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ARTICLE

The effect of childhood cancer therapy on ovarian reserve and pubertal development

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Dr Bertan Akar was born in Adana, Turkey in 1977. He studied medicine at Erzurum Atatürk University between 1996 and 2003, and worked as a research assistant in the Department of Obstetrics and Gynaecology at Kocaeli University Faculty of Medicine between 2008 and 2013. He currently works at the IVF unit at Kocaeli University, and has conducted research in the area of reproductive endocrinology.

Abstract The effects of childhood cancer therapy on ovarian reserve tests and on pubertal development within 5 years were compared with a control group. The study group was composed of 41 patients who underwent chemotherapy during pre-menarche (subgroup A; $n = 15$) and after menarche (subgroup B; $n = 26$); the control group was composed of 44 patients admitted with non-cancer related diseases (in total $n = 85$). Mean total ovarian volume and total antral follicle counts on ultrasound examination were significantly lower in the study group compared with the control group (3.5 ± 2.3 versus 5.2 ± 2.4 ml; $P = 0.001$; and 3.4 ± 3.3 versus 8.6 ± 3.5; $P < 0.001$, respectively). Mean FSH level was significantly higher in the study group (13.5 ± 16.2 versus 7.3 ± 2.7 mIU/ml; $P = 0.017$). Anti-Müllerian hormone levels in subgroup A were significantly higher than in subgroup B (1.8 ± 0.1 versus 1.5 ± 0.08 pg/dl; $P = 0.034$). In conclusion ovarian volume, antral follicle count and FSH can be used for evaluating the harmful effect of cancer chemotherapy on ovarian follicles. Post-menarche, Anti-Müllerian values reveal that ovarian follicles are more sensitive to the devastating effects of cytotoxic treatment.

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KEYWORDS: childhood cancer therapy, ovarian reserve, puberty

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Introduction

Survival rates have increased by 80% in many types of cancer owing to recent modern multi-agent cytotoxic chemotherapy and radiotherapy methods for treating childhood cancers (Ries et al., 2006). The increase in survival, and the health problems that these children will encounter in later years owing to the long-term effects of cancer therapy, have become a major concern. Early and massive loss of primordial follicles in girls receiving cancer therapy results in loss of fertility, impairment of endocrine functions, early menopause and an increased risk of associated early ageing, osteoporosis and cardiovascular disease (Green et al., 2009a; Robison et al., 2002). The damaging effect of cancer therapy on long-term ovarian reserve, and the stimulation of an early menopause, has been proven (Byrne, 1999; Green et al., 2009b; Hamre et al., 2012; Sklar, 2005; Sklar et al., 2006; Thomas-Teinturier et al., 2013). In addition, knowledge about acute ovarian damage and ovarian failure during and shortly after cancer therapy is limited (Chemaitilly et al., 2006; Green et al., 2009b; Lantigna et al., 2006; Mörse et al., 2013).

The purpose of this study was to evaluate the effects of therapy within a follow-up period of 5 years on follicle pool in female adolescents who had received chemotherapy for the treatment of childhood cancer. Biochemical markers of ovarian function were used, such as FSH, LH, oestradiol, anti-Müllerian hormone (AMH) and inhibin B, as well as ultrasonographic indicators such as total antral follicle count (AFC) and total ovarian volume measurements.

Materials and methods

This case-control study was conducted between 2009 and 2013 following local ethics committee approval (approval granted on 26 June 2009, reference number 2009/125). Written informed consent was obtained from all participants and legal guardians.

Inclusion criteria were determined as follows: female childhood cancer survivors who had a follow-up period of 6 months to 5 years after completing chemotherapy; already in the postmenarche period; 16 years or older at diagnosis of cancer; and signed informed consent obtained for participation. Forty-one females meeting these criteria constituted the study group. Criteria for exclusion was being terminally ill or having a history of bilateral or unilateral oophorectomy. In the study group, 15 patients received chemotherapy before menarche and 26 patients received chemotherapy after menarche. The patients who had received cancer therapy in the pre-menarche period (group A) and who had received cancer therapy in the post-menarche period (group B) were also grouped and used in a subgroup analyses. Forty-four female adolescent volunteers 16 years or younger, and in the postmenarche period, were enrolled as the control group. Their admissions to the outpatient department were a result of acute diseases, such as vaginitis ($n = 2$), cystitis ($n = 11$), upper respiratory tract infection ($n = 23$), dermatitis ($n = 5$) and orthopedic disorders ($n = 3$) that were assumed not to affect hormone levels. History of a chronic disease, cancer and autoimmune disease were exclusion criteria. Demographic data, physical examination findings, sexual maturity (breast and pubic hair ratings as defined by Tanner, 1969), characteristics of chemotherapy and radiotherapy, history of gonadotrophin-releasing hormone agonist or antagonist treatments, and whether they used combined oral contraceptive pills, were recorded.

