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Clinical and biochemical amenorrhea in premenopausal patients with breast cancer treated with chemotherapy - a prospective cohort study

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Abstract

Background Patients with breast cancer receiving chemotherapy frequently experience amenorrhea due to the cytotoxic effects of drugs, resulting in ovarian suppression and impacting reproductive health. This study aimed to prospectively investigate the incidence of clinical amenorrhea defined as the cessation of menstrual bleeding for at least three consecutive months and and biochemical amenorrhea, characterized by serum estradiol levels < 20 pg/mL and FSH levels ≥ 40 mIU/mL, using clinical and biochemical methods to determine their relation and identify the predictive factors for early amenorrhea.

Methods This longitudinal study was conducted at a tertiary care hospital with 76 premenopausal patients with breast cancer who received chemotherapy. The patients were followed up for two years with monthly clinical assessments of amenorrhea status for a year and biannual hormonal assessments of serum estradiol and follicle-stimulating hormone (FSH) levels. The incidence of clinical and biochemical amenorrhea was estimated and explored to determine any association between the two factors and the impact of risk factors.

Results The rates of clinical and biochemical amenorrhea were 84.2% and 78.9%, respectively. The median time for clinical amenorrhea was 8 (95% CI, 7.83–8.17) months and 18 (95% CI, 17.90–18.10) months for biochemical amenorrhea. A significant association was observed between clinical and biochemical amenorrhea (P =.0022). The estradiol and FSH levels were initially in the premenopausal range and reached postmenopausal values by the end of the study period. Age, BMI, chemotherapy regimen, hormonal treatment, and biochemical amenorrhea were not predictive of the time to clinical amenorrhea.

Conclusions The occurrence of chemotherapy-related amenorrhea in premenopausal patients with breast cancer was high. Clinical amenorrhea is a reliable early indicator of biochemical amenorrhea. Regular menstrual and hormonal assessments are essential in patients with breast cancer undergoing chemotherapy. Future studies with larger cohorts are required to explore predictive factors associated with chemotherapy-related amenorrhea.

Keywords Breast neoplasms, Amenorrhea, Estradiol, Follicle stimulating hormone, Incidence

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Introduction

Recent cancer facts show that Breast cancer is the leading cause of cancer in women, constituting 23.8% of new cases of cancer and a leading cause of cancer-related mortality [1]. Global statistics show that the prevalence and incidence of breast cancer among young women are increasing at an alarming rate, with 645,000 premenopausal women diagnosed with breast cancer and 130,000 deaths reported [2]. Although the premenopausal disease burden is mostly found in countries with a low Human Development Index (HDI), there is a rising trend in age-standardized incidence rates in young premenopausal age groups in countries with high incomes [2, 3]. Breast cancer occurring at an extremely young age is associated with an increased risk of locoregional recurrence and distant metastasis [4]. Suppression of ovarian function of ovaries is a time-tested strategy to decrease the risk of breast cancer recurrence in young adults. In the SOFT trial, the addition of ovarian suppression improved treatment outcomes in patients who required adjuvant chemotherapy and remained premenopausal during treatment [5]. An 8-year follow-up of the ASTRRA trial also suggested the addition of ovarian suppression to tamoxifen in young premenopausal patients with breast cancer who remained premenopausal [6]. Chemotherapeutic drugs cause direct damage to ovarian follicles due to the depletion of germ cells, leading to the loss of ovarian reserve, premature ovarian failure, and infertility [7]. Despite the adverse effects of chemotherapy, ovarian suppression can be beneficial in premenopausal patients with breast cancer.

Recently, the American Society of Clinical Oncology (ASCO) recommended measuring the ovarian toxicity of anticancer drugs in clinical trials that enrolled prepubertal and premenopausal patients using clinical and biochemical methods because of concerns about premature menopause and the consequent long-term effects [8]. Ovarian function can be assessed by clinical methods, including menstrual status, or by biochemical estimation of hormones that influence the ovaries, including serum estradiol and follicle-stimulating hormone (FSH). Newer biomarkers like Anti-Mullerian hormone (AMH) and inhibin-B are also valuable tools for ovarian function assessment. These new biomarkers have the advantage over traditional assessment by providing reliable and long-term status information on ovarian reserve. Close monitoring of amenorrhea is essential following chemotherapy to estimate the incidence of amenorrhea because of its potential impact on reproductive health, quality of life, and treatment outcome [9–11]. The exact incidence of amenorrhea following chemotherapy is unknown, but its reported rate is high in many studies, exceeding 75% of patients [12, 13]. Although many high-risk factors for chemotherapy-related amenorrhea have been identified, conflicting data exist regarding most high-risk variables, including age, chemotherapy regimen, Body Mass index (BMI), and tamoxifen use [14]. These conflicting reports underscore the need for a more reliable way to assess chemotherapy-related amenorrhea and risk factors. The current assessment of amenorrhea focuses on clinical assessment alone; adding biochemical methods could enhance diagnostic accuracy, provide an in-depth understanding of ovarian function for comprehensive management of breast cancer patients. This study aimed to address these gaps by investigating the incidence of amenorrhea associated with chemotherapy in patients with breast cancer, to determine whether there is an association between clinical and biochemical assessments, and to identify any risk factors related to early amenorrhea.

