RESEARCH ARTICLE

Gonadotrophin Releasing Hormone Analogues for Ovarian Function Preservation in Young Females Undergoing Chemotherapy

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Abstract

Chemotherapy has significantly improved the prognosis of cancer patients with various malignancies. However, female patients, especially those whoich are premenopausal, suffer from significant chemotherapy induced ovarian function impairment, which decreases their quality of life. Many new techniques for ovarian preservation have been established in recent years. Although the use of gonadotrophin releasing hormone analogues (GnRHa) for this purpose is not a new concept, its effectiveness in protection of ovarian function is still debatable. This article deals with studies and metaanalyses which have been undertaken in the past, demonstrating the impact of GnRHa in ovarian function preservation, and whether their use can be implemented in routine practice.

Keywords: Hemotherapy - gonadotrophin releasing hormone analogues - ovarian failure - gonadotoxicity

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Introduction

The introduction of newer drugs and sophisticated regimens has resulted in improved survival rates and thus, an increase in the lifespan in many cancer patients. Over the last 30 years, the 5-year survival rate for children with cancer has improved from 58% to approximately 80%; and for adults from 50% to almost 70% (Maltaris et al., 2007). As a result, the treatment related side effects and quality of life are now being increasingly recognized and addressed. Ovarian failure following chemotherapy is an unfortunate but unavoidable consequence of potentially lifesaving chemotherapy. Up to two-thirds of adult female patients undergoing chemotherapy for malignancies eventually develop premature ovarian failure (POF) (Kreuser et al., 1990). Loss of ovarian function may have a significant impact on the social, emotional, physical and functional well being in young female patients.

Several approaches of ovarian/fertility preservation have been tried in cancer patients undergoing chemotherapy. These are embryo cryopreservation, oocyte cryopreservation, ovarian cryopreservation and transplantation and ovarian suppression with gonadotrophin releasing hormone analogues (GnRHa) or antagonists (Lamar et al., 2009). Though embryo cryopreservation is the most established technique for fertility preservation in women, its use is asscociated with many limitations. In this technique, ovarian stimulation must be started at the onset of menstruation, which takes two weeks, therefore, a delay of 2 to 6 weeks in chemotherapy initiation may be required if reproductive specialists do not see women early in their menstrual cycle. Most insurance companies do not offer assisted reproductive techniques as benefits, so this approach may be associated with high out-of-pocket costs for most women. A partner or sperm donor is also required. Except for embryo cryopreservation, all the techniques are still in experimental phase.

GnRHa were first isolated and characterized in 1971.The effectiveness of a GnRHa before and during chemotherapy to preserve ovarian function was first demonstrated in animal studies on rodents and monkeys in 1980s (Ataya et al., 1993).

The mechanism of action of GnRHa in ovarian protection is via shutting down the hypothalamic -pituitary-ovarian (HPO) axis and inducing a prepubertal state (Shalev et al., 2003). Natural human GnRH is released in a pulsatile fashion from hypothalamus, leading to ovarian steroidogenesis. When chemotherapy drugs are used for tumor control, there occur initial wave of follicle loss due to the cytotoxic effects of these drugs. This leads to increased levels of Follicle stimulating hormone (FSH), which cause recruitment of more follicles. Continuous use of drugs causes lose of more and more follicles until there occur complete depletion, which leads to POF. The primary target of chemotherapy drugs are pre-granulosa cells of the primordial follicle. Ovarian damage occurs by apoptosis or by injury to blood vessels and focal ovarian

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cortical fibrosis.

Sustained-release synthetic GnRHa binds the GnRH receptors on the pituitary, and the ovary is briefly hyperstimulated (flare reaction). Pituitary GnRH receptors thereby get downregulated, and gonadotrophin release is prevented, resulting in complete ovarian suppression and postmenopausal estrogen levels. Hence, by using GnRH analogues, the recruitment and maturation of primordial follicles is prevented, thereby decreasing the number of follicles vulnerable to the cytotoxic effect of chemotherapy (Shalev et al., 2009).