For mensturating patients in both the study and control groups, serum FSH, LH, oestradiol, AMH and inhibin B levels were measured on day 2 or 3 of their menstrual cycle, and total ovarian volume and AFC were evaluated by pelvic ultrasonography. Blood hormone levels and ultrasonographic measurements were taken during enrollment in non-menstruating patients.

Anti-Müllerian hormone levels were measured by E90228Hu 96 test (Uscn Life Science Inc., China), which is sandwich enzyme immunoassay for in-vitro quantitative measurement of AMH (intra-assay coefficient of variation less than 10%, inter-assay coefficient of variation less than 12%, detection range 0.156–10 ng/mL, minimum detectable concentration less than 0.055 ng/mL). Inhibin B was measured by E90760Hu 96 test (Uscn Life Science Inc., China), which is a sandwich enzyme immunoassay for the in-vitro quantitative measurement of inhibin B (intra-assay coefficient of variation less than 10%, inter-assay coefficient of variation less than 12%, detection range 15.6–1000 pg/mL, minimum detectable concentration less than 7.6 pg/mL). Serum FSH was measured by direct immunoenzymatic determination test (Diametra SRL, Italy) (intra-assay coefficient of variation 5.1% or less, inter-assay coefficient of variation 7.6% or less, minimum detectable concentration 0.22 mIU/ml). Serum LH was measured by solid phase enzyme immunoassay (Diametra SRL, Italy) (intra-assay coefficient of variation 9.21% or less, inter-assay coefficient of variation 7.91% or less, minimum detectable concentration 0.22 mIU/ml). Oestradiol was measured by competitive binding immune test (DIAsource E2-EASIA; diasource immunoassays, Belgium) (intra-assay coefficient of variation 3.9–4.6%, inter-assay CV 6–6.1%, minimum detectable concentration 5 ± 2 pg/ml).

Ovarian volume and AFC measurements were taken abdominally in patients with full bladder by using Sonoace-x8 ultrasonography device and 2–6 MHz abdominal probe (Samsung-Medison Inc., South Korea). Three orthogonal diameters were measured to determine ovarian volume and calculated using a formula for prolate ellipsoid ($\pi/6 \times D1 \times D2 \times D3$). Then, data were recorded in milliliter (ml) after the collection of results belonging to both ovaries. Follicles with a diameter of 2–10 mm were counted in both ovaries and combined for total AFC.

Data obtained from the study were analysed using the Statistical Pakcage for Social Sciences (SPSS) version 16.0 (SPSS Inc, Chicago, Illinois, USA). Mean numerical data were presented as standard deviation (mean ± SD). Mann–Whitney U and Student's t-tests were used to compare continuous variables between groups. Chi-squared test and Fisher's exact test were used to compare classified data between the two groups. $P < 0.05$ was considered statistically significant.

Results

Mean age (years) was 15.02 ± 2.44 in the study group and 15.36 ± 0.9 in the control group at enrollment. Mean age of

Table 1 Distribution of chemotherapeutics, doses and radiotherapy treatments among study participants according to malignancy group.

Treatment agent and dose	*Solid tumours* n = *11 (%)*	*Leukaemia and lymphoproliferative tumours* n = *30 (%)*	P-*value*
Alkylating agent	9 (81.8)	22 (73.3)	NS
Cumulative dose of alkylating agent (mg/m^2)	30.880 ± 31.598	4431 ± 9768	0.0002
Antimetabolite	6 (54.5)	29 (96.7)	0.0015
Cumulative dose of Antimetabolite (mg/m^2)	17.545 ± 37.339	25.191 ± 20.409	NS
Anthracyclins	8 (72.7)	27 (90.0)	NS
Cumulative dose of anthracyclins (mg/m^2)	184 ± 184	219 ± 108	NS
Microtubule inhibitors	8 (72.7)	28 (93.3)	NS
Cumulative dose of microtubule inhibitors (mg/m^2)	51 ± 131	10.9 ± 10	NS
Other chemotherapeutics[a]	4 (36.4)	21 (70.0)	NS
Tumour radiotherapy	4 (36.4)	17 (56.7)	NS

[a]Other chemotherapeutics: topoisomerase II inhibitors in four cases with solid tumours, and seven cases with leukaemia or lymphoproliferative tumours group, one case of imitanip and 13 cases of asparaginase.
NS = not statistically significant.

survivors exposed to cancer therapy pre-menarche (group A) and post-menarche (group B) were 13 ± 1.81 and 16.19 ± 1.96 years, respectively at enrollment ($P < 0.001$). At the beginning of the cancer treatment, mean age of group A and B was 9.46 ± 1.88 and 14.46 ± 1.45 years; and the follow-up period was 42.26 ± 15.24 and 20.53 ± 13.62 months, respectively ($P < 0.001$).