Methods

This hospital-based prospective observational study investigated the association between clinical and biochemical amenorrhea and its incidence and risk factors in premenopausal patients with breast cancer. The Institutional Human Ethics Committee approved this study. All patients were observed for two years from the date of enrollment. Informed consent was obtained from all participants. The study population consisted of premenopausal patients with stage I to III breast cancer who had at least one menses within three months at the time of enrollment. Histopathological evidence of invasive cancer, hormone receptor status, and Her2 neu status were required before initiating treatment. All patients were required to undergo a cardiac function assessment by a cardiologist before initiating chemotherapy. Patients were required to receive at least six cycles of chemotherapy, including anthracycline and taxane. Patients with metastatic disease, an Eastern Cooperative Oncology Group (ECOG) performance score of ≥ 3, altered hepatic or renal function, those who received chemotherapy for other malignancies, and those who could not recollect their menstrual status were excluded. Clinical amenorrhea was estimated based on self-reported menstrual status. Patients who could not recall their menstrual status were excluded as precise classification of amenorrhea status was required along with time to achieve amenorrhea. Patients who underwent oophorectomy, hysterectomy, ovarian suppression therapy, or pelvic field radiation treatment were excluded. For all patients, the pathological evaluation included estimation of estrogen receptor (ER), progesterone receptor (PR) and Her2 neu status. Those who tested Her2 neu 3 + received Trastuzumab for one year, and those who tested positive for ER or PR received hormonal treatment with tamoxifen and were scheduled to continue for five years or longer, depending on the risks and benefits.

The chemotherapy regimen consisted of four cycles of Adriamycin and Cyclophosphamide (AC) and four cycles of Docetaxel, four cycles of AC and four cycles of paclitaxel, or six cycles of Docetaxel, Doxorubicin, Cyclophosphamide (TAC). The treating oncologist selected a specific chemotherapy regimen, dose, and number of cycles. For AC chemotherapy, doxorubicin (60 mg/m^2) and cyclophosphamide (600 mg/m^2 intravenously every three weeks) were administered. Docetaxel was administered intravenously at a dose of 75 mg/m^2 every three weeks. Paclitaxel was administered at 175 mg/m^2 intravenously every three weeks. For TAC chemotherapy, the doses of docetaxel were 75 mg/m^2, doxorubicin 50 mg/m^{2,} and cyclophosphamide 500 mg/m^2. Patients receiving different chemotherapy regimens were grouped together to reflect real-world clinical practice and capture the overall incidence of chemotherapy-related amenorrhea.

Premenopausal status was clinically defined as continuing menstrual cycles irrespective of irregularities at diagnosis [15]. Menopause was defined as the absence of menses for one year in the absence of chemotherapy, tamoxifen, or surgical removal of the ovaries [16]. Clinical amenorrhea in patients treated with chemotherapy is the cessation of menstrual bleeding for at least three consecutive months during or after the completion of chemotherapy [17]. Serum FSH ≥ 40 mIU/mL and estradiol < 20 pg/mL are reliable indicators of biochemical amenorrhea [18]. Menstrual status was assessed by asking patients questions directly at the time of chemotherapy initiation and every month after that for one year. Following the first cycle of chemotherapy, hormonal estimation of amenorrhea was performed by periodically checking serum FSH and Estradiol levels at an interval of 6 months for two years using standardized assays. The biannual hormonal assessment was chosen to maintain accuracy, reliability, and resource preservation, thereby avoiding variability from frequent assays and capturing long-term changes in ovarian function, whereas the monthly clinical assessment provides early detection of amenorrhea.

The sample size for this study was calculated based on a previous study that reported a 95% incidence of chemotherapy-induced amenorrhea depending on chemotherapy regimens and patient demographics [19]. With a desired margin of error of 5%, the required sample size was estimated to be 75 participants.

Statistical analysis

Descriptive statistics were used to summarize demographic variables and patient characteristics. Mean and standard deviation (SD) expressed continuous variables with a normal distribution, whereas median and 95% confidence intervals (CI) were used for those that followed a non-normal distribution. Categorical variables were expressed as frequencies and percentages. The association between clinical and biochemical amenorrhea was examined using the chi-square test. Degrees of freedom have been used to ensure transparency. The time required to achieve clinical and biochemical amenorrhea was estimated, along with a 95% confidence interval. Cox proportional hazards regression was used to estimate the influence of age, BMI, chemotherapy regimen, hormonal treatment, and biochemical amenorrhea on the time to clinical amenorrhea. This model was selected as it properly could deal with censored observations and examine the effect of several covariates over the study period. Although survival analysis could enhance the understanding of the findings, its application in this study was limited due to the sample size; thus, the Cox model was the best option for our analysis. Hazard ratios (HR) and 95% confidence intervals (CIs) were reported. All statistical analyses were performed using R Software (version 4.1), and a P value < 0.05 was taken as statistically significant [20].

