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Menstrual and Hormonal Changes in Breast Cancer Patients Treated By Adjuvant, Adriamycin and Cyclophosphamide Chemotherapy

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Abstract

Background and objectives: The long term effects of adjuvant chemotherapy are very important in patients with breast cancer. Cytotoxic chemotherapy may induce changes in menstrual cycle to variable extents and even may induce amenorrhea. This study was designed to find out the effect of adjuvant chemotherapy, Doxorubicin and Cyclophosphamide, on ovarian function in patients with early stages breast cancer. **Methods:** Thirty premenopausal women with newly diagnosed early breast cancer enrolled in this study. In addition to a proper menstruation history, the following parameters FSH, LH, Estrogen, Progesterone, were estimated before and after 4 cycles of chemotherapy with another follow up for menstrual history after 6 months, in each patient. **Results:** After 4 cycles of adjuvant Doxorubicin and Cyclophosphamide chemotherapy the mean level of FSH and LH was increased and Estrogen level was decreased, significantly. While, Progesterone level was decreased non-significantly. Menstrual changes detected in most patients after receiving chemotherapy but only %47 of them developed amenorrhea which was persistent after 6 months in %33 of the patients. The induced amenorrhea has found to be related positively with the age of the patient but has no any relation with receptor status, estrogen, progesteron and Her2 (human epidermal growth factor receptor 2), of the tumor and neither with the menstrual phase at which chemotherapy has been given nor with the body mass index. **Conclusions:** Adjuvant chemotherapy changes ovarian hormonal levels significantly and induces transient and permanent amenorrhea in patients with early stages breast cancer.

Keywords: Doxorubicin and Cyclophosphamide, Hormonal changes, Amenorrhea.

Introduction

According to the National Comprehensive Cancer Network (NCCN) guide line, breast cancer can be treated by one or more of the following steps: surgery, radiation therapy, chemotherapy (CT), hormone therapy, and monoclonal antibody therapy. Several large studies have shown, conclusively, that adjuvant CT improves survival, especially in premenopausal women [1-5]. One of the beneficial adverse effect of chemotherapy for premenopausal women is the induction of amenorrhea [6-7]. Chemotherapy induced amenorrhea (CIA) is the term used to describe the cessation of menses for several months during or soon after the use of chemotherapy due to ovarian atrophy and loss of primordial follicle which leads to ovarian failure, however, these effects is not an “all or nothing phenomenon” [7-8]. A review of the literature reveals inconsistencies regarding the definition of chemotherapy-related amenorrhea, with some authors defining it as a cessation of menses lasting ≥ 3–6 months and others defining it as a cessation lasting 12 months [8]. The impact of various definitions is illustrated by Padmanabhan et al, 1986, who reported the incidence of amenorrhea from the beginning of CMF (Cyclophosphamide, Methotrexate and 5-Fluorouracil) chemotherapy at 3 months, 6 months, and 12 months later as 50%, 70%, and 80%, respectively [9].

It is well known that evaluation of ovarian function can be assessed by different method like menstrual history taking and laboratory measurements. So, history of menstrual bleeding is a convenient method of assessment in determining ovarian function. However, menstrual status cannot thoroughly reflect actual ovarian function. The serial measurement of serum ovarian biochemical markers is an acceptable method to reflect ovarian function [10-11]. Previous studies preferred FSH as a biochemical marker to predict ovarian reserve, but currently Other than FSH, anti-Müllerian

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hormone (AMH) and inhibin B are used as predictive capacity of ovarian function, they appear to be superior to FSH because they are not affected by the administration of tamoxifen. Moreover, AMH and inhibin B reflect subtle changes in menstrual transition, which has been compared with FSH. AMH is also strongly recommended for its sensitivity in predicting ovarian function and stable expression over the menstrual cycle [12-16].

The incidence of CIA differs with respect to patient age and chemotherapeutic regimens used 13-14. For this reason the incidence of CIA has varied across studies from 27% to 94% in patients treated with older cyclophosphamide based or doxorubicin-based regimens. The CIA can be regarded as an important clinical sequences of adjuvant chemotherapy because it produces menopausal symptoms like hot flushing, night sweats, sleep disturbance, palpitation, depression, agitation and vaginal atrophy, also might cause cardiovascular morbidity, early bone mineral loss and fertility impairment [12-13,17]. The objectives of the study were to evaluate hormonal changes after adjuvant chemotherapy (AC), find out the incidence of menstrual pattern changes in early stage breast cancer patients treated with AC and evaluate the effect of the molecular characters of the tumor on CIA.

