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GENERAL INFERTILITY

Assessment of ovarian reserve following ovarian tissue banking and/or GnRH-a co-treatment prior to chemotherapy in patients with Hodgkin's disease

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Abstract

Purpose To examine ovarian reserve following chemotherapy in women with Hodgkin's disease.

Methods The study included nine patients who underwent ovarian tissue cryopreservation (OTCP) prior to chemotherapy consisting of the ABVD regimen (Adriamycin, bleomycin, vinblastine, and dacarbazine) and co-treatment with gonadotropin-releasing hormone agonist (GnRH-a) (Group A), and 13 patients treated by the ABVD protocol only without GnRH-a (Group B). The average age was 25.2±2.7 years for the women in Group A and 31.8± 6.8 years for those in Group B.

Results Six months following the end of chemotherapy, the menstrual cycle resumed in all Group A patients and in four Group B patients who had amenorrhea. Eight Group B patients had regular menses during and after chemotherapy. None of the patients suffered from ovarian failure. Two Group A patients conceived in the first year after completing chemotherapy.

Conclusions Co-treatment with GnRH-a has little effect on ovarian protection in women with Hodgkin's disease.

Keywords Hodgkin's disease · Chemotherapy · Ovarian tissue cryopreservation · GnRH-a · Ovarian reserve

Capsule Ovarian tissue banking with combined GnRH-a and ABVD given to patients with Hodgkin's disease minimally affects ovarian function, similar to ABVD administered alone.

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Introduction

Hodgkin's disease (HD) is the most common malignancy among individuals aged 15–24 years. A prolonged survival rate of >80% is now expected for a high proportion of young patients treated with cytotoxic chemotherapy [1]. The ABVD (Adriamycin, bleomycin, vinblastine, dacarbazine) regimen is currently regarded as the modern therapeutic "gold standard". It has more anti-tumor activity than the MOPP (mechlorethamine, vincristine, procarbazine, prednisolone) regimen [2]. Furthermore, ABVD is less sterilizing and less leukemogenic, since it does not contain alkylating agents or procarbazine as does the MOPP protocol. Gonadal toxicity is among the long-term sequelae of chemotherapy or radiotherapy. It has been reported that the chances of maintaining gonadal function following combined modality treatment are significantly greater among girls than boys [2]. Because dividing cells at rest are more resistant to chemotherapy, it has been suggested that inhibition of the hypothalamic pituitary axis would reduce the rate of oogenesis. This can be achieved by using gonadotropin-releasing hormone agonist (GnRH-a), which reduces the number of vulnerable cells in mitosis and temporarily creates a prepubertal milieu in women before and during the chemotherapeutic insult. Blumenfeld and Haim [3] reported that co-treatment with GnRH-a significantly reduced the rate of premature ovarian failure from 55% to 5% ($P<0.01$). These findings were challenged by the only published prospective study [4] whose results showed no such benefit. Furthermore, primordial follicles lack gonadotropin receptors [5]. The results obtained in animals have been conflicting [6].

In addition to co-treatment with GnRH-a, the presently available options for preserving female fertility include

ovarian transposition and oophoropexy [7], cryopreservation of mature M-II oocytes [8], embryo cryopreservation and ovarian tissue cryopreservation (OTCP) [9]. The last options are available for preservation of fertility in HD.

In many studies, resumption of the menstrual cycle was considered as being an indicator for assessing ovarian reserve. It had, however, been shown that the menstrual cycle alone is not an accurate tool [10]. Many other tests have also been suggested, among them basal follicle-stimulating hormone (FSH) and estradiol (E2), inhibin B, anti-Mullerian hormone (AMH), and antral follicle count. Further assessments include dynamic tests, among them the gonadotropin releasing hormone agonist test and the clomiphene citrate challenge test [11, 12]. Basal FSH levels >10 IU/L, E2 levels >80 pg/ml and a low antral follicle count indicate low ovarian reserve, but many studies showed a limited predictive value of these parameters [10].

AMH is a glycoprotein of the transforming growth factor-B super family. It is secreted by Sertoli cells in the early embryonic stage and inhibits the development of the Mullerian system. During the postnatal period, it is secreted by granulosa cells from preantral and antral follicles. Under the effect of AMH, those follicles become less sensitive to FSH and, consequently, fewer are recruited. Low levels of AMH indicate low ovarian reserve and they stay stable throughout the menstrual cycle [13–15].

The current study was designed to examine ovarian reserve following chemotherapy in a cohort of patients with Hodgkin's disease who were treated by either ABVD protocol in combination with GnRH-a and ovarian tissue cryopreservation or by ABVD only.

Patients and methods

Patients

All the participating patients signed an informed consent after the risk of chemotherapy and the various treatment options had been explained to them. The OTCP procedure was approved by the Institutional Ethical Committee. The subjects were women who underwent OTCP and were treated by monthly injections of GnRH-a prior to chemotherapy (Group A, age range 19–34 years) and 13 who did not undergo OTCP and did not receive GnRH-a (Group B, age range 21–44). Following clinical staging, OTCP, and GnRH-a administration, the Group A patients were treated by the ABVD protocol. The Group B patients were treated by the ABVD protocol only. Two patients were additionally treated by radiotherapy above the diaphragm and the MOPP protocol was added. GnRH-a was not administered and OTCP was not performed in one patient.

The OTCP procedure

The ovarian tissue was removed by laparoscopy, placed in sterile phosphate buffer saline (PBS; Biological Industries, Beit Haemek, Israel) and transferred to the laboratory. The ovarian cortex was sliced into 1- to 2-mm thickness in PBS medium at room temperature (25°C) and cut into 1-2 X 5-mm sections. We followed the cryopreservation and thawing protocol described by Gosden et al. [16].