Results

A total of 76 breast cancer patients who were premenopausal and received chemotherapy with or without hormonal treatment after meeting the inclusion criteria were included in this study. The mean patient age was 41.6 years (SD 4.4). Mean height was 154.8 cm (SD, 9.9) and a mean weight of 61.0 kg (SD, 10.3), resulting in a mean BMI of 25.3 kg/m² (SD, 3.2). The mean duration of symptom onset before diagnosis was 29.1 months (SD, 39) months. Only a few patients had hypertension (2.63%) or diabetes mellitus (3.95%). The majority of the patients had histopathological diagnoses of invasive ductal carcinoma (98.7%), predominantly grade 2 (52.0%) and grade 3 (41.3%) tumors. Stages II and III disease constituted the majority of patients. Among those with a positive hormone receptor status, ER receptor positivity was predominant (46%). Most patients received the chemo regimen AC, followed by docetaxel (68.4%). Patients who tested positive for ER or PR receptor (47.3%) received hormonal treatment with tamoxifen. The median age at menarche was 13.0 years. Before treatment, most patients had regular menstrual cycles (90.8%), and 97.4% were multiparous. Most of the patients (96.1%) breastfed their infants.

Post-chemotherapy, 64 patients (84.2%; 95% CI, 74.3–90.8) developed clinical amenorrhea;12 patients (15.8%; 95% CI, 9.2–25.7) did not develop clinical amenorrhea (Table 1). The mean age of patients was higher for patients who had clinical amenorrhea compared to those who did not have it: 42.1 (SD, 4.3) vs. 39.0 (SD, 4.1) years. Following chemotherapy, 60 patients (78.9%) experienced biochemical amenorrhea, while 16 (21.1%) did not (Supplementary Table 1).

Table 1 Baseline characteristics and incidence of clinical amenorrhea

Characteristic	[ALL] *N* = 76	Clinical Amenorrhea (YES) *N* = 64	Clinical Amenorrhea (NO) *N* = 12
Age, years	41.6 ± 4.4	42.1 ± 4.3	39.0 ± 4.1
Height, cm	154.8 ± 9.9	155.2 ± 10.3	152.4 ± 7.2
Weight, kg	61.0 ± 10.3	61.0 ± 10.2	60.9 ± 10.9
BMI[1], kg/m^2	25.5 ± 3.2	25.3 ± 3.3	26.1 ± 3.0
Duration of symptoms months	29.1 ± 39.0	31.3 ± 41.7	17.2 ± 15.7
Age at menarche	13.0 [13.0;14.0]	13.0 [13.0;14.0]	13.5 [13.0;14.0]
Regular cycles:			
regular cycles	69 (90.8%)	58 (90.6%)	11 (91.7%)
not	7 (9.21%)	6 (9.38%)	1 (8.33%)
Nulliparous:			
no	74 (97.4%)	63 (98.4%)	11 (91.7%)
nulliparous	2 (2.63%)	1 (1.56%)	1 (8.33%)
Breastfed:			
breastfed	73 (96.1%)	61 (95.3%)	12 (100%)
no	3 (3.95%)	3 (4.69%)	0 (0.00%)
Socioeconomic status:			
Low	42 (55.3%)	37 (57.8%)	5 (41.7%)
High	34 (44.7%)	27 (42.2%)	7 (58.3%)
Hypertension:			
Hypertensive	2 (2.63%)	2 (3.12%)	0 (0.00%)
not	74 (97.4%)	62 (96.9%)	12 (100%)
Diabetic:			
diabetic	3 (3.95%)	3 (4.69%)	0 (0.00%)
no	73 (96.1%)	61 (95.3%)	12 (100%)
T: Status			
T1	10 (13.2%)	8 (12.5%)	2 (16.7%)
T2	41 (53.9%)	35 (54.7%)	6 (50.0%)
T3	17 (22.4%)	13 (20.3%)	4 (33.3%)
T4	8 (10.5%)	8 (12.5%)	0 (0.00%)
N: Status			
N0	29 (38.2%)	22 (34.4%)	7 (58.3%)
N1	26 (34.2%)	22 (34.4%)	4 (33.3%)
N2	14 (18.4%)	13 (20.3%)	1 (8.33%)
N3	7 (9.21%)	7 (10.9%)	0 (0.00%)
M: M0	76 (100%)	64 (100%)	12 (100%)
stage:			
stage IA	5 (6.58%)	4 (6.25%)	1 (8.33%)
stage IIA	21 (27.6%)	16 (25.0%)	5 (41.7%)
stage IIB	18 (23.7%)	15 (23.4%)	3 (25.0%)
stage III A	19 (25.0%)	16 (25.0%)	3 (25.0%)
stage III B	6 (7.89%)	6 (9.38%)	0 (0.00%)
stage III C	7 (9.21%)	7 (10.9%)	0 (0.00%)
Histopathology:			
mixed	1 (1.32%)	1 (1.56%)	0 (0.00%)
pure	75 (98.7%)	63 (98.4%)	12 (100%)
Grade:			
grade 1	5 (6.67%)	5 (7.94%)	0 (0.00%)
2	39 (52.0%)	34 (54.0%)	5 (41.7%)
3	31 (41.3%)	24 (38.1%)	7 (58.3%)
ER[2]			
ER + ve	35 (46.1%)	31 (48.4%)	4 (33.3%)
-ve	41 (53.9%)	33 (51.6%)	8 (66.7%)