Another proposed mechanism of ovarian protection by GnRHa is via decrease in ovarian blood flow, causing a decrease in the chemotherapy drugs reaching the ovary (Dada et al., 2001). However, the studies on the effect of GnRHa on blood flow are few and contradictory.

There have been inconsistent results on the benefits of the use of GnRHa as an ovarian protectant. Most of the studies lacked an appropriate control arm and others were not well randomized. The purpose of this article is to review the studies and metaanalysis published in literature so far, that have compared GnRHa co-therapy during chemotherapy, with chemotherapy alone to determine if GnRHa can improve ovarian preservation and maintain fertility.

Materials and Methods

We carried out a literature search from 1966 to July 2013, without language restrictions, through the Medline database at the National center for biotechnology information (NCBI) website (http://www.ncbi.nlm.nih.gov/pmc). The search terms used were 'chemotherapy', 'gonadotrophin releasing hormone analogues', 'ovarian failure', 'gonadotoxicity', 'fertility preservation'. We selected those articles that reported ovarian function after chemotherapy with GnRH agonist protection. Included studies met the following criteria: *i*) females less than 50 years undergoing potentially ovarian-toxic therapy for malignancy *ii*) studies that included a control group of women with similar illness and chemotherapy who did not receive GnRHa therapy, and *iii*) an acceptable definition of ovarian function was included in patient assessment using menstrual history, FSH levels, or antral follicle counts. We excluded articles that met any of the following criteria: *i*) Articles reporting data which were reported once again in subsequent articles, *ii*) Reports in which the data were presented only as an abstract and not a full peer-review article, *iii*) Articles that combined treatment with GnRH antagonist and agonist.

Results

Six studies were prospectively randomized (Cobleigh et al., 1995; Laml et al., 2000; Dada et al., 2001; Walshe et al., 2006; Maltaris et al., 2007; Megan et al., 2009) and six were case series with control (Bonadonna et al., 1985; Kreuser et al., 1990; Ortin et al., 1990; Ataya et al., 1993; Shalev et al., 2003; Lamar et al., 2009). The randomized controlled studies included 340 patients (173 in the study group and 167 in the control group). The case series with control included 470 patients (261 study and 209 control) (Table 1).

Disease and the treatment

Among the 12 identified and selected studies, nine presented data of women with malignant hematological diseases (Hodgkin's disease and non-Hodgkin's lymphoma). Three of the studies included patients with breast cancer and one study was on patients with ovarian cancer. The GnRHa used were tryptorelin (seven articles), goserelin (three articles), leuprolide (one article), diphereline (one article) and buserelin (one article). The chemotherapy protocols differed and in some studies, radiotherapy was also used.

Most women received GnRHa every 4 weeks throughout chemotherapy administration. Most studies described starting treatment about 2 weeks prior to chemotherapy to avoid the subsequent cycle of chemotherapy during the expected ovarian flare that follows GnRHa therapy by 5-10 days.The patients' ages varied widely, between 14 and 50 years. There was also a great variability in the period which elapsed between the time chemotherapy was administered to the time ovarian reserve was determined (between less than a year and more than 8 years) (Table 2).

Outcome measurements

Ovarian preservation and POF were defined differently in the studies. Most used regular menstruation after cessation of chemotherapy as an indicator of continued ovarian function. Some included hormone levels such as

Table 1. Preserved Ovarian Function and Pregnancy Rate

	Preserved Ovarian function, N (%)		No. of pregnant patients	
	Study	Control	Study	Control
Waxman et al. (1987)	4 (8) 50%	3 (9) 33%	0 (8)	1 (9)
Pereyra Pacheco et al. (2001)	12 (12) 100%	0 (4) 0%	2 (12)	0 (4)
Blumenfeld and Eckman (2005)	70 (75) 93%	38 (82) 46%	21 (75)	13 (82)
Dann et al. (2005)	7 (7) 100%	5 (6) 83%	5 (7)	3 (6)
Castelo-Branco et al. (2007)	27 (30) 90%	6 (26) 23%	1 (30)	0 (26)
Loverro et al. (2007)	14 (14) 100%	7 (15) 47%	0 (14)	2 (15)
Gilani (2007)	15 (15) 100%	10 (15) 67%		
Blumenfeld et al. (2008)	63 (65) 97%	29 (46) 63%	19 (26)	12 (20)
Huser et al. (2008)	57 (72) 79%	13 (45) 29%		
Badawy (2008)	35 (40) 88%	13 (40) 33%		
Gerber (2009)	21 (30) 70%	17 (30) 57%		
Sverrisdottir et al. (2009)	10 (66) 15%	5 (57) 9%		

