Original Article-I

Post-treatment Testicular Activity in Lymphoma Patients

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ABSTRACT

Objectives: **For young patients receiving cancer chemotherapy, the major concern is gonadal dysfunction with impaired reproductive capacity. The impact of such therapy in patients with lymphoma was focus of this study.**

Methods: **Semen analysis and serum FSH and LH levels were determined pretreatment, immediately after completion of therapy and at follow-up in 60 patients of lymphoma receiving chemotherapy.**

Results: **Pretreatment infertility was present in 13.2% of patients. After completion of treatment, 76.9% developed azoospermia. However, after a mean follow up of 56.66 months, percentage of normospermic patients declined to 43.3%, recovery being better in patients of age less than 30 years.**

Conclusion: **The effects of chemotherapy on testicular activity were significant with combination containing cyclophosphamide and procarbazine. Recovery in spermatogenesis and FSH levels to normal was seen in patients receiving cyclophosphamide, vincristine and prednisolone but was least when procarbazine was added to this. 90% patients continued to have azoospermia at 5 years in the group which received cyclophosphamide in combination with procarbazine.**

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INTRODUCTION

Currently, a significant proportion of young patients have long term survival following chemotherapy. This is true for lymphomas, germ cell tumours and acute leukemia etc. However, the major concern for young patients is gonadal dysfunction with impaired reproductive capacity.

Most of these chemotherapy protocols use the drugs in large doses and may be toxic to gonads. The degree to which testicular function is affected is dose and agent dependent .[1,2]

In men treated for lymphoma, recovery of spermatogenesis has to begin at the beginning after drug induced azoospermia occurs that continues until the germ cells repopulate the testes adequately for the spermatogenic process of differentiation and maturation. Azoospermia lasting only weeks to months reflects transient damage to late differentiating spermatogonia whereas a delay of years for recovery point to widespread damage of even early spermatogonia and stem cells.[2] Patients having the largest reductions in their sperm concentration after treatment require the longest recovery period for spermatogenesis.[3]

Germinal epithelium is particularly susceptible to injury by cytotoxic drugs and radiation due to its high mitotic rate although Leydig's and Sertoli's cell function is often preserved due to their very low proliferation index. This results in halted spermatogenesis, leaving testosterone function unaffected.[4] As the gonads and pituitary gland function in feedback mechanism style, serum assays of FSH and luteinizing hormone (LH) may well be useful markers for assessing the functional status of the germinal epithelium. Considerable evidence suggests that depletion of germinal epithelium leads to decrease in secretion of "INHIBIN" from Sertoli cells with subsequent elevation of serum FSH levels.[4] LH is a feasible marker for assessing Leydig cell function.[5]

In the earlier retrospective studies, determination of gonadal dysfunction following lymphoma treatment has been documented with the help of semen analysis and/or hormonal assays of FSH and LH. We studied hormonal assays-FSH and LH with sperm count to assess testicular function both before and after completion of the treatments to determine the effects of disease and the early and delayed effects of treatment.

METHODS

This study was conducted in the departments of Medical and Radiation Oncology at our institute between Jan 2002 - Dec 2003. The prospective group comprised of thirty newly diagnosed male patients of lymphoma. A written, informed consent was taken from the patients and ethical approval was sought from the institute ethical committee. Patients mean age was 29.45 years ranging from 18 to 42 years. All of them had histopathological confirmation of diagnosis of lymphoma and were staged as per Ann-Arbor staging. Besides a detailed sexual history, routine complete blood counts, ESR, liver and kidney function tests, serum LDH and uric acid, semen analysis and serum assays for FSH and LH were done by radio-immune assay prior to initiation of treatment. Prior to collection of semen and hormone profile, patients were asked for sexual abstinence for 72 hours, and blood samples were collected at 10.00 AM (to avoid diurnal variation). Semen was analyzed within half an hour of collection, for volume, consistency, sperm count, motility and morphology. On the basis of sperm count, patients were divided into normospermic with sperm count of $> 20 \times 10^6$/ml; oligospermic with sperm count $< 20 \times 10^6$/ml to 0.1×10^6/ml; severe oligospermic $< 0.1 \times 10^6$/ml; azoospermic with zero sperm count.

Patients received combination chemotherapy, radiotherapy (none received infra-diaphragmatic radiotherapy) as per the indications. During the treatment period, they were followed monthly to see the response and monitor adverse effects, if any. On completion of treatment, patients were reassessed for the disease as well as gonadal activity. All baseline investigations were repeated along with semen analysis and hormonal assay.