Table 1 (continued)

Characteristic	[ALL] *N* = 76	Clinical Amenorrhea (YES) *N* = 64	Clinical Amenorrhea (NO) *N* = 12
PR[3]			
PR + ve	22 (28.9%)	20 (31.2%)	2 (16.7%)
-ve	54 (71.1%)	44 (68.8%)	10 (83.3%)
Chemo regimen:			
AC[4] > docetaxel	52 (68.4%)	43 (67.2%)	9 (75.0%)
AC > paclitaxel	20 (26.3%)	18 (28.1%)	2 (16.7%)
TAC[5]	4 (5.26%)	3 (4.69%)	1 (8.33%)
Hormonal treatment:			
No	39 (52.7%)	32 (50.8%)	7 (63.6%)
Yes	35 (47.3%)	31 (49.2%)	4 (36.4%)
Tamoxifen	35 (100%)	31 (100%)	4 (100%)

[1]Body Mass Index

[2]Estrogen Receptor

[3]Progesterone Receptor

[4]Doxorubicin, Cyclophosphamide

[5]Docetaxel, Doxorubicin, Cyclophosphamide

Hormonal values, including serum estradiol and FSH levels, showed significant alterations as this study progressed. In the beginning, serum estradiol was 138.76 pg/mL (SD, 116.97); by 24 months, it had decreased to 17.65 pg/mL (SD, 35.15). At the start of the study, serum FSH was 14.62 mIU/mL (SD, 13.73), which increased to 51.92 mIU/mL (SD, 17.47) by the end.

The median time to clinical amenorrhea was 8 months (95% CI, 7.83–8.17 months) (Fig. 1). The median time to biochemical amenorrhea was 18 months (95% CI, 17.90–18.10) (Fig. 2). Of the 76 patients, 55 patients (72.4%; 95% CI, 61.4–81.2) had both clinical and biochemical amenorrhea; 9 patients (11.8%; 95% CI, 6.2–21.5) had clinical amenorrhea without biochemical amenorrhea. Only five patients (6.6%; 95% CI, 2.9–14.3) had biochemical amenorrhea without clinical amenorrhea, and seven patients (9.2%; 95% CI, 4.5–17.9) had neither clinical nor biochemical amenorrhea. There was a significant association between clinical and biochemical amenorrhea ($P = .0022$), with a calculated effect size (Cramér's V) of 0.352, indicating a moderate strength of association. The mean age of patients with clinical amenorrhea was 42.1 years, compared to 39 years for those without clinical amenorrhea; the difference was not statistically significant ($P = .599$). The mean age of patients with and without biochemical amenorrhea was 44.5 years vs. 42.8 years, respectively, which was not significantly different ($P = .227$). The impact of age, BMI, chemotherapy regimen, hormonal treatment, and biochemical amenorrhea on the time to clinical amenorrhea, when analyzed using Cox proportional hazards regression, did not show any statistically significant association. A summary of the results is presented in Table 2. The univariate Cox hazard regression analysis for the effect of individual predictors is given in (Supplementary Table 2).

Discussion

This study aimed to estimate the prevalence of chemotherapy-related amenorrhea and its associated factors in premenopausal patients with breast cancer undergoing chemotherapy. In this study, the incidence of chemotherapy-related clinical amenorrhea, as estimated by the menstrual status, was 84.2%. The reported incidence of chemotherapy-related amenorrhea varies widely from 21 to 98% [21, 22]. There is wide variation in the incidence of chemotherapy-related amenorrhea, which could be due to the different criteria defined. In the definition of chemotherapy-related amenorrhea, the time required for the absence of menstruation varies widely, from 3 months to more than two years [21]. In this study, we defined cessation of menses for three months to be labeled as clinical amenorrhea, as this is a commonly used criterion. As women experience cessation of menses at different time points following chemotherapy, the variation in time requirement across studies could have influenced the reported incidence of amenorrhea and limited comparability. The high incidence of chemotherapy-related amenorrhea in this study aligns with that reported in the existing literature [23]. In addition, chemotherapy can suppress ovarian function in patients with breast cancer, and serum estradiol and FSH levels were used to determine whether the patients were in the postmenopausal range [24]. Hormonal levels change rapidly following the administration of chemotherapy, gradually approaching postmenopausal values owing to damage to the ovaries and interruption in the hypothalamic-pituitary axis feedback mechanism [25]. In this study, the serum estrogen and FSH levels were initially in the premenopausal range. After chemotherapy and during the follow-up period, they reached postmenopausal values, confirming the findings of another study that evaluated hormonal