Table 2. Dose and Duration of GnRHa Used

GnRHa	Dose and duration
Triptorelin	-3.75mg IM four wkly (1st dose 1–2 wks before chemotherapy) and some studies have used Tibolone four times a day, along with GnRHa to decrease low estrogen effects.
	-11.25 mg, 3 monthly for duration of chemotherapy
Depot triptorelin	
Goserelin	-3.6 mg four weekly for 6 months
Leuprolide	-3.75mg IM 4 wkly. Short-acting leuprolide given daily the 1st 2 weeks of treatment so that y chemotherap could be started immediately
Buserelin	-200 mg thrice daily. intranasal, starting 1 week before chemotherapy
Diphereline	-3.75 mg four weekly for duration of chemotherapy

Table 3. Characteristics of the Studies

	Study design	Evaluated patient no.		Disease And treatment given	The GnRH agonist used	Age (years, mean/median)		Follow-up duration (years, mean/median)		Outcome measurements
		Study	Control			Study	Control	Study	Control	
Waxman et al. (1987) UK	PR	8	10	HL Up to 6 cycles of MVPP	Buserelin	28.5	25.9	2.3	2	Amenorrhea
Pereyra Pacheco et al. (2001) Argentina	CSC	12	4	Hematological oncology (Leukemia) BMT/CVPP/ ABVD	Leuprolide acetate	16.8	17.8	5	6	Amenorrhea
Blumenfeld and Eckman (2005) Israel	CSC	75	82	HL, NHL. Age 14-40 Various CCT regimens XRT in 65%	Tryptorelin acetate	25.5	26.7	NM	NM	Amenorrhea, estradiol <100 pmol=L, and FSH >25 IU=L
Dann et al. (2005) Israel	CSC	7	6	NHL Age 18-40 (median 27) Cumulative CYC dose: 8,000-12,000mg/m^2 10: CYC 3,000mg/m^2 over 2 days + doxorubicin 50mg/m^2, vincristine 1.4mg/m^2, prednisone 1: CYC 2500mg/m^2; 2: CYC 2000mg/m^2	Tryptorelin acetate	25.6	26.5	5.34	6.23	Cyclic ovarian Function =regular menses¦normal gonadotropin and sex steroid levels or follicles on US or pregnancy
Castelo-Branco et al. (2007) Spain	CSC	30	26	HL. Age 14–45 Various: ABVD: 10 of each group ABVDþXRT: 10 GnRH, 7 control	Tryptorelin	14–45*		NM	NM	No regular Menses
Loverro et al. (2007) Italy	PR	14	15	HL 13 patients: ABVD_6 13 patients: ABVD_5 alternating with C(M)OPP 3 patients: C(M)OPP alternative ABV, then DHAP 24 patients: Supradiaphragmatic radiation	Tryptorelin	24.3±6.6		2.4±1.7 5.9±4.5		No menses within 12 months
Gilani et al. (2007) Iran	PR	15	15	Ovarian Malignancy Surgery: Conservative with preservation of one or two ovaries CCT: Up to 6 cycles of alkylating or alkylating-like MCT	Diphereline	21	22	0.5	0.5	Definition of POF: Early, permanent cessation of menstruation after 6
Blumenfeld et al. (2008) Israel	CSC	65	46	HL	Tryptorelin	23	24	8		Regular menstruation, FSH, LH, estradiol, progesterone, sonography
Huser et al. (2008)	CSC	72	45	HL	Tryptorelin	29	32.5	1	1	Regular menstruation, FSH, LH, sonography
Badawy et al. (2009) Egypt	PR	40	40	Unilateral adenocarcinoma of the breast Surgery: Modified radical mastectomy or breast-conserving surgery plus full axillary lymph node dissection Chemotherapy: Up to 6 cycles of FAC regimen Radiotherapy: Not used	Goserelin	30	29.2	0.66	0.66	Early cessation of menstruation, ovulation and increased serum FSH level (hypergonadotropic amenorrhea)
Gerber et al. (2009) Germany	PR	30	30	Receptor-negative breast cancer Surgery: NA, Chemotherapy: Up to 12 cycles of anthracycline/taxane polychemotherapy Radiotherapy: NA	Goserelin	35.1	38.2	0.5	0.5	Cessation of menstruation
Sverrisdottir et al. (2009) Sweden	PR	66	57	Node-positive breast cancer Surgery: breast conserving Chemotherapy: Up to 6 cycles of CMF regimen _ tamoxifen Radiotherapy: Performed in patients with breast conserving surgery and/or four or more positive lymph nodes	Goserelin	45	45	1	1	Cessation of menstruation