Out of 41 survivors, 18 had leukaemia, 12 had lymphoproliferative tumours and 11 had solid tumours. Malignancy diagnoses were acute lymphoblastic leukaemia ($n = 14$, 34.1%), Hodgkin's lymphoma ($n = 7$, 17.1%), acute myeloblastic leukaemia ($n = 3$, 7.3%), osteosarcoma ($n = 3$; 7.3%), Ewing's sarcoma ($n = 3$, 7.3%), non-Hodgkin's lymphoma ($n = 4$, 9.8%), Wilm's tumour ($n = 2$, 4.9%), chronic myeloid leukaemia ($n = 1$, 2.4%), Langerhans cell histiocytosis ($n = 1$, 2.4%), giant cell bone tumour ($n = 1$, 2.4%), ependymoma ($n = 1$, 2.4%) and nasopharynx cancer ($n = 1$, 2.4%). Alkylating agent was used in 31 cases (75.6%), agents affecting cell metabolism were used in 35 cases (85.4%), antracyclines were used in 35 (85.4%) and microtubule inhibitor was used in 36 cases (87.8%) in the study group. A total of 21 (51.2%) survivors (one abdominal and 20 extra abdominal site) underwent radiotherapy (Table 1).

Mean FSH value in the study group was significantly higher compared with the control group (13.5 ± 16.2 versus 7.3 ± 2.7 mIU/l; $P = 0.017$). Mean total ovarian volume in the study group was significantly lower compared with the control group (3.5 ± 2.3 versus 5.2 ± 2.4 ml; $P = 0.001$). Similarly, mean total AFC in the study group was found to be significantly lower compared with the control group (3.4 ± 3.3 versus 8.6 ± 3.5; $P < 0.001$). Serum levels of LH, oestradiol, AMH and inhibin B were similar in both study and control groups (Table 2). No correlation was found between study (r = 0.05) and control (r = 0.01) groups in age and AMH levels. In subgroup analyses, serum AMH values of group A was significantly higher than group B (1.8 ± 0.1 versus 1.5 ± 0.08; $P = 0.034$). Other biochemical ovarian reserve markers were similar between groups (Table 3).

In the study group, transient amenorrhoea was detected in 10 patients, permanent amenorrhoea in two patients, oligomenorrhoea in nine patients and regular menstrual cycles in 20 patients after therapy. Although mean menarche ages of study and control groups were similar (12.4 ± 1.06 versus 12.08 ± 1.4 years), Tanner breast and pubarche stages of the study group were significantly more advanced than the control group ($P = 0.002$ and $P = 0.003$, respectively) (Table 4).

Discussion

Acute ovarian failure in young women exposed to chemotherapy, radiotherapy, or both, because of childhood cancer can be recognized by the emergence of amenorrhoea during or shortly after therapy, changes in biochemical markers that show ovarian reserve and a decrease in ovarian volume and AFC that is measured by ultrasonography (Chemaitilly et al., 2006; Lantigna et al., 2006; Letourneau et al., 2012; Mörse et al., 2013). It is relatively difficult to show acute ovarian failure in prepubertal girls. When the emergence of amenorrhoea during chemo-radiotherapy and 5-year follow up was considered as AOF, it was reported in 6.3%; and if it was evaluated based on FSH and oestradiol, it was reported in 22.6% of the patients (Chemaitilly et al., 2006; Green et al., 2009b; Lantigna et al., 2006).

In addition, acute ovarian failure was seen in 70–85% of the girls who were exposed to dose- and age-dependent abdominal-pelvic irradiation (Chemaitilly et al., 2006). It was suggested that ovarian damage was a result of treatment-induced apoptosis, mitochondrial DNA damage, damage in

Table 2 Ultrasonographic and biochemical ovarian reserve test results in the study and control groups.

