Gonadotropin-releasing hormone agonists for prevention of chemotherapy-induced ovarian damage: prospective randomized study

Ahmed Badawy, M.D., Aboubakr Elnashar, M.D., Mohamed El-Ashry, M.D., and May Shahat, M.D.

Department of Obstetrics & Gynecology, Mansura University, Mansoura, Egypt; and Department of Radiotherapy & Nuclear Medicine, Benha University Hospital, Banha, Egypt

Objective: To determine whether GnRHa administration before and during combination chemotherapy for breast cancer could preserve posttreatment ovarian function in young women or not.

Design: Prospective randomized controlled study.

Setting: Department of Obstetrics and Gynecology, Mansura University Hospital, Mansura, Egypt.

Patient(s): Eighty patients with unilateral adenocarcinoma of the breast and with no metastasis who had undergone modified radical mastectomy or breast-conserving surgery plus full axillary lymph node dissection were included in the study. Patients were assigned randomly to receive combined GnRHa and chemotherapy or chemotherapy alone. One woman in each group dropped out.

Main Outcome Measure(s): Return of spontaneous menstruation and ovulation. Hormonal changes (FSH, LH, E2, P) during and after the course of treatment.

Result(s): In the study group, 89.6% resumed menses and 69.2% resumed spontaneous ovulation within 3–8 months of termination of the GnRHa/chemotherapy cotreatment; 11.4% experienced hypergonadotrophic amenorrhea and ovarian failure 8 months after treatment. In the control group (chemotherapy without GnRHa), 33.3% resumed menses and 25.6% resumed normal ovarian activity. The median FSH and LH concentrations, 6 months after completion of the GnRHa/chemotherapy cotreatment group, were significantly less than the control group. During the GnRHa/chemotherapy cotreatment the concentrations of FSH, LH, and P decreased to almost prepubertal levels. However, within 1–3 months after the last GnRHa injection, an increase in LH and FSH concentrations was detected, followed several weeks later in by an increase in P concentrations to within normal levels.

Conclusion(s): GnRHa administration before and during combination chemotherapy for breast cancer may preserve posttreatment ovarian function in women <40 years. Long-term studies are required.

Key Words: GnRHa, chemotherapy, ovarian function

Materials and Methods

The study included 80 patients who were diagnosed with unilateral adenocarcinoma of the breast, with positive or negative
lymph node status, and with no metastasis that had undergone modified radical mastectomy or breast-conserving surgery plus full axillary lymph node dissection. All patients were menstruating normally and between the ages of 18 years and 40 years (premenopausal status with basal follicle-stimulating hormone [FSH] levels <10 mU/mL). Macroscopic metastatic spread of the disease was excluded by the usual criteria. All patients provided written informed consent before inclusion in the study. Patients were assigned randomly to receive combined GnRHa and chemotherapy or chemotherapy alone by using sealed, dark envelopes. Sample size was calculated using GraphPad Instnt software, version 3.01. Twenty-four patients in each group were required to give the study a power of 80% and α of 0.05.

All patients were treated with an FAC regimen (a combination of 5-Fluorouracil 500 mg/m² i.v., Doxorubicin 500 mg/m² i.v., and Cyclophosphamide 500 mg/m² i.v.) on day 1 of therapy to be repeated every 6–8 weeks for 6 cycles. No patients received radiotherapy as a cotreatment.

Two weeks before the initiation of chemotherapy, patients in the study group received goserelin at a dose of 3.6 mg subcutaneously (Zoladex, Zeneka Pharma International, UK) and then every 28 days for 6 months.

All women had a hormonal profile including: FSH, luteinizing hormone (LH), estradiol (E₂), progesterone (P), and prolactin, before starting treatment, and monthly thereafter (for FSH, LH, E₂, and P) until resuming spontaneous ovulation and menses up to 8 months after. Serial ultrasound scans were by a transvaginal probe (except for nonmarried patients) at each visit for evaluating the ovary and endometrial thickness. Eighty patients were randomized, 40 in each group. One woman in each group dropped out.

Outcome Measures
Outcome was return of spontaneous menstruation and ovulation. Hormonal changes (FSH, LH, E₂, and P) were measured during and after the course of treatment.

Statistical Analysis
The proportion of menstruation, ovulation, and premature ovarian failure (POF) in each group was compared by using Fisher’s exact test. A P level of <.05 was considered significant.

RESULTS
Table 1 for the patients’ characters showed that there were no differences between both groups regarding age, body weight, height, number of dissected lymph nodes, and serum LH. The serum FSH was significantly less in the chemotherapy + GnRHa group, but the mean values were well below 10 mIU/mL (4.3 ± 1.11 vs. 5.7 ± 1.3). Serum E₂ was significantly more in the chemotherapy + GnRHa group (306 ± 21.22 vs. 344 ± 25.67). The mean total dose of cyclophosphamide, doxorubicin, and 5-fluorouracil was 1249 ± 120.1 mg/m² in the study group and 1284 ± 99.3 mg/m² in the control group, without significant differences between the two groups.