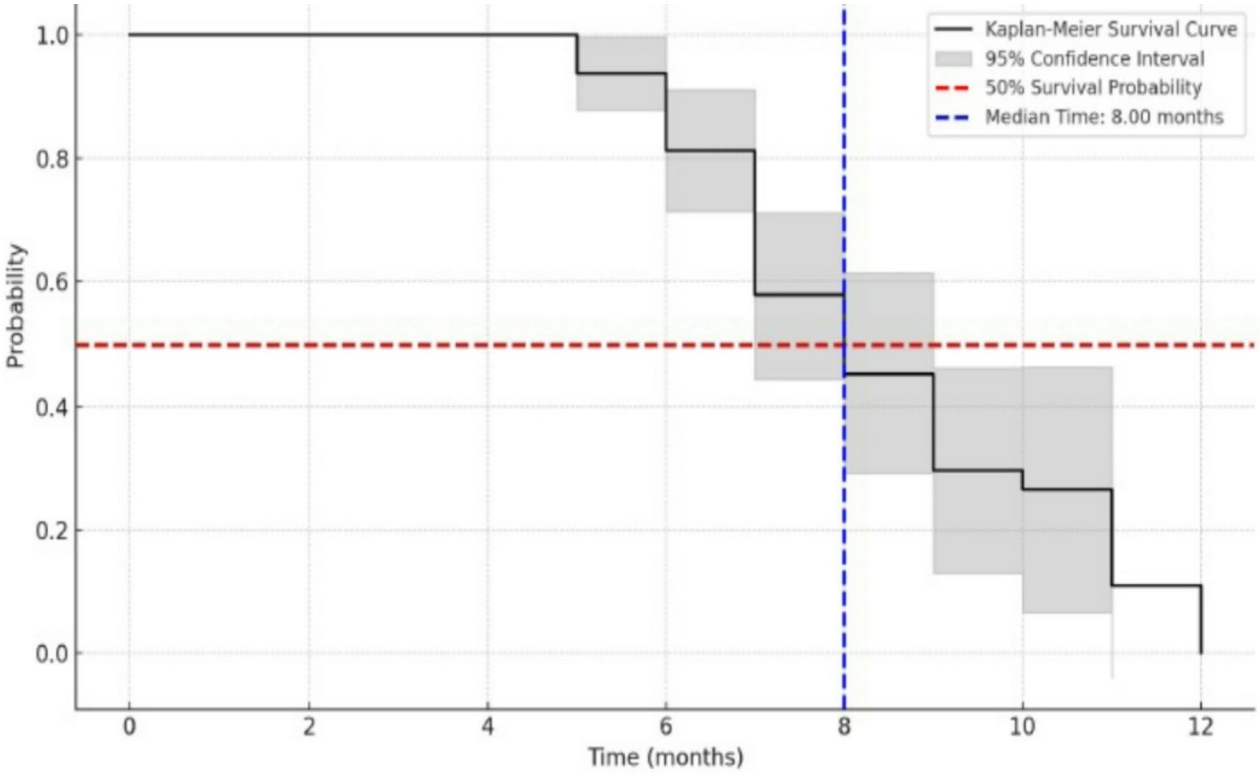

Fig. 1 Kaplan-Meier survival estimates showing the time to clinical amenorrhea in premenopausal breast cancer patients following chemotherapy. The median time to clinical amenorrhea was 8 months, which indicates that half of patients experience cessation of menstruation within this time. The chance of not attaining clinical amenorrhea decreased as time passed; most patients had clinical amenorrhea with the progression of chemotherapy cycles. The shaded area in the curve is the 95% confidence interval, which is the range of variability in survival estimates. These findings highlight that early onset clinically reported amenorrhea may serve as an initial indicator of chemotherapy-induced ovarian suppression

function in patients with breast cancer treated with chemotherapy [26]. A higher percentage of patients (78.9%) developed biochemical amenorrhea in this study, which is similar to the findings of other studies [25, 26].

Patient age is an important indicator of the risk of developing amenorrhea after chemotherapy [24]. Studies comparing different age ranges of patients have shown that the chance of developing chemotherapy-related amenorrhea increases with age [27, 28]. In this study, we included premenopausal patients with a mean age of 41.6 years, similar to other studies that evaluated the incidence of chemotherapy-related amenorrhea [23, 29]. The mean age of the patients with clinical amenorrhea was higher than that of the patients without amenorrhea. However, the lack of statistical significance indicates that other factors, such as body mass index, hormonal variations, chemotherapy drugs, and hormonal drugs, can influence the onset of amenorrhea. The duration of the onset of the clinical symptoms of breast cancer before diagnosis was high in this study, which means that there was a delay in seeking medical advice. Such challenges are common in this region, may have an impact on treatment outcomes, and can only be prevented by increasing awareness among young patients with breast cancer. Histopathology analysis showed that the majority of the patients were stage II and III, with the vast majority of the tumors being high-grade, indicating that this study population consisted mainly of patients with locally advanced breast cancer, which is the most common presentation of breast cancer in this region. Nearly half of the patients were ER or PR positive and received adjuvant tamoxifen, which could also have contributed to the high incidence of chemotherapy-related amenorrhea observed in this study. Similarly, several studies have shown that the incidence of amenorrhea is high when tamoxifen is added as adjuvant hormonal therapy in patients with breast cancer [19, 30].