Marker	*Study group* n = *41*	*Control group* n = *44*	P-*value*
Total ovarian volume (ml)	3.5 ± 2.3	5.2 ± 2.4	0.001
Total antral follicle counts (n)	3.4 ± 3.3	8.6 ± 3.5	<0.001
Anti-Müllerian hormone (pg/dl)	1.57 ± 0.41	1.67 ± 0.33	NS
Inhibin B (pg/ml)	15.0 ± 9.5	15.0 ± 9.5	NS
Oestradiol (pg/ml)	47.2 ± 35.0	48.2 ± 40.3	NS
FSH (mIU/mL)	13.5 ± 16.2	7.3 ± 2.7	0.017
LH (mIU/mL)	8.03 ± 10.4	8.7 ± 7.5	NS

For menstruating patients, all measurements were at the follicular phase. For 12 amenorrheic patients, measurements were taken at enrollment.
NS = not statistically significant.

Table 3 Biochemical and ultrasonographic ovarian reserve test results of survivors who were treated during pre-menarche period (group A) and post-menarche period (group B).

Marker	*Group A* (n = *15*)	*Group B* (n = *26*)
Total ovarian volume (ml)	3.2 ± 2.4	3.7 ± 2.2
Total antral follicle counts (n)	3 ± 1.1	4.4 ± 0.6
Anti-Müllerian hormone (pg/dl)[a]	1.8 ± 0.1	1.5 ± 0.08
Inhibin B (pg/ml)	12.2 ± 1.1	15.7 ± 2.1
Oestradiol (pg/ml)	31.4 ± 10.4	51.5 ± 7
FSH (mIU/ml)	13 ± 5.2	11.1 ± 1.8
LH (mIU/ml)	6.7 ± 1.4	7.5 ± 1.7

[a]*P* = 0.034.

Table 4 Breast and pubarche development of study and control groups based on Tanner stages.

Tanner stage	*Study group* (n = *41*) (%)	*Control group* (n = *44*) (%)	P-*value*
Stage1 breast	4 (9.8)	–	0.002
Stage 2 breast	8 (19.5)	24 (54.5)	
Stage 3 breast	29 (70.7)	20 (45.5)	
Stage 1 pubarche	–	–	0.003
Stage 2 pubarche	10 (24.4)	24 (54.5)	
Stage 3 pubarche	31 (75.6)	20 (45.5)	

– = denote the number zero.

blood vessels, neovascularization and focal fibrosis (Ben-Aharon et al., 2012).

In the present study, measurements of AMH, inhibin B, FSH, LH and oestradiol levels and of ultrasonographic markers, such as ovarian volume and AFC were obtained from cancer survivors exposed to chemo-radiotherapy in the past 5 years so that acute ovarian damage can be evaluated, and these parameters were compared with the control group. When AMH, LH, inhibin B and oestradiol levels were compared, no significant difference could be deteceted. Mean FSH level was significantly higher and mean ovarian volume and AFC were significantly lower in the study group compared with the control group.

In the present study, the AMH levels in the study and control groups were between mean and 1 SD below the mean. This is the normal range presented in a recent study by Kelsey et al. (2011). The mean ovarian volume in the control group was between mean and 1 SD below the mean and mean ovarian volume of the study group was between 1 SD and 2 SD below the normal validated model as calculated by Kelsey et al. (2013). Mörse et al. (2013) assessed acute ovarian failure by measuring AMH, inhibin B, FSH, LH and oestradiol levels at 3–4 month periods during and after childhood cancer therapy; they reported a sharp decrease in AMH and inhibin B levels and a significant increase in FSH and LH levels at 3 months after therapy regardless of AMH level, age, menarche status or therapy and dose received at the time of diagnosis. At 1–3 years after chemotherapy, AMH levels increased except in those who received sub-diaphragmatic radiotherapy, but this increase did not reach its previous levels. Similarly Brougham et al. (2012) showed that AMH fell rapidly during cancer treatment in both prepubertal and pubertal girls with no AMH recovery in the high-risk chemotherapy group, whereas AMH recovery was observed in the low-to-medium risk groups after completing treatment. Rosendahl et al. (2010) reported that AMH acutely decreased after one two two regimens of chemotherapy in women aged between 19 and 35 years. Although inhibin B and AFC decreased after the third regimen of chemotherapy, FSH reached menopausal levels after the fourth regimen. Although FSH level returned to normal within the first months after therapy, an earlier increase was shown in inhibin B and a later increase was shown in AFC and AMH. In addition, Lie Fong et al. (2009) found no significant difference between study and control groups in AMH levels, which is similar to our findings. In patients who underwent three or more regimens of procarbazine, and who were exposed to abdominal or whole-body irradiation, AMH levels were found to be lower compared with the control group. No patients had undergone whole-body irradiation for bone marrow transplantation in our study group, and abdominal radiotherapy was administered to only one patient. This may show that standard childhood cancer chemotherapy has a low potential for damaging pre-antral follicles. In our study, FSH was a good marker of ovarian damage within 5 years after therapy. However, FSH is improves most quickly after acute ovarian damage, and its ability to predict residual ovarian reserve and long-term results in patients who are taking and who have

received chemotherapy is unclear (Letur-Könirsch and Guis, 1996).

