (P=0.02) after CT. Women receiving CT experienced severe NV (P<0.001), were upset over hair loss (P<0.001), and had more AS (P<0.01) and systemic effects (P<0.001). Adjuvant radiotherapy resulted in deterioration in social functions (P=0.02) during treatment, with significantly more BS (P<0.001) as compared with women not receiving adjuvant radiotherapy. Another study from south India by Dubashi et al. on the assessment of QoL issues in young patients <35 years using EORTC QoL C30 and BR23 reported that the overall QoL in younger patients with breast cancer appeared to be good. The QoL and sexual function were marginally worse

in the breast conservation group when compared to the mastectomy group.¹⁴

In our study, there were no effects of hormone therapy (tamoxifen or anastrozole) on QoL or sexual function, consistent with published literature.^{13,14}

Limitations of the study

The drawback of our study is an inability to ascertain changes over a long period, especially considering the long-term late effects due to the sequelae of treatment or disease relapse.

CONCLUSION

QoL and sexual function in post-mastectomy breast cancer patients and their alteration during adjuvant treatment are of paramount importance, but unfortunately, they are often underestimated. Indian women's population differs in ethnic, social, cultural, and economic aspects as compared with Western women, so it is necessary to study the factors affecting QoL, especially for Indian patients. From this study, it can be concluded that treatment with CT and radiation therapy significantly affects QoL and psychosexual function, along with physical problems. Psychological support and patient education are the most significant factors for a newly diagnosed breast cancer patient and should be encouraged by healthcare professionals.

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BB, ABU- Conceptualization, methodology, data collection, data interpretation, statistical analysis, and reviewing of the final manuscript; **SA, AB-** Conceptualization, methodology, data interpretation, statistical analysis, and reviewing the final manuscript; **DMA, DM, DC-** Conceptualization, methodology, data interpretation, statistical analysis, writing of the manuscript, and reviewing of the final manuscript.

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