Gonadal function and fertility in males survivors treated for Hodgkin's disease in Iran

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ABSTRACT

Objective: To investigate the effect of chemotherapy on gonadal function of young men cured of childhood Hodgkin's disease.

Methods: Young adult males surviving Hodgkin's disease, aged 17 and over at least 2 years after therapy were studied in Ali Asghar Children's Hospital, Tehran, Iran from March 2000 to March 2005. Clinical evaluation for secondary sexual characteristics, semen analysis, follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone was studied in 33 survivors of Hodgkin's disease.

Results: The age at diagnosis was 5-15 years, median 9 years, age at study 17-29 years, median 19 years old. The median duration off therapy was 7 years (2-20 years). All 33 patients received chemotherapy as follows: 32 patients received nitrogen mustard (mechlorethamine), vincristine (Oncovin), procarbazine, prednisone (MOPP) / doxorubicin (adriamycin), bleomycin, vinblastine, dacarbazine (ABVD) 6-8 cycles, 5 of whom after relapses received other protocols. One received only MOPP. Twenty-seven (81.8%) had azoospermia, 2 had severe oligospermia, 3 had oligospermia, and one had normal sperm count (58000,000). All patients had normal secondary sexual characteristic. The FSH, and LH in 6/33 patients were above normal. Testosterone in 3/33 was below normal.

Conclusion: A prepubertal status does not protect the gonads from the harmful effect of chemotherapy, and approximately 87% of male survivors of Hodgkin's disease develop azoospermia or severe oligospermia.

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Since the introduction of the effective combination of a non-cross-resistant antineoplastic agent with non-overlapping toxicity chemotherapy, more than 80% of children diagnosed with Hodgkin's disease (HD) will achieve long-term disease free survival.[1] Sterility, alteration in fertility, and potential gonadal injury after staging and treatment are important issues that should be addressed at the time of diagnosis and before therapy is instituted. However, the use of chemotherapy and radiation therapy has become associated with late sequels such as organ damage,[2] second malignancy,[3] and infertility.[4] Testicular dysfunction is the most common long–term side effect of chemotherapy in men. The testis has 3 principle cell types: Leydig cells that are responsible for testosterone synthesis, Sertoli cells that support developing germ cells, and germ cells or spermatogonia that develops into mature sperm.[5] The germ cells are susceptible to injury by cytotoxic drugs. In contrast, Leydig cells are resistant to the effects of chemotherapy.[6] In summary, males survivor are more susceptible to infertility than females, due to testosterone deficiency. The extent and reversibility of cytotoxic germ cell damage depends on the specific agents used and the cumulative dose, alkylating agents are the most common agents effecting testicular function. The introduction of the MOPP [nitrogen mustard (mechlorethamine), vincristine (Oncovin), procarbazine, prednisone] regimen was a crucial event in the treatment of HD.[7,8] However, MOPP was associated with toxic effects including sterility, in both males and females, so the Milan Cancer Institute in 1974 developed an alternative treatment based on the ABVD combination [doxorubicin (Adriamycin), bleomycin, vinblastine, dacarbazine]. The efficacies of ABVD and MOPP in adults were equivalent to the MOPP, but with a lower incidence of gonadal toxicity for ABVD combination.[9] The aim of this study was to investigate the impact of therapy on gonadal function of young men cured of childhood HD and assess whether a prepubertal state during treatment protects the gonads from chemotherapy late effect.

Methods. From March 2000 to 2005 all HD male survivors who had received chemotherapy at the Ali Asghar Children's Hospital, Tehran, Iran, age ≥17 years who had least 2 years off therapy were eligible for this study. Patients who had undergone pelvic irradiation and bone marrow transplantation were excluded, and 33 patients agreed to and completed all study requirements. All were invited to participate following approval of the study by the Institutional Review Board and informed consent being obtained. Overall survival rates at the time of the study were 86.3±1 for Hodgkin's disease (96.3% for stage I-II, and 82.7% for stage III-IV). A complete history including age at diagnosis, stage, primary site, pathology, chemotherapy regimens, duration of treatment and off therapy, age at the time of study, and physical examination was performed. Testicular size was assessed by a pediatric oncologist with a ruler and with a Prader orchidometer. Because of the frequent occurrence of varicocele among infertile men and its possible causal role in infertility the testis should be carefully palpated while the patient is standing. Laboratory studies included serum luteinizing hormone (LH), follicle–stimulating hormone (FSH), and testosterone. A semen sample was analyzed for concentration, morphology, and motility. The subjects were asked not to ejaculate for 72 hours before assessment. Semen analysis results were defined as follows: normospermia = sperm count >20,000,000 Sp/ml, oligospermia = sperm count of 100,000-20,000,000 Sp/ml, severe oligospermia = sperm count of 1-100,000, azoospermia = sperm count 0 Sp/ml. Normal values for testosterone are: 2.3-9.9 ng/ml, FSH: 1.7-15 mIU/ml, and LH: 1.2-7.8 mIU/ml.