In this study, the median time to achieve clinical amenorrhea appears to be shorter than that required to achieve biochemical amenorrhea, indicating that chemotherapy has a strong influence on clinical amenorrhea. The difference in assessment frequency of clinical and biochemical amenorrhea assessment may have contributed to the observed difference in timing. The discrepancy in assessment intervals was due to practical challenges in frequent biochemical testing. Another reason could be that, in the absence of ovarian function, chemotherapy drugs do not have much influence on

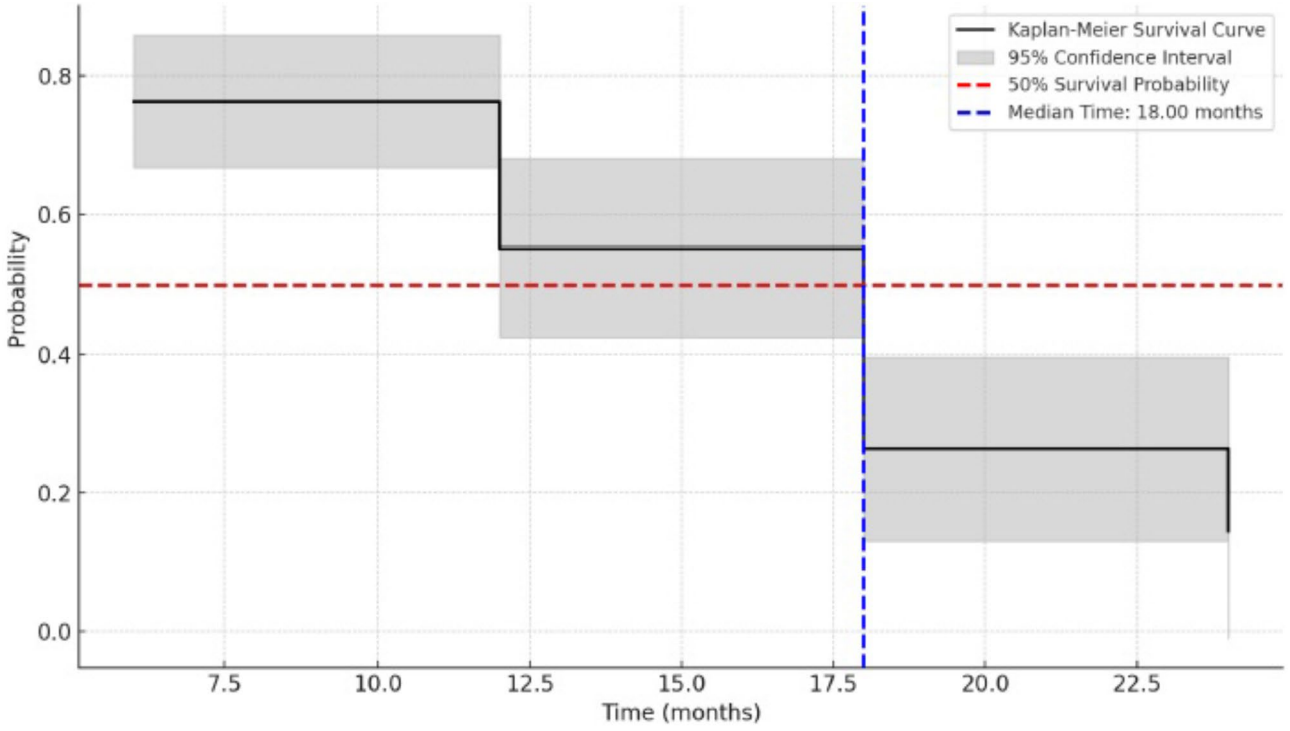

Fig. 2 Kaplan-Meier survival estimates showing the time to biochemical amenorrhea in premenopausal breast cancer patients following chemotherapy. The median time to biochemical amenorrhea was 18 months, which indicates that half of the patients had hormonal changes suggestive of ovarian insufficiency within this period. The extended median duration for biochemical amenorrhea presumably indicates the delayed hormonal response in relation to clinical manifestations, possibly affected by varying evaluation intervals and the intrinsic hormonal fluctuations. This delayed onset, in comparison to clinical amenorrhea (8 months), implies that cessation of menstruation alone may not be a reliable indicator of ovarian suppression; continuous monitoring of hormonal status is necessary in these patients. The shaded area is the 95% confidence interval, which is the uncertainty in biochemical amenorrhea estimates. These findings highlight the need for combining biochemical assessments along with clinical evaluation for precisely assessing ovarian function following chemotherapy

Table 2 Cox proportional hazards regression analysis for time to clinical amenorrhea

Predictor	Coefficient (log HR)	Standard Error	Hazard Ratio (HR)	*P* value	95% CI for HR
Age	-0.012	0.0414	0.9881	0.7725	[0.9111, 1.0716]
[z,2] Body Mass Index BMI[z,2]	0.0317	0.0521	1.0322	0.5432	[0.9320, 1.1431]
Chemotherapy Regimen	-0.2568	0.2216	0.7736	0.2467	[0.5010, 1.1944]
Hormonal Treatment	-0.0787	0.2681	0.9244	0.7692	[0.5466, 1.5632]
Biochemical Amenorrhoea	-0.0808	0.3828	0.9224	0.8329	[0.4356, 1.9533]