Of the 39 women in the study group, 35 (89.6%) resumed menses and 27 (69.2%) resumed spontaneous ovulation within 3–8 months of termination of the GnRHa/chemotherapy cotreatment. Only four (11.4%) patients experienced hypergonadotrophic amenorrhoea and ovarian failure 3 months after treatment. In contrast, only 13 of the 39 (33.3%) in the control group (chemotherapy without GnRHa) resumed menses and 10 (25.6%) resumed ovulation (P <.001) (Table 2). The median FSH and LH concentrations, 6 months after

| TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>Study group</th>
<th>Control group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(chemotherapy + GnRHa)</td>
<td>(chemotherapy)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n = 39)</td>
<td>(n = 39)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>30 ± 3.51</td>
<td>29.2 ± 2.93</td>
<td>.76</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>158 ± 12.33</td>
<td>164 ± 14.55</td>
<td>.54</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>68 ± 3.20</td>
<td>65 ± 4.21</td>
<td>.12</td>
</tr>
<tr>
<td>Married patients</td>
<td>32 (82.0%)</td>
<td>31 (79.4%)</td>
<td>.91</td>
</tr>
<tr>
<td>Parity</td>
<td>1.9 ± 0.32</td>
<td>1.6 ± 0.43</td>
<td>.04 a</td>
</tr>
<tr>
<td>Lymph node excision</td>
<td>25 (64.1%)</td>
<td>30 (76.9%)</td>
<td>.21</td>
</tr>
<tr>
<td>Serum FSH (mIU/mL)</td>
<td>4.3 ± 1.11</td>
<td>5.7 ± 1.3</td>
<td>.04 a</td>
</tr>
<tr>
<td>Serum LH (mIU/mL)</td>
<td>3.9 ± 1.20</td>
<td>4.2 ± 1.43</td>
<td>.14</td>
</tr>
<tr>
<td>Serum E₂ (pg/mL)</td>
<td>306 ± 21.22</td>
<td>344 ± 25.67</td>
<td>.03 a</td>
</tr>
<tr>
<td>Serum P (ng/mL)</td>
<td>6.7 ± 1.21</td>
<td>7.1 ± 1.34</td>
<td>.12</td>
</tr>
</tbody>
</table>

a P value <.05 was significant.

completion of the GnRHa/chemotherapy cotreatment group, were significantly less than the control group ($P < .009$ and $.004$, respectively). Serum P concentration was higher in the study group ($P < .04$). During the GnRHa/chemotherapy cotreatment the concentrations of FSH, LH, and P decreased to almost prepubertal levels. However, within 1–3 months after the last GnRHa injection, an increase in LH and FSH concentrations was measured, followed by several weeks later in an increase in P concentrations to within normal levels (Fig. 1). Eight months after start of GnRHa/chemotherapy cotreatment, FSH and LH rose from 4.3 to 8.3 mIU/mL and from 3.9 to 7.5 mIU/mL, respectively.

**DISCUSSION**

Many drugs used in the treatment of cancer have profound and lasting effects on gonadal function (4). Both germ cell production and endocrine function may be affected, not infrequently in an irreversible manner. The magnitude of the effect varies with the drug class, the total dose administered, and the age and pubertal status of the patient at the time of therapy. Drugs most frequently associated with ovarian failure are divided into three classes: [1] those that definitely are associated with gonadal toxicity: cyclophosphamide, L phenylalanine mustard, busulfan, and mitrogen mustard; [2] those that are unlikely to cause gonadal toxicity: methotrexate, 5-fluorouracyl, and 6-mercaptopurine; and [3] those drugs where the gonadal toxicity is unknown: doxorubicin, bleomycin, vinca alakloids (vincristine and vinblastin), cisplatin, nitrosoureas, and cytosine, arabinoside (4). It has been reported that 64% of adult female patients undergoing cancer therapy experienced one or more of the symptoms of ovarian failure (7).

There are a limited number of prospective studies in humans, which are flawed because of short-term follow-up and/or because of a lack of control subjects. Reechia et al. (8) investigated the protective role of goserelin in 64 premenopausal patients with early breast cancer. After a median follow-up of 55 months, 86% of the patients resumed normal menses. There was no control group. Blumenfeld et al. (9) administered a GnRHa concurrently with chemotherapy in 18 women with Hodgkin’s or non-Hodgkin’s lymphomas. The study group was compared with a historic control group of 18 women treated with similar regimens. The percentage of patients who resumed spontaneous ovulation and menses was significantly higher in the study group than in the control group (93.7% vs. 37%). In a similar study, a GnRHa appeared to be beneficial in both cancer patients ($n = 54$) and non-cancer patients ($n = 8$) receiving chemotherapy (10). The control group ($n = 55$) was chosen retrospectively from patients who were treated with similar regimens. The percentage of patients who resumed menses and ovulation was significantly higher in the study group than in the control group ($< .001$ vs. <.50%). However, the length of follow-up was not stated for the treatment group.