Patients and methods

This prospective study; was carried at Rizgary Teaching Hospital, from January 2014 to February 2015, after obtaining scientific committee approval from college of medicine. Thirty newly operated breast cancer women aged (23-50 years old), who had indication for adjuvant chemotherapy, were enrolled in this study after obtaining their consent. All of these patients were planned to receive an adjuvant Cyclophosphamide and Doxorubicine (AC) chemotherapy protocol for 4 cycles. The criteria of inclusions were recent breast cancer, pre-menopause, no history of previous chemotherapy, hormonal therapy, oral contraceptive. Postmenopausal women, recurrence breast cancer, patients with gonadotropin-releasing hormone (GnRH) agonist administration or hysterectomy/bilateral oophorectomy histories, and history of chemotherapy for other malignancy were excluded. Before starting first cycle of adjuvant chemotherapy AC and after finishing four cycles, peripheral venous blood was drawn from each patient, exactly at the day after her menstruation (if she was menstruating). The blood has been centrifuged at 2500 rpm for 10 min, the sera were divided into two aliquot and kept in tubes without anticoagulant. One tube used for hormonal assay including serum: (FSH, LH, Progesterone, Estradiole), and other tube used for biochemical assays including: (liver function test, renal function test and lipid profile). The obtained results were evaluated and comparison was done between pre and post chemotherapy values. Another follow up was carried out after 6 months to evaluate the menstrual pattern of the patients.

All results were expressed as mean ± SE. the data were analyzed by using student t test and chi square. Software SPSS program version 20 was used. P values ≤ 0.05 were considered statistically significance.

Results

In this study, giving 4 cycles of adjuvant AC to patients with early stages breast cancer significantly increased both FSH and LH levels and decreased the level of estrogen, while did not affect the level of progesterone as shown in Table 1.

Table (1): Mean FSH, LH, Estrogen and Progesterone in pre- chemotherapy (basal value) and after administration of four cycles of AC.

Parameter	Before chemotherapy	After chemotherapy
(Mean FSH (mIU/ml	2.982 ± 11.295	*6.28 ± 42.61
(Mean LH (mIU/ml	2.27 ± 12.04	*3.93 ± 31.99
(Estrogen (pg/ml	11.20 ± 77.37	*9.779 ± 32.56
(Progesterone (ng/ml	0.557 ± 1.978	0.852 ± 1.136

(Note: * is sign of significance, P values < 0.05).

Among a total of 30 patients enrolled in this study, 14 cases developed CIA and 16 cases were menstruating normally, after finishing four cycles AC. However, six months later we found that 4 patients (28.57%) among CIA resumed their menstruation (temporary CIA) and only 10 patients (71.42%) stayed with definite CIA. While between 16 patients with no CIA, 7 patients (43.75%) stayed with normal menstruation and 9 patients (56.25%) were suffering from oligomenorrhea Table 2.

Table (2): Number and percent of patients with different pattern of menstruation. Number and percent of patients with different pattern of menstruation.

CIA 14 patients		No CIA 16 patients	
Definite CIA	**Temporary CIA**	**Normal menstruation**	**Oligomenorrhea**
10 (71.42%)	4 (28.57%)	7 (43.75%)	9 (56.25%)
33.3%	13.33%	23.33%	30%

The mean FSH, LH and progesteron levels in 14 patients with CIA were significantly greater than the mean FSH and LH of 16 patients without CIA. While the mean Estrogen level in patients with CIA was significantly less than the mean Estrogen level of menstruating patients, as shown in Table 3.

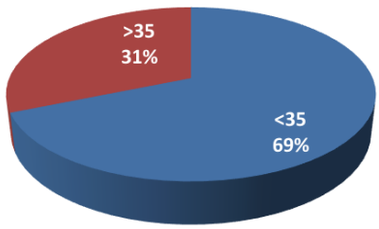
Table (3): Mean FSH, LH, Estrogen and Progesterone level in (CIA) patients and patients with no (CIA) after administration of four cycles of adjuvant AC chemotherapy.

Parameters	CIA patients	Patients with no CIA
(Mean FSH(mIU/ml	63.01±9.96	*24.75±4.71
(Mean LH(mIU/ml	42.73±6.32	*22.6±3.62
(Mean Estrogen(pg/ml	10.81±4.83	*51.6±16.65
(Mean progesterone(ng/ml	2.07±1.82	*0.31±0.48

(Note: * is sign of significance, P values < 0.05).