Cryopreservation

The tissue blocks were incubated in medium containing 1.5 M dimethylsulphoxide (DMSO), 0.1 M sucrose and 20% serum at 4°C for 30 min. The tissue was then cooled to −7°C at 2°C/min rate in a programmable freezer (Kryo 10; Planer Instruments, Middlesex, United Kingdom). After manual seeding, the cooling continued at 0.3°C until −40°C and then at 10°C/min until it reached −150°C. The vials were then plunged into liquid nitrogen.

Ovarian reserve assessment

Basal FSH, luteinizing hormone (LH), and E2 were measured on day 3 of the menstrual cycle using radioimmunoassay. AMH levels were measured in the plasma using AMH/MIS Enzyme Immunoassay Kit by Immunotech. Follicular ovarian count was assessed using a vaginal ultrasound probe. The presence of at least three follicles was considered as representing good ovarian reserve.

Statistical analysis

The non-parametric Mann Whitney and the Fisher exact tests were used for comparison between the parameters in the two groups. A P value <0.05 was considered as being significant.

Results

The demographic data of the two study groups are presented in Table 1. Six months following the end of chemotherapy, the menstrual cycle resumed in all Group A patients and in 12 Group B patients (including four patients who suffered from amenorrhea during treatment). Eight Group B patients had regular menses during and after chemotherapy. None of the patients suffered from ovarian failure with the exception of the 44-year-old (Table 1). Two Group A patients conceived during the first year following chemotherapy, one patient underwent termination of pregnancy because of personal reasons and the other gave birth to healthy twins. There was no significant difference

Table 1 Comparison of clinical features of patients in each study group

Marital status	Group A			Marital status	Group B			
	Age, y	Clinical stage	Menstrual cycle		Age, y	Clinical stage	Menstrual cycle	Comments[a]
Married +2	24	II-A	Regular	Divorced	34	II-A	Regular	Irradiation
Single	25	II-A	Regular	Married +3	39	II-A	Amenorrhea	
Single	29	II-A	Regular	Single	24	II-A	Regular	
Single	20	III-A	Regular	Single	30	II-A	Regular	Irradiation
Single	28	II-A	Regular	Married	44	IV-B	Amenorrhea	
Married +1	27	II-A	Regular	Married	35	II-A	Irregular	Irradiation, MOPP
Married +2	24	II-A	Regular	Single	32	III-A	Regular	
Married	26	II-A	Regular	Married +2	37	II-B	Amenorrhea	
Single	24	III-A	Regular	Single	25	III-A	Regular	
				Single	31	II-A	Regular	Irradiation
				Single	36	II-A	Regular	Irradiation
				Single	24	II-A	Regular	
				Single	23	III-B	Irregular	Irradiation

[a] Irradiation directed to the supradiaphragmatic-involved field only between 3,000–3,400 Rads.

between the two study groups for any of the parameters used for assessing ovarian reserve (Table 2).

Discussion

The results of our study showed that the majority of patients resumed menstruation during the 6 months that followed the end of chemotherapy. Evaluation of ovarian reserve by means of several measurements demonstrated that ovarian reserve was not affected in most of them. Group B included some women older than those in Group A, but sole treatment with ABVD without GnRH-a did not negatively affect the ovarian reserve in either group. Although the AMH levels are very different between the two groups (6.07±1.3 vs. 24.2±19.6), the difference did not reach a level of significance because of the wide range of values, which may be mainly attributed to the small number of patients.

These findings cast doubt on the additive protecting effect attributed to GnRH-a and contradict those of Blumenfeld and Haim [3] who reported resumption of menstruation in 95% of the patients treated by chemotherapy and GnRH-a compared to 45% in the control group treated by chemotherapy only. Pacheco et al. [17] compared three groups of patients comprised of five premenarche girls treated by chemotherapy only, 12 women after menarche treated by chemotherapy and GnRH-a and four women after menarche treated by chemotherapy only. Those authors concluded that GnRH-a may protect ovaries, basing their conclusion on the resumption of menstruation. In their prospective study, however, Waxman et al. [4], showed that GnRH-a was not useful. In the current study, we used several parameters for evaluating ovarian reserve and none of them showed a deleterious effect upon the ovary in either study group. Our having used several parameters for assessment strengthens these findings.

In summary, our findings demonstrate that patients with HD who are treated with the ABVD protocol have minimal risk of ovarian damage and diminished ovarian reserve. Co-treatment with GnRH-a has negligible effect in terms of ovarian protection and ovarian reserve. These findings raise the question of the need for OTCP among women with HD. Based on our current findings, we have changed our

Table 2 Comparison of age, basal gonadotropins, E2, AMH, and follicular count of the two study groups

Parameter	Group A		Group B		
	Average ± SD	Range	Average ± SD	Range	P Value
Age (years)	25.2±2.7	19–34	31.8±6.8	21–44	NS
E2 (pg/ml)	97.7±10	10–200	95±36.6	50–140	NS
FSH (Iu/l)	16.5±17.4	5.7–45.3	12±5.7	8–16	NS
AMH (ng/ml)	6.07±1.3	5.5–9	24.2±19.6	5.5–67	NS
Number of follicles	4.7±2.7	2–9	6±3.7	2–11	NS

approach and do not perform OTCP in this population, especially in young patients. Since the number of study patients was small and our study was retrospective, larger prospective studies for assessing the effect of GnRH-a on ovarian reserve are warranted.

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