*PR: Prospective randomized study, CSC: Case series with control, HL: Hodgkins lymphoma, NHL: Non hodgkins lymphoma, CCT: Chemotherapy

FSH, Leiutinizing hormone (LH), and estradiol levels. Others included ultrasound-derived follicle counts (ovarian sonography) to define ovarian function.

Pregnancy following chemotherapy was described in seven of the nine included studies, as a criterion for fertility.

In total, data on ovarian function (Table 3) were obtained for 810 women who received chemotherapy, including 432 women who received GnRH agonist co-treatment with chemotherapy. These are referred to as the study group and the 376 women who did not receive agonists comprised the control group. Within the study group, ovarian function was reported as preserved in 335 women (77.54%). Premature ovarian failure or persistent amenorrhea was reported in 97 women (22.46%). In the control group, ovarian function was reported as preserved in 146 women (38.82%). Premature ovarian failure or persistent amenorrhea was reported in 230 women (61.18%). The relative risk for preserved ovarian function with the use of GnRHa comes out to be 1.99.

Only seven studies out of 12, determined the pregnancy rate (Table 3). Pregnancy was far less frequent than ovarian function preservation in all studies. Out of 172 patients in study group, 48 became pregnant (27.92%), while only 31 patients (19.13%) had pregnancy out of 162 patients on control arm. The relative risk for pregnancy rate with the use of GnRHa is 1.45.

Discussion

On an average, 40% of women undergoing chemotherapy develop ovarian failure. The rate of ovarian failure depends largely on the age of the woman receiving the treatment, type of chemotherapy and cumulative dose (Laml et al., 2000). The risk of ovarian failure increases with increasing patient's age at the time of therapy. In a study by Ortin in 1990 in 240 children (15 years of age or less) receiving chemotherapy for hodgkin's disease, POF occurred only in 13% girls, compared with azoospermia in 83% boys (Ortin et al., 1990). This showed that pre-pubertal female gonads are much less vulnerable to the effect of chemotherapy. The possible explanation is that the majority of primordial follicles are in immature, resting and non-growing state. As 83% of children diagnosed with cancer will survive into adulthood, GnRHa administration to female childhood cancer patients in combination with chemotherapy might represent a valuable attempt to preserve future ovarian function and fertility when ovarian tissue preservation is not an option (Osborne et al., 2013).

Bonadanna et al studied the effect of GnRHa on early stage breast cancer patients (Bonadonna et al., 1985). He found that 54% women less than 40 years of age developed amenorrhea after 6-12 cycles of (CMF) and menstruation resumed in 23% of these young women. However in women more than 40 years of age, 96%

developed premature ovarian failure during therapy. 92% never resumed menstruation.

Historically, non cell cycle-specific alkylating agents (i.e. cyclophoshamide, iphosphamide, nitrosoureas, melphalan, bulsulfan, chorambucil, and procarbazine) have proven to be directly toxic to the ovarian granulosa cell, leading to depletion of finite ovarian follicles such that permanent menopause ensues (Walshe et al., 2006). Cobleigh et al retrospectively analyzed 100 women with breast cancer, co treated with Goserelin 3.5mg every 4 weeks or 11.25mg every 12 weeks, along with CMF/ Anthracycline based regimens (Cobleigh et al., 1995). After a median follow-up of 75 months, 33% of the women developed ovarian failure following chemotherapy. Normal menstruation was resumed by all patients less than age 40 years, but only 56% of patients older than age 40 years. None of the 25 women, less than age 40 had ovarian failure. However, it was difficult to determine if the administration of a GnRH analog, which may preserve ovarian function, can also preserve fertility, since study reported on only three pregnancies.