In the retrospective group, thirty previously diagnosed and treated male patients of lymphoma (mean age 26.58 years range, 18 to 40 years) were included. Patients were disease free for a minimum period of 3 years. These were taken up for studying the delayed response of treatment on testes side effects, congenital defects in the off-spring born after treatment and development of second malignancy. The data was compiled to find the recovery of spermatogenesis, treatment and effects of various factors at presentation, drug regimens, duration of follow-up and histology. To determine the effect of lymphoma on testicular activity, pretreatment results were compared with thirty age matched healthy males constituting control group. Patients with bulky disease received involved field radiation in the dose of 3000 to 3500 cGy in 3 weeks in addition to chemotherapy. However, no patient in the study group received, infradiaphragmatic radiation.

Statistics: Evaluation of the data was done using students 't' test (unpaired) for comparison of two groups where as analysis of variance test was used for comparing the two groups. For quantitative data chi square test was applied.

RESULTS

The study comprised of two groups with thirty patients in each group. Of the thirty patients in prospective group, all completed treatment and were available for post treatment analysis. All patients in retrospective group having achieved remission were under follow up and available for analysis. None of the patients had any evidence of second malignancy. The mean age of patients in prospective group was 29.45 years (range 18 to 42 years), and was comparable with retrospective group, mean age 26.58 years (range 18 to 40 years). In prospective group, stage I comprised seven patients, stage II fourteen, stage III four and Stage IV five, whereas in retrospective group, stage I comprised of four patients, stage II twenty, stage III and IV, three patient each.

Twelve patients in prospective group had B-symptoms compared to 18 patients in retrospective group. Twelve patients in prospective group had HL, 18 NHL compared to retrospective group, where seventeen had HL and 13 NHL. At completion of treatment, all the thirty patients in prospective group were evaluated, 90% (n=27) had achieved remission. Two patients had evidence of disease on ultrasonography and one patient had CNS relapse (infiltration by high grade immunoblastic lymphoma) who expired after two months.

The mean pretreatment FSH was 5.72 IU/L, increased five fold with treatment and declined gradually thereafter. The variation in FSH was associated with a parallel variation in sperm count, but the effect on LH was not significant (table 1). The mean FSH in patients with lymphoma (pre-treatment) was 5.72 IU/L compared to 3.45 IU/L in control group. Pretreatment sperm count was 52.59 $x10^6$/ml compared to 87.90 $x10^6$/ml in control group, the difference being significant where-as mean serum LH in patients was 6.72 IU/L compared to 6.32 IU/L, normal controls, p=ns (Table-1)

Difference in the testicular activity after treatment of HL and NHL is shown in Table 2. Various drug regimens used for the treatment on testicular activity had varied effect (table 3). COPP regimen being most gonado toxic. 100% patients were normospermic before treatment, at completion 91% had azoospermia and after follow-up of almost five years 90% continued with that. The effect of cyclophosphamide and procarbazine combination on sperm count and hormone levels (FSH) was significant. With CHOP regimen, recovery rate of normospermia at follow-up in our study was 25%.

When effects of chemotherapy were analysed according to age (< 30 years vs > 30 years) the difference in FSH, LH levels and sperm counts were significant (table 4). On follow-up sperm counts recovered in 61.11% of patients of < 30 years age and in 16.67% of > 30 years of age, $p<.05$.

Patients were also divided in two groups on the basis of their follow-up period. One with a follow-up of > 5 years and 2nd group with a follow-up of < 5 years. Fifteen patients had a follow up of > 5 years. Results are depicted as per table 5. The FSH and sperm count has significantly improved in patients with a follow-up of more than 5 years as compared to those having a follow-up of less than 5 years. Eighty percent of patients of less than five year follow-up period were azoospermic, compared to 33.3% patients having more than five year follow-up.

DISCUSSION

Hodgkin's and non-Hodgkin's lymphoma are important malignancies for studying the gonadal toxicity of chemotherapy and radiotherapy because it commonly affects the persons of reproductive age groups and is potentially curable in the majority of the patients.[6] Pretreatment infertility is a significant problem in males having Hodgkin's lymphoma, about 20 to 30% of patients have either azoospermia or oligospermia and large number of patients with Hodgkin's lymphoma have normal spermatogenesis with 24.5% having total sperm count of < 40 x 10^6/ ejaculate. There is decrease in semen quality in patients with stage III and IV Hodgkin's lymphoma.[7]

Table 1: Overall effects of chemotherapy on testicular activity (n= 60)

Values (Mean)	Pretreatment	After completion of Treatment	Follow-up (2 yrs)	p value
FSH (IU/L)	5.72	31.68	14.83	<0.05
LH (IU/L)	6.72	6.07	6.59	p=ns
Sperm count X 10^6/ml	52.59	10.89	19.62	<0.05

Normal values FSH (1.0 to 12.0 U/L) LH (2.0 to 12.04 U/L) Sperm count (20 – 150 x $10^{6/}$ /ml)

Table 2. Effect of chemotherapy on testicular activity in relation to type of lymphoma