Note: None of the predictors, notably age, BMI, chemotherapy regimen, hormonal treatment, and biochemical amenorrhea, demonstrated statistically significant associations with the risk of clinical amenorrhea (all $P > .05$). Hazard ratios (HR) near 1 imply minimal effect sizes, with 95% confidence intervals including 1, highlighting the lack of significance. For the chemotherapy regimen, the reference group is Regimen A (e.g., anthracycline-based regimen). For hormone treatment, the reference group is "no hormonal treatment."

hormonal levels, as seen in another study [25]. Chemotherapy-related amenorrhea, as measured by clinical status alone, may overestimate the incidence of amenorrhea when used as the sole method for estimating amenorrhea status [24]. The difference in the onset of amenorrhea could also be due to the different mechanisms by which cytotoxic drugs affect the ovaries, with clinical amenorrhea manifesting early and biochemical changes that reflect the ovarian reserve appearing later [8]. The early onset of clinical amenorrhea compared to biochemical amenorrhea underscores the need of continued monitoring of these patients to reliably estimate the amenorrhea status following clinical amenorrhea. This type of temporary cessation of menstruation has clinical implications including the decisions on fertility preservation approaches and selection of hormonal treatment following chemotherapy. In this study, there was a significant association between chemotherapy and biochemical amenorrhea, indicating that patients who developed clinical amenorrhea were more likely to develop biochemical amenorrhea. The majority of patients with clinical amenorrhea also developed biochemical amenorrhea, with only a few patients without clinical amenorrhea having

biochemical amenorrhea, indicating that the association between these two is strong. It is possible to predict biochemical amenorrhea by estimating serum estradiol and FSH levels, as estimated in a previous study [25]. These hormonal responses to cytotoxic drugs may indicate early and sustained amenorrhea in patients with breast cancer receiving chemotherapy. The strong association between clinical and biochemical amenorrhea and early onset of clinical amenorrhea indicates that cessation of menses during chemotherapy is a reliable early indicator of amenorrhea. Simultaneously, hormonal levels provide additional clinical information for continuous monitoring of patients with breast cancer treated with chemotherapy.

Several factors influence chemotherapy-related amenorrhea, including age, BMI, chemotherapy regimen, and hormonal treatment. In a previous study that analyzed the risk factors for chemotherapy-associated amenorrhea, only age was found to be a significant risk factor [31]. In contrast, this study did not find a significant difference in the mean age of the patients with or without amenorrhea. The reason for the insignificance of age in this study could be the higher mean age of the patients in the study population and the fact that the majority of patients achieved clinical amenorrhea. As age advances beyond 40 years, the chance of cessation of menses increases following chemotherapy [12]. Another significant factor affecting menstrual status is the type of chemotherapy administered to patients with breast cancer. It has been shown that the addition of taxanes to the chemotherapy regimen increases the risk of amenorrhea compared with anthracyclines alone [32]. Anthracyclines alone, without taxanes, can have a significant influence on clinical amenorrhea [33]. This study included both anthracyclines and taxanes in the chemotherapy regimens; however, different chemotherapy regimens did not influence the onset of clinical amenorrhea. The insignificant results with different chemotherapy regimens could be due to the minor differences between these regimens. The effect of BMI on chemotherapy-associated amenorrhea varies. In a study of young patients with breast cancer, lower BMI was found to be a predictor of treatment-related amenorrhea [34]. In another study, the relationship between BMI and chemotherapy-associated amenorrhea was not nonsignificant [35]. This study showed a slight increase in clinical amenorrhea with high BMI, although there was no significant association with clinical amenorrhea, similar to another study that did not confirm an association between chemotherapy-related amenorrhea and BMI [36]. However, the effects of tamoxifen on chemotherapy-related amenorrhea remain controversial. Studies have reported that the use of tamoxifen after chemotherapy increases the incidence of chemotherapy-related amenorrhea [27, 37]. Other studies have shown that tamoxifen decreases chemotherapy-induced menopause or leads to early resumption of menstruation [12, 38]. In this study, there was a 7.56% reduction in the risk of clinical amenorrhea with the use of tamoxifen; however, the difference was not significant. The observed reduction in risk with tamoxifen may be due to its complex endocrine actions, including the control of estrogen levels and feedback mechanisms. Tamoxifen has a dual mechanism of action, predominantly acting as an antiestrogen in the breast tissue, while exhibiting partial agonist activity in tissues such as the ovaries. This could stimulate the ovaries, resulting in follicular growth and sustaining lower estrogen levels, reducing the probability of amenorrhea in some patients. Tamoxifen also interferes with the hypothalomo-pituitary axis, leading to altered gonadotropin secretion, impacting ovarian function. Nonetheless, individual variability in age, ovarian reserve, and the type of chemotherapy drugs used could affect this outcome. The effect of biochemical amenorrhea on the time to clinical amenorrhea indicates a slight reduction in the risk of clinical amenorrhea. In addition, it did not have a significant influence on this study, indicating that hormonal levels alone may be an insufficient predictor of clinical amenorrhea. Although low estrogen and high FSH levels indicate postmenopausal status, tamoxifen increases estrogen levels and decreases FSH levels [39]. Also, the threshold level chosen for hormonal assessment could vary across the population and within patients receiving various chemotherapy regimens. The results of this study may also have been influenced by the use of tamoxifen, leading to uncertainty in accurately predicting amenorrhea using hormonal levels alone.