Categorical data was tabulated using frequency table. Continuous data was analyzed using Univariate procedure. Values are expressed as the mean ± standard deviation (SD). Comparison and correlation were made between LH and testosterone, age at diagnosis and chemotherapy protocol. All data were analyzed with software SPSS Version 11.5.

Results. The median age at the time of diagnosis was 9.1 years (range, 5-15 years), median age at study 19.2 years (range, 17-29). The median years off therapy were 7 years (range, 2-20 years). The median level of FSH was 8 mIU/ml (range, 1-32), 6/33 cases were above normal. The median level of LH was 5 mIU/ml (range, 0.1-14), 6/33 were above normal. The median level of testosterone was 4.10 ng/ml (range, 0.1-14.10), 3/33 were below normal. All patients with low level of testosterone had normal LH (**Table 1**). A strong correlation was found between severe oligospermia and higher FSH levels (r=1) (**Table 1**). Twenty-nine (87.8%) patients received 6-8 cycles of MOPP/ABVD, 5 (15.1%) patients relapsed, and received other protocols. Three patients received MOPP/ABVD + radiotherapy, and one patient received MOPP (10 cycles) (**Table 2**). The primary sites of diagnosis were the neck 42.4%, and supraclavicular plus mediastinal (12.1%). Staging of the patients was stage IA-36.4%, IIA-24.2%, and IIIB-18.2%. The mixed cellularity subtype was the most common type (n=23; 69.7%), followed by nodular sclerosis (n=4; 12/1%), lymphocyte predominant (n=4; 12.1%), and lymphocyte depletion (n=2; 6%). All patients had normal secondary sexual characteristics. The normal testicular size in adult men is greater than 15 ml in volume, however, testicular size in our patients was slightly less than the normal limit

Table1 - Main clinical data and laboratory result.

Clinical data	Mean	Median	Standard deviation	Minimum	Maximum
Sperm	2.56	0	10.58	0	58
FSH	10.5	8	7.50	1	32
LH	5.37	5	3.49	1	14
Testosterone	4.70	4.10	2.62	0.10	14.1
Age at diagnosis	9.01	9.10	2.45	5	15
Age at study	19.90	19.20	3.32	17	29

FSH - follicle stimulating hormone, LH - luteinizing hormone

Table 2 - Protocol of treatment.

Chemotherapy protocol	n	(%)
MOPP/ABVD	23	(69.7)
MOPP/ABVD+XRT	3	(9.1)
MOPP/ABVD+CCNU,VP16,Prednisolone	1	(3.0)
MOPP/ABVD+Vinbastin, Leukeran	1	(3.0)
MOPP/ABVD+COPP/ABVE	1	(3.0)
MOPP+Splenectomy	1	(3.0)
MOPP/ABVD+CCNU,VP16,MTX,CPA, Nathulane	1	(3.0)
MOPP/ABVD+CCNU,VP16,MTX	1	(3.0)
MOPP	1	(3.0)
Total	**33**	**(100.0)**

MOPP - mechlorethamine, oncovin, procarbazine, and prednisone,
ABVD - adriamycin, bleomycin, vinblastine, and dacarbazine,
XRT - radiotherapy, CCNU - lomustine, VP16 - vincristine, platinol
COPP - cyclophosphamide, Oncovin, procarbazine, prednisone
ABVE - Adriamycin, bleomycin, vincristine, etoposide
MTX - methotrexate, CPA - cyclophosphamide

for all study participants (sexual maturation rate [SMR] Tanner 4). The mean testis size was 4.5 cm (range, 3.5-5 cm) in length, with a volume of 17.5 ml (range, 14-20ml). Twenty-seven patients had azoospermia, 2 patients had severe oligospermia, and one case had a count of 6,000,000, and another case of 20,000,000, this case was diagnosed at the age of 6 years old and received MOPP/ABVD in 6 cycles, relapsed at age 16 and then received lomustine, methotrexate (MTX), and vincristine, platinol 16 (VP16) in 5 cycles, semen analysis was carried out at age 24 years. Another case had a normal sperm count (58,000,000), and received MOPP/ABVD in 6 courses, after 13 years off therapy relapsed and received ABVD in 7 cycles, and the sperm count was carried out 2 years after the second time off therapy. Two tailed pearson correlation was significant at level 0.01 between LH and testosterone, age at diagnosis with chemotherapy protocol.