Clowse et al in 2009 performed a systematic review of studies examining whether a GnRHa administered during chemotherapy is protective for ovarian function and fertility (Clowse et al., 2009). He included nine studies that reported an association between GnRHa and ovarian preservation in women receiving chemotherapy. Three studies included women with autoimmune disease receiving cyclophosphamide; six included women with hematologic malignancy receiving combination chemotherapy. Studies without a control group were excluded. Ovarian preservation was defined as the resumption of menstrual cycles and a premenopausal FSH after chemotherapy. Fertility was determined by a woman's ability to become pregnant. He estimated the summary relative risk (RR) and associated 95% confidence intervals (95%CI) using a random-effects model. In total, 178 women were treated with GnRHa during chemotherapy, 93% of whom maintained ovarian function. Of the 188 women not treated with GnRHa, 48% maintained ovarian function. The use of a GnRHa during chemotherapy was associated with a 68% increase in the rate of preserved ovarian function compared with women not receiving a GnRHa (summary RR 1.68, 95%CI 1.34-2.1). Among the GnRHa-treated women, 22% achieved pregnancy following treatment compared with 14% of women without GnRHa therapy (summary RR 1.65, CI 1.03-2.6). Based on this, the study concluded that GnRHa appear to improve ovarian function, as well as improve ability to achieve pregnancy following chemotherapy. Premenopausal women facing chemotherapy should be counseled about ovarian preservation options, including the use of GnRHa therapy.

In our study, the use of GnRHa is associated with 99% increase in the rate of ovarian preservation and 45% increase in the rate of pregnancy, compared to those who donot receive GnRHa along with chemotherapy.

Bedaiwy et al in 2011 assessed the efficacy of GnRH analogues to prevent chemotherapy-related ovarian damage in premenopausal women (Bedaiwy et al., 2011). Only RCTs were eligible for inclusion in the review. Both the incidence of women with spontaneous menstruation and incidence of spontaneous ovulation demonstrated a statistically significant difference in favor of the use of GnRHa (OR 3.46; 95%CI, 1.13-10.57; and OR 5.70; 95%CI, 2.29-14.20, respectively). There was no statistically significant difference between treatment and the control groups in the incidence of a spontaneous pregnancy (Odds ratio (OR) 0.26; 95%CI, 0.03-2.52). The study concluded that though the use of GnRH agonists has shown potential benefit in reproductive age women receiving chemotherapy, still more well-designed, powered, and reported trials are needed to strengthen the body of evidence.

Balkenende et al (Balkenende et al., 2011) in a letter to editor commented on the systematic review and meta analysis by Bedaiwy et al. According to the author, one of the largest studies published in 2008 by Bedaiwy et al included in the metanalysis in Bedaiwy et al had many limitations. Firstly, there was a significant methodologic weakness in the study. Second, the study group consisted of very young women compared with European and American women with breast cancer, who had an unlikely low resumption of menstruation (namely, only 33% of the control subjects). Third, the follow-up time was short (maximum 8 months after the last dose of chemotherapy). Therefore, he performed a new meta-analysis excluding this controversial study. According to this new meta-analysis, at 24 months follow up, the incidence of POF was not significantly different anymore ([OR] 2.25, 95% [CI] 0.65-7.78) compared with the original meta-analysis in which a potential benefit of GnRH analogs was suggested (OR 3.46; 95%CI 1.13-10.57).