On the basis of our findings, we consider that AFC and measurements of ovarian volume may be also used to evaluate ovarian reserve during the post-menarche period. In one study, a reduction in AFC after three regimens of chemotherapy was reported (Rosendahl et al., 2010). Other studies have reported that ovarian volume measurements and AFC were lower after chemotherapy compared with pre-treatment values, and that AFC after FSH administration was lower during early and late follow-ups after chemotherapy compared with pre-treatment values (Bath et al., 2003; El-Shalakany et al., 2013; Larsen et al., 2003a, 2003b). In addition, a few studies have evaluated ovarian reserve in cancer survivors by using ovarian volume and AFC counts, and threshold values of these parameters that represent the reduction in ovarian reserve are not known.

Anti-Müllerian hormone levels of survivors in the post-menarche period during cancer treatment were significantly lower than patients who were in the pre-menarche period during treatment. Similarly, Chemaitilly et al. (2006) and Lantigna et al. (2006) have reported that acute ovarian failure was more frequent in survivors who were exposed to cancer therapy during and shortly after menarche compared with survivors who were exposed before menarche (45% and 16.4%, respectively). In these studies, however, acute ovarian failure was evaluated only by clinical data obtained from patient expressions and survey results or by FSH and oestradiol measurements. To assess, acute ovarian failure, we also measured AMH and inhibin B levels. In a five-centre study and a recent study evaluating the effects of childhood cancer chemo-radiotherapy (Byrne, 1999; Thomas-Teinturier et al., 2013), it was proposed that prepubertal girls were more resistant to the toxic effects of chemotherapy with alkylating agents, and the possibility of early menopause was higher in girls who were treated during post-puberty period. In the five-centre study, the rate of menopause of patients in their twenties who were diagnosed between 0 and 12 years and treated before puberty was similar to controls; however, it was increased up to 2.32-fold in patients who were treated after puberty. It is also known that infertility problems occur more frequently in women treated after menarche (Byrne, 1999). These results may suggest that growing follicles are the target of chemotherapy and ovaries become more sensitive against gonadotoxic agents with puberty. Follicles at the preantral stage by the start of gonadotrophin releasing hormone pulsation may be more sensitive to chemotherapy and radiotherapy compared with primordial follicles. It is known that AMH prevents recruitment of primordial follicles from the pool of growing follicles (Durlinger et al., 1999, 2002). Repeated acute decreases in AMH level make follicles more sensitive to the effects of chemotherapy by increasing the recruitment of primordial follicles from the new cohort and the start of their maturation, and they may cause a more rapid exhaustion in primordial pool. This condition reveals that additional precautions should be taken in order to protect the follicle pool in the patients for whom chemotherapy is planned after puberty. Some studies, however, have reported no relationship between puberty status during treatment, AMH levels and early menopause (Green et al., 2009b; Krawczuk-Rybak et al., 2008; van Beek et al., 2009). Childhood cancer therapy may cause a delay in pubertal development and impairment in regular menstrual cycles in adolescent girls (Armstrong et al., 2009; Siris et al., 1976). It was shown that puberty progress was delayed in 5.8% of the cases who were given cancer therapy and in 33.3% of the patients who were given cancer therapy during puberty (Siris et al., 1976). It was also reported that reversal of ovarian functions was not possible in patients whose amenorrhoea occurred during cancer therapy and lasted more than 3 months (Green et al., 2009b). This retardation observed in pubertal development was associated with thyroid hormone deficiency, growth hormone deficiency and malnutrition in relation to chemotherapy-associated thyroid, hypophysial and gonadal dysfunctions (Dvorak et al., 2011). Although age and menarche age of the study and control groups were similar in our study, their pubertal development was significantly different; breast development and pubarche Tanner stages were significantly higher compared with the control group, which reflects the wider age distributions of the study groups. Endocrinological and gynaecological consultation should be recommended. Hormone replacement therapy for the development of secondary sex characteristics in the girls whose pubertal development is delayed may provide psychosocial benefits during transition from childhood to adulthood (Hershlag et al., 2011).

In conclusion, female survivors of childhood cancer, especially those exposed to chemotherapy after menarche, are under the risk of ovarian failure. Increase in serum FSH levels and decrease in ovarian failure and AFC detected by ultrasonography are good markers for ovarian failure.

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