In this study, the relation between age and CIA has been studied and statistical analysis showed that the age was affecting the induction of amenorrhea significantly (p value 0.04). Among a total of 14 patients with CIA only 2 (14.3%) patients were younger than 35 years old, while 12 (85.7%) patients were older than 35 years. In contrast, among 16 patients with no CIA 11 (68.7%) patients were younger than 35 and only 5 (31.3%) of the cases were more than 35 years old, Figure (1).

A



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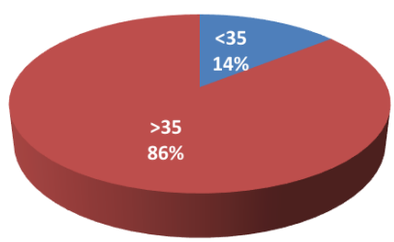

Figure (1): Percent of patients with (A) and without (B) CIA within each age group after four cycles of chemotherapy

In CIA patients, chemotherapy has been given during the follicular phase of the menstruation in eight (57.1%) patients and during luteal phase in six (42.9%) of them. However, in those 16 patients who maintained their menstruation, AC has been adminis-

tered during follicular face in six (37.5%) and during luteal phase in ten (62.5%), patients. No significant association was found between CIA and menstrual phase at which chemotherapy been given, Table 4.

Table (4): Number and percent of patients developed (CIA) related to the phases of menstruation at which chemotherapy has been administered.

Menstrual state	Follicular phase	Luteal phase
CIA	(57.1%) 8	(42.9%) 6
No CIA	(37.5%) 6	(62.5%) 10

In this study we assessed the association between CIA and hormonal receptors and Her2 receptor status. The statistical analysis showed that there is no significant association between amenorrhea induced by chemotherapy and receptor status (data not shown-negative results)

Other factor which has been studied in this work was the effect of BMI of the patient with the possibility of developing CIA. Our findings show that BMI has no statistically significant effect on CIA. We have found that in 14 patients with CIA the mean BMI was (30.13 ± 1.83), 4 patients (28.6%) were within normal range BMI and 10 patients (71.4%) had abnormal BMI (over normal range). Almost the same results have been found in other 16 patients with no CIA, the mean BMI was (30.07 ± 1.49), 3 (18.8%) of them were within normal range (BMI) and 13 (81.3%) had high BMI (data not shown-negative results).

Discussion

Of more than 230,000 new diagnoses breast carcinoma in the US each year, 25% occur before menopause, and 15% of women are diagnosed in the reproductive age group (age 45 yrs. or younger)[18]. It has been found that adjuvant chemotherapy prolongs disease-free and overall survival for patients with breast carcinoma [3-4] but also can induce long-term side effects, such as suppression of ovarian function with subsequent premature menopause. This results in loss of childbearing potential and prolonged exposure to the risks of menopause and psychological distress [19-21].

It is well known that chemotherapy decreases ovarian reserve rapidly and dramatically, although, ovarian function may recover to some extent and menses may return after the completion of chemotherapy treatment [7]. In this study, we assessed ovarian function deterioration in response to adjuvant AC by taking menstrual history and measuring serum biomarkers of ovarian function like FSH, LH Estrogen and Progesterone levels, before and after completion of chemotherapy. We have found that adjuvant AC causes CIA in a good percentage of the patients particularly those above 35 years old. In accordance to Peterek J et al, 2006, the diagnosis of CIA was applied to those patients who developed cessation of menstruation for 3 consequent months after receiving chemotherapy AC [13]. As well as, we found that AC significantly increased the levels of FSH and LH, while Estrogen level decreased significantly, the mean level of above hormones except progesterone reached to menopausal range after giving four cycles of AC. These results are in agreement with other studies, showing that adjuvant chemotherapy induces CIA [22-26]. Furthermore, as in Park et al [15], in current study other follow up done to know if there was recovery from CIA and resumption of menstruation, therefore the history of menstruation without estimation of hormones has been used after 12 months of starting chemotherapy. We have found that between the 14 CIA patients 4 of them resumed their menstruation and the remained 10 were still in amenorrhea, while regarding patients with no CIA 7 patients were still has normal menses and 9 patients have developed oligomenorrhea during follow up time. Many studies used menstrual calendar to evaluate ovarian function [5, 8, 22-24].