Cochrane metaanalysis (Chen et al., 2011) also assessed the efficacy and safety of GnRH analogues. Four RCTs included in this review showed that intramuscular/ subcutaneous administration of GnRH agonists was effective in protecting menstruation and ovulation after chemotherapy; (resumed menses: RR 1.90, 95%CI 1.30 to 2.79; amenorrhoea: RR 0.08, 95%CI 0.01 to 0.58; ovulation: RR 2.70, 95%CI 1.52 to 4.79). Intranasal administration of GnRH agonists had no protective effect on ovaries (resumed menses: RR 0.75, 95%CI 0.33 to 1.72; ovulation: RR 1.13, 95%CI 0.20 to 6.24). Pregnancy rates were not significantly different between groups (intramuscular/subcutaneous GnRH agonist: RR 0.21, 95%CI 0.01 to 4.09; intranasal GnRH agonist: RR 0.41, 95%CI 0.02 to 8.84). Ultrasound antral follicular count (AFC) was not significantly different between groups (SMD 1.11, 95%CI 0.32 to 1.90). The authors' concluded that the use of GnRH agonists should be considered in women of reproductive age receiving chemotherapy. Intramuscular or subcutaneous GnRH analogues seem to be effective in protecting ovaries during chemotherapy and should be given before or during treatment, although no significant difference in pregnancy rates was seen.

Del Mastro (Del Mastro et al., 2013) in a metaanalysis, included nine studies with 225 events of POF occurring in 765 analyzed patients. The pooled OR estimate indicates a highly significant reduction in the risk of POF (OR=0.43; 95%CI: 0.22-0.84; p=0.013) in patients receiving GnRHa. There was statistically significant heterogeneity among

studies (p=0.012). Subgroups analyses showed that the protective effect of GnRHa against POF was similar in subgroups of patients defined by age and timing of POF assessment, while it was present in breast cancer but unclear in ovarian cancer and lymphoma patients.

However, there are many limitations of the studies published so far in literature on the role of GnRHa in ovarian preservation undergoing chemotherapy. There is paucity of data, as very limited number of prospective studies has been published so far. Most of the studies lack truly randomized control subjects, and utilized short and different follow-up periods for study and control groups. No statistical significant analysis has been used in most of the studies due to small number of patients. Also, end-points were poorly defined and inconsistent between the studies.

Besides, different methods were used in various studies to assess ovarian function and fertility preservation. It is now well established that continuation or resumption of menstruation alone, is not a reliable indicator of ovarian function and fertility, because pregnancy rates even in the presence of menstruation are extremely low, when FSH measurements on 2nd or 3rd day of the menstrual period exceed 20 mIU/ ml (Licciardiet al., 1995). Likewise, elevation of estradiol levels above 75 pg/ ml on the 2nd or 3rd day of the menstrual period is also associated with compromised fertility (Scott et al., 1989).

Premature ovarian failure (POF) was defined previously as the condition of hypergonadotropic, hypoestrogenic oligomenorrhea or secondary amenorrhea lasting for greater than 6 months that occurs in women after menarche and before 40 years of age (Del Mastro et al., 2006). Serum FSH level greater than 40 mIU/ml on two occasions drawn more than 1 month apart defines the hypergonadotropic state. In the past, the absence of ovarian follicles on an ovarian biopsy was necessary for the diagnosis of POF. However, it has been shown that 40-60% of women who meet the criteria noted above may still have ovarian follicles visible on ultrasound. Thus nowadays, POF is diagnosed by at least two measurements of FSH >40 mIU/ml one month apart, regardless of menstrual bleeding.

In the above studies, it is also possible that more women interested in future childbearing elected to receive GnRHa co therapy. It is also unclear if pregnancies in the GnRHa groups were spontaneous or assisted, which may also lead to bias and overstatement of the overall fertility benefits. Moereover, none of the studies reported the number of women who tried unsuccessfully to become pregnant. Several of the studies, however, have longer follow-up periods for women without GnRHa cotherapy, giving this cohort of women the unfair advantage of more time to become pregnant.

Oktay et al in his article mentioned that for a new medical treatment to be proven effective, the following three conditions should be met (Oktay et al., 2007). There must be a biological plausibility of the effect of the drug or treatment. Multiple prospective, controlled studies must show consistent results. Potential risks of the treatment should not exceed potential benefits.