The strengths of this study are its single-institution and prospective nature, which allowed for continuous evaluation and eliminated the variability in clinical and hormonal assessments. This study is one of the few to assess amenorrhea status using both clinical and hormonal assessments following chemotherapy in patients with breast cancer. We studied multiple risk factors known to influence amenorrhea in breast cancer patients undergoing chemotherapy. We mainly focused on clinical amenorrhea but additionally performed a biochemical estimation of hormones to add another layer of evidence to estimate the association between these two factors. Our results have broad applicability in oncology practice and the reproductive health of patients with breast cancer. We recommend that clinicians use dual clinical and biochemical assessments to monitor ovarian function for individualized fertility preservation strategies.

The main limitation of this study was the small sample size, which made it difficult to find any association between some predictors of amenorrhea (e.g., BMI, age, hormonal treatment) and the time to clinical amenorrhea. As this was a single-center study, the generalizability of this study to a larger population may be limited.

This study used heterogeneous chemotherapy regimens, and individual drugs varied among individuals, which could have led to variations in ovarian suppression and resulting amenorrhea. Stratified analysis examining age, BMI, and chemotherapy agents could have yielded a better understanding of the independent effects of these variables; however, the limited sample size prevented such analysis. Previous research shows that substantial subgroup analyses require larger cohorts to identify significant differences [28, 34]. Confounding factors, such as cumulative doses of chemotherapy drugs and genetic (BRCA mutations), autoimmune diseases that predispose women to early menopause, were not considered. As a short-term study with resource constraints and primary focus on clinical amenorrhea, we could not estimate ovarian reserve biomarkers, e.g., Anti-Mullerian Hormone (AMH), for amenorrhea that sets late or amenorrhea that recovers late. There was a risk of recall bias in this study because the outcome of clinical amenorrhea was based on patient-reported menstrual status and also the influence on tamoxifen on amenorrhea was not fully disentangled. A potential bias may arise due to a discrepancy in the assessment intervals for clinical (assessment every month) and biochemical (assessment every six months) amenorrhea, as clinical amenorrhea may be picked up sooner due to more frequent assessments being undertaken. Variations in menstrual cycle phases may have affected hormone levels because hormonal assessments were scheduled at standardized intervals to minimize variability. The absence of statistically significant associations with all available predictors may be due to insufficient statistical power to detect small to moderate associations, which should be addressed in future studies using larger sample sizes. Future studies with objective tracking of menstrual status could consider stratified analyses and include monthly biochemical assessments, specific chemotherapy regimens, genetic predispositions, such as BRCA mutations, ovarian reserve markers like AMH, and the use of tamoxifen.

Conclusion

In conclusion, the incidence of chemotherapy-related clinical and biochemical amenorrhea in patients with breast cancer is high. There is an association between clinical and biochemical amenorrhea. Hormonal assessments remain the gold standard for evaluating ovarian function. The detection of clinical amenorrhea before biochemical amenorrhea indicates that clinical amenorrhea serves as an early indicator of biochemical amenorrhea. It is important to monitor the menstrual status and hormonal levels at fixed intervals to accurately detect amenorrhea in patients with breast cancer treated with chemotherapy, but it needs further validation through larger multicenter studies for personalized management. Dual clinical and biochemical assessment will provide early insights into the timing of amenorrhea, ovarian function, and predictors. Due to the ease of the evaluation, clinical amenorrhea could serve as an early tool to estimate amenorrhea. In contrast, biochemical methods could be used for confirmation of ovarian function, where decisions on fertility preservation and endocrine therapy are required. The limited sample size and single-center design of this study are constraints, necessitating multi-center studies with larger populations with long-term follow-up to determine predictive factors for chemotherapy-related amenorrhea.

Abbreviations

HDI	Human Development Index
ASCO	American Society of Clinical Oncology
FSH	Follicle-stimulating Hormone
BMI	Body Mass Index
ECOG	Eastern Cooperative Oncology Group
ER	Estrogen Receptor
PR	Progesterone Receptor
AC	Adriamycin and Cyclophosphamide
TAC	Docetaxel, Doxorubicin, Cyclophosphamide
SD	Standard Deviation
CI	Confidence Interval
HR	Hazard ratio
AMH	Anti-Mullerian Hormone

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Not applicable.

Author contributions

CR developed the study concept and was involved in the study design. CR and SSM acquired the data. IY and SSM statistically analyzed the data. CR and IY interpreted data. CR drafted the main manuscript. IY prepared Figs. 1 and 2. SSM and IY made essential revisions to the intellectual content of the draft. CR, SSM, and IY reviewed and approved the final draft.

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Data availability

The datasets used and/or analysed during the current study are included in the manuscript and supplementary file. Further access to data will be given by the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

This prospective study was conducted after approval from the institutional Human Ethics Committee of Government Medical College Thiruvananthapuram. Written informed consent was obtained from all participants of this study. All the methods followed in this study are in accordance with the declaration of Helsinki. The authors are responsible for all aspects of the work in ensuring that questions related to the accuracy or integrity of any aspects of the work are appropriately investigated and resolved.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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