Discussion. Treatment for HD has been very gonadotoxic. Azoospermia is seen in 80-90% of patients after 6 courses of MOPP. This testicular toxicity rate of the MOPP protocol can be attributed to alkylating agents (mechlorethamine and procarbazine).[10,11] The duration of azoospermia appears to be related to the proportion of stem cells killed. If all stem cells are eradicated, azoospermia will be irreversible.[12] Germinal aplasia documented by testicular biopsy and high serum FSH levels were found in patients treated by MOPP during childhood or puberty.[13] Substituting MOPP with MOPP/ABVD does not improve the MOPP-related oligo/ azoospermia according to this study, and to a previous study by Ben Arush et al.[14] Adult studies substituting cyclophosphamide with mustin (COPP) showed similar efficacies to MOPP and was associated with less testicular damage.[15] Hobbie et al[1] used COPP/ ABV, and 9 of 11 HD survivors were infertile, most likely because of procarbazine. Hassel et al[16] studied testicular function after OPA/COMP, vincristine, prednisone, Adriamycin/cyclophosphamide, vincristine, MTX, and prednisone. These patients showed no major testicular damage compared to males who had received OPPA/ COPP (including procarbazine), which is a potent gonadotoxic agent. Aubier et al[17] studied the testicular function of 30 adolescents or adult males who received chemotherapy during childhood, the mean follow up time was 9 years, 20 cases had azoospermia and all of them received MOPP or cyclophosphamide. Treatment during prepuberty did not protect the gonads from long-term azoospermia. Contrary to Aubier et al,[17] Ben Arush et al[14] and our findings, Pennisi et al,[18] found that prepubertal states protect the gonad from the harmful effects of cyclophosphamide in nephrosis patients, however, this finding for HD is not the rule. Dhabhar et al[19] suggested that ABVD should be used in young patients to minimize gonadal damage.

Follicle stimulating hormone stimulates spermatogenesis at several levels.[20] For example, it stimulates mitosis of Sertoli cells, increasing their number during puberty, however, the principle role of FSH in spermatogenesis is a quantitative one, and in suppressed FSH activity, spermatogenesis can be restored by LH alone.[21] This suggests that FSH is essential for initiation but not maintenance of spermatogenesis, and there may be normal FSH and LH in low sperm counts.[21] Treatment with an alkylating agent can cause temporary elevated LH in pediatrics but not adults, with normal testis size. Low level testosterone implying the presence of subclinical Leydig cell dysfunction.[5,22]

A multi center study of 110 children with clinically staged I-II A non bulky disease has become a model of success.[23] When using 4 cycles of VAMP (vinblastine, Adriamycin [doxorubicin], MTX, prednisone] and 15–25 Gy involved– field radiation to treat those children in this group with fewer than 3 nodal sites, there is a 5-year projected survival of 100%, and event–free survival of 97%. This combined modality regimen is associated with no serious toxicity, and confirms that clinically staged children with favorable risk HD can be cured with limited therapy that does not include an Alkylating agent bleomycin, etoposide, or high-dose extended-field radiation therapy.[24] The determination of elevated FSH may offer the most practical approach for predicting subsequent testicular damage. However, a normal FSH level in this group did not predict fertility as it did in our patients, and approximately 27 patients had FSH in the normal range with a sperm count of zero. However, lower sperm count correlates with high normal FSH. A semen analysis remains the gold standard for the prediction of fertility.[1]

In summary, among 33 HD survivors, 27 patients had azoospermia. The World Health Organization (WHO) says a normal sperm count consists of 20 million sperm per ejaculate, with 50% motility, and 60% normal morphology,[25] with adequate forward motility concentrations as low as 5-10 million that can produce pregnancy. With this definition, 4 cases (12.1%) had fertility, sperm count above 5,000,000 with 35-40% abnormal form and activity near normal. Despite normal secondary sexual characteristics, 3 cases had low levels of testosterone.

In conclusion, the study shows that a prepubertal status does not protect the gonads from the harmful effects of chemotherapy. Further attempts must be made to design regimens that are equally effective as standard combination chemotherapy, but have less late toxicity and with a lower risk of infertility. Advances

in reproductive technology have shown that sperm can exist in the testis but not in the ejaculate.[26] With testis sperm extraction (TESE) followed by intracytoplasmic sperm injection (ICSI), it is now possible for patients who did not bank sperm and have azoospermia on semen analysis to be evaluated for TESE/ICSI.

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