Reproductive aging in women is a natural progression through 3 stages: reproduction, the menopausal transition and post menopause. The early stage of menopausal transition begins on around age 42 and late stage which begins on average around age 46 and ends on average at age 51 with the final menstrual period [25-26]. In sight of the above physiology of menstruation pattern and age of women, in this study association of age and CIA has been studied. In agreement with other studies [22, 27-30], we have found that the age of the patient at the time of diagnosis was significantly associated with the occurrence of CIA. The significant increase in incidence of amenorrhea seen in older women more than 35 yrs. treated with chemotherapy, this may be due to the relatively low-

er number of existing oocytes. Approximately 2 million oocytes are present at birth; they have decreased to 200,000 by puberty and to 400 at menopause [25-26], following treatment with chemotherapy, the ovaries have a decreased number of oocytes available for follicular recruitment, along with evidence of fibrosis, these changes are similar to those observed in natural postmenopausal ovaries [10, 31].

In accordance with our study, other researchers have found that different types of chemotherapy are associated with different rate of menopausal incidence. Bines et al (1996) reported that 40% of women younger than age 40 years and 76% of women older than age 40 years become menopausal during adjuvant chemotherapy with cyclophosphamide plus methotrexate and 5-fluorouracil.Other authors reported a lower risk with anthracycline-containing regimes, such as doxorubicin and cyclophosphamide (AC) or 5-flurouracil, epirubicin, and cyclophosphamide combinations [31]. This difference in the incidence rate of CIA may be related to that, in addition to the age of the patient, cumulative dose and type of the cytotoxic agents are the most important factors that determine the likelihood of gonadal failure [28, 32]. It is not clear if the duration and dose intensity of chemotherapy independently affect the risk for gonadal failure. Alkylating agents, such as cyclophosphamide, are extremely gonadotoxic because they are not cell cycle-specific and can damage resting primordial follicles, whereas cycle-specific agents such as methotrexate and 5-fluorouracil do not have any effect on ovarian reserve. In a mouse study, cyclophosphamide induced follicular damage in a dose-dependent manner through a dose range of 20–100 mg/kg, whereas destruction of primordial follicles occurred even at the lowest cyclophosphamide dose [10]. With each additional dose of cyclophosphamide administration, an incremental number of primordial follicles are lost and the incidence of ovarian failure increases. Patients who receive cyclophosphamide have a 4- to 9.3-fold greater risk for the development of premature ovarian failure than healthy controls [10, 31-33]. In this study, AC typically consists of 4 cycles delivered over 12 weeks with a lower cumulative dose of cyclophosphamide compared with CMF and less anthracycline exposure than many other typical combination regimens.

The relation of CIA and phases of menstruation during which chemotherapy has been given was studied by Di Cosimo et al, 2004, they have found that the incidence of CIA higher between the patients received first cycle CT during follicular phase of menstrual cycle, because chemotherapy administered within the follicular phase could be responsible for the follicular maturation impairment, primordial follicle depletion and amplification of physiological apoptotic mechanisms occurring in this phase [34]. However, in the current study, we did not find any significant association between incidence of CIA and phase of menstruation at which first cycle of chemotherapy has been administered. This opposite result might be due to small sample size of the current study and or might be due to patient's uncertainty of their menstrual cycle dates. Although, the effect of molecular characters of the tumor on the CIA has not been studied widely, in this work we tried to find out relation between hormonal status (ER and PR) and Her2 with CIA. However, we did not find any positive effect of these molecular features of the tumor on the incidence of CIA. These results are in agreement with those found by Perez-Fidalgo et al, 2010, [35]. Furthermore, in this study, the association between number of cycles of CT at which patients developed amenorrhea and persistence of amenorrhea, although it has been noted that the majority of the patients with permanent amenorrhea developed cessation of menstruation during first two cycles of chemotherapy but the result was not statistically significant (data not shown). Unfortunately there is no enough study about this relation in the literature and this issue needs more study with a larger sample size to be clarified.

Conclusions

Through this study, it has been found that adjuvant chemotherapy, adriamycin and cyclophosphamide, in early breast cancer induces menstrual changes and even permanent amenorrhea in some patients associated with the ovarian hormonal changes. However, these chemotherapies did not cause any significant biochemical changes. This study delights the way for further investigation, with a bigger sample size and for a longer period of follow up, to find out the prognostic effects of CIA on the overall survival and progression free survival.

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