In 2006, Sonmez and Oktay (6) reviewed the protective role of GnRHa against chemotherapy-induced gonadal damage.

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**TABLE 2**

Outcome 8 months after therapy.

<table>
<thead>
<tr>
<th></th>
<th>Study group (chemotherapy + GnRHa) ($n = 39$)</th>
<th>Control group (chemotherapy) ($n = 39$)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menstruating</td>
<td>35 (89.6%)</td>
<td>13 (33.3%)</td>
<td>&lt; .001 $^a$</td>
</tr>
<tr>
<td>Ovulating</td>
<td>27 (69.2%)</td>
<td>10 (25.6%)</td>
<td>&lt; .001 $^a$</td>
</tr>
<tr>
<td>POF</td>
<td>4 (11.4%)</td>
<td>21 (66.6%)</td>
<td>&lt; .001 $^a$</td>
</tr>
<tr>
<td>Serum FSH (mIU/mL)</td>
<td>$8.3 \pm 2.10$</td>
<td>$15.2 \pm 5.31$</td>
<td>&lt; .009 $^a$</td>
</tr>
<tr>
<td>Serum LH (mIU/mL)</td>
<td>$7.6 \pm 2.34$</td>
<td>$16.3 \pm 2.43$</td>
<td>&lt; .004 $^a$</td>
</tr>
<tr>
<td>Serum E$_2$ (pg/mL)</td>
<td>$279 \pm 23.32$</td>
<td>$75.43 \pm 18.98$</td>
<td>&lt; .001 $^a$</td>
</tr>
<tr>
<td>Serum P (ng/mL)</td>
<td>$6.3 \pm 1.01$</td>
<td>$3.7 \pm 1.21$</td>
<td>&lt; .004 $^a$</td>
</tr>
</tbody>
</table>

*Note: POF = premature ovarian failure.

$^a$ $P$ value < .05 was significant.


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**FIGURE 1**

Hormonal changes during the course of GnRHa/chemotherapy cotreatment.
They stated that: in the absence of a prospective randomized study with sufficient power, we do not rely on ovarian suppression as an effective means of fertility preservation. The present study is a prospective randomized controlled study. The results of the present study are promising; spontaneous ovulation was resumed in 69.2% within 8 months in the GnRHa/chemotherapy group. In contrast, only 25.6% resumed spontaneous ovulation in the chemotherapy (without GnRHa) group. The outcome measures of the present study included both the changes in menstrual history and hormonal profile, before and after the treatment. Depending on the menstrual history alone has some limitations (11).

Several possibilities to explain the beneficial effect of GnRH agonists to minimize chemotherapy-associated gonadotoxicity are suggested: [1] the hypogonadotropic milieu decreases the number of primordial follicles entering the differentiation stage, which is more vulnerable to chemotherapy; [2] the hypoestrogenic state decreases ovarian perfusion and delivery of chemotherapy to the ovaries; [3] a direct effect of the GnRH agonist on the ovary occurs independently of the gonadotropin level; [4] GnRH agonists may up-regulate an intragonadal antiapoptotic molecule such as sphingosine-1-phosphate; [5] the GnRH agonist may protect ovarian germ-line stem cells (12).

Two-thirds of the chemotherapy group experienced hypergonadotropic amenorrhea and POF. This rate of POF is in keeping with the findings of previous publications (4, 7, 9). However, another GnRHa, buserelin failed to preserve fertility in four of eight women treated with cytotoxic treatment for Hodgkin’s disease (13). A possible explanation to the differing results between this study and ours is the possibility that the pituitary–ovarian desensitization achieved by buserelin in Waxman et al.’s (13) study was incomplete; thus, the prepubertal milieu was not adequately imitated. In our study the hormonal profile (FSH, LH, and P) decreased to almost prepubertal levels during the GnRHa and chemotherapy. This profound ovarian suppression possibly explains the different results.

Other options for fertility preservation range from the well-established techniques such as embryo cryopreservation to experimental ones, such as oocyte or ovarian tissue cryopreservation (6). Embryo cryopreservation require approximately 2 weeks of ovarian stimulation beginning with the onset of the patient’s menstrual cycle. Thus, it is crucial that these patients are referred to appropriate assisted reproductive technology centers as soon as they are diagnosed with breast cancer. Breast cancer patients may be single or have no sufficient time to undergo ovarian stimulation. Based on data from the current study, it appears that the administration of GnRH analogue in combination with chemotherapy is feasible, well tolerated, and protects long-term ovarian function. The GnRHa cotreatment should be considered in every woman in the reproductive age receiving chemotherapy. It is an effective method of fertility preservation.

REFERENCES