Primordial follicles (constituting 90% of ovarian reserve) lack Gnrh/LH/FSH receptors i.e they are Gonadotrophin unresponsive (Oktay et al., 1997). Thismeans the presumed protective effect of GnRH analogues is also gonadotropin independent. GnRH creates a hormonal milieu similar to the prepubertal state and thereby protects ovary, but all prepubertal children are not protected against the gonadal- damaging effects of chemotherapy, therefore hypogonadism alone cannot be a means to preserve fertility. GnRHa protect ovarian reserve by reducing blood flow is also not supported by scientific evidence. If GnRHa were to cause reduced blood flow to the ovary, one would expect this to happen with the tumor also, thus resulting in an overall lower effectiveness of the drug.

Fox et al studied 24 young women with early stage breast cancer, treated with adjuvant chemotherapy and cotherapy with GnRHa (Fox et al., 2001). Twenty three of 24 women resumed menstruation and only 1 woman developed amenorrhea. Only 6 pregnancies occurred in 5 patients; 3 resulted in miscarriage,1 was terminated because of Down's syndrome, 1 pregnancy was ongoing, and 1 delivered. The study concluded that GnRHa treatment reduces the incidence of amenorrhea in a population of relatively older reproductive-age women, but reproductive outcome was very poor.

Besides, GnRHa may also decrease the effectiveness of the chemotherapy. A variety of human cancers like breast, ovary and endometrium, express GnRH receptors. These receptors mediate anti proliferative and antiapoptotic activity in tumor cells (Emons et al., 2003). Thus, GnRH agonist therapy concomitant with cytotoxic chemotherapy might reduce the efficacy of chemotherapy, specifically among hormone-sensitive malignancies, such as ER positive breast cancer.

GnRHa may increase the gonadotoxicity associated with chemotherapy. Antioxidant enzymes (glutathione S-transferases) present in granulosa cells of follicles of various stages in the ovary, play a role in detoxifying chemotherapeutics (Rahilly et al., 1991). Ovarian suppression by GnRHa may reduce the expression of these enzymes, rendering follicles more vulnerable to the toxic effects of chemotherapy.

Above all, the use of GnRHa is associated with hot flushes, hypoestrogenic symptoms (97%), bone loss which is non reversible.

Lee et al in 2006 provided American society of clinical oncology (ASCO) guidelines to practicing oncologists about available fertility preservation methods in females (Lee et al., 2006). According to the guidelines, embryo cryopreservation is the standard practice and is widely available; other available fertility preservation methods should be considered investigational and be performed in centers with the necessary expertise. Since there is insufficient evidence regarding the safety and effectiveness of GnRH analogs, women interested in ovarian suppression for this purpose should be encouraged to participate in clinical trials.

Huser et al in 2012 evaluated 154 young female cancer patients who were offered fertility preservation counseling (Huser et al., 2012). It was found that the administration of GnRH analogues (n=123, 79.9%) and

ovarian tissue cryopreservation (n=15, 9.7%) were the most commonly used fertility preservation strategies. 20 cases (16.1%) were offered the combination of several fertility preservation techniques. The study concluded that the combination of several fertility preservation techniques gives young cancer patients the best chance for future fertility and should be concentrated in specialized centers.

Considering all the above studies, it can be concluded that there is not enough evidence yet to consider co treatment with GnRH analogues in premenopausal women receiving chemotherapy. At present, the use of GnRHa cannot be considered as a standard practice as the biological plausibility is lacking and its safety for use in cancer patients undergoing chemotherapy remains questionable.

In conclusion, GnRHa administration has been used widely due to ready availability. However, there is still insufficient evidence that ovarian suppression protects fertility from gonadotoxic therapies. Various studies-including the SWOG study in U.S., Zoladex Rescue of Ovarian Function study in Germany, Italian multicenter study for breast cancer patients, GHLG study, and PREGO (Prevention of gonadal toxicity and preservation of gonadal function and fertility in young women with SLE treated by cyclophosphamide) in Europe are under way, and more rigorous evaluations will be reported in the near future. A large well designed randomized clinical study with long-term follow-up is required before GnRHa can be routinely recommended for use along with chemotherapy to protect ovarian function.

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