Parameter	Hodgkin's lymphoma Mean (Range)	Non-Hodgkin's lymphoma Mean (Range)
FSH (IU/L)		
Pre-treatment	5.62(2.21 – 22.4)	5.80 (2.41 – 16.0)
Post treatment	39.92(4.56 – 99.24)	26.86 (2.58 – 99.6)
Follow up (2 yrs)	17.31(2.21 – 78.12)	9.06 (2.50 – 24.89)
LH (IU/L)		
Pre-treatment	5.37(3.0 – 9.4)	7.68(1.20 – 12.40)
Post treatment	4.96 (3.1 – 10.5)	6.82 (1.24 – 17.00)
Follow-up (2yrs)	7.72(2.0 – 23.35)	4.90(1.21 – 25.88)
Sperm count x 106/L		
Pre-treatment	57.91(0 – 90)	49.05(0 – 156)
Post treatment	6.42 (0-49)	13.86(0 – 86)
Follow-up (2 yrs)	14.47 (0 – 84)	29.84(0-96)

Difference between the pre-treatment, post treatment and follow-up (2 years) in Hodgkin's and non-Hodgkin's lymphoma being insignificant.

In our study 13.20% patients had azoospermia at presentation, four times more as compared to normal population. The mean FSH and LH also showed significant variation from that of normal population, comparable to other studies. Whitehead et al found sperm count of <20 x 10^6/ml in 20% of lymphoma patients [8,] and Cigessky noticed decreased sperm count in 40% of men with Hodgkin's lymphoma.[9] Comparatively low incidence of azoospermia in our study may be related to the selection of a particular age group (20 to 40 years) and majority of the patients had early stage disease (Stage I and II). The pathogenesis of pretreatment infertility is unclear and complex perhaps because of involvement of both pituitary and gonads.[9]

Ageing has been associated with hormonal changes leading to decreased spermatogenesis. In our study major difference in two groups was found at follow up in FSH levels, being significant in favor of age <30 years. More patients (61.11%) achieved normal sperm count having age <thirty years as compared to (16.67%) in age group of > 30 years (Table 4).

Difference in histology between Hodgkin's and non-Hodgkin's lymphoma did not reveal any significant variation in testicular activity, before or after treatment. Comparing the mean FSH,

Table 3. Effects of various drug regimens on testicular activity

Parameter	COPP Mean (range)	COP Mean (range)	CHOP Mean (range)	MOPP* Mean (range)
FSH (IU/L)				
Pre treatment	4.11(2.21-5.9)	3.95(2.41-5.6)	8.24(3.1-22.4)	-
Post treatment	34.12(4.56-69.41)	10.95(2.58-37.7)	41.52(4.68-99.6)	-
Follow-up (2 yrs)	22.71(5.4-78.12)	3.81(2.54-6.11)	12.34(2.5-24.89)	4.34(2.0-7.7)
P value	<0.05	>0.05	<0.05	
LH (IU/L)				
Pre treatment	5.39(3-11)	7.85(3.12-10.2)	7.37(1.2-12.4)	-
Post treatment	4.86(3.1-10.0)	7.98(3.12-17.0)	6.08(1/24-11.0)	-
Follow-up (2yrs)	9.58(3.12-23.35)	3.30(3.12-4.02)	5.88(1.21-25.88)	3.25(2.0-4.9)
P value	>0.05	>0.05	>0.05	
Sperm count $x10^6$/ml				
Pretreatment	56.90(18-90)	70.00(25-156)	38.50(0-107)	-
Post treatment	2.55(0-28)	37.14(0-86)	3.21(0-29)	-
Follow-up (2 yrs)	2.0(0-24)	54.80(24-96)	14.25(0-85)	44.4(29-84)
P value	<0.05	>0.05	<0.05	

COPP: Cyclophosphamide 650mg/m2 d1-d8(total 7.8 gm/m2), vincristine 1.4 mg/m2 d1, d8, procarbazine 100 mg/m2/d d1-d14, prednisolone 60 mg/m2/d d1-d14) [Total 6 cycles]

COP: Cyclophosphamide 750 mg/m2 (total 4.5 gm/m2), vincristine 1.4mg/m2 (total 8.4 mg/m2), prednisolone 60 mg/m2/d d1-d5 [Total 6 cycles]

CHOP: Cyclophosphamide 750 mg/m2 (total 4.5 gm/m2), Adriamycin 50 mg/m2 (total 300 mg/m2), vincristine 1.4mg/m2, prednisolone 100 mg/m2 x 5 days (total 3 gms)

MOPP: Mechlorethamine 6 mg/m2 d1, d8 (total 72 mgs/m2), procarbazine 100 mg/m2 d1-d14 (total 8.4 mg/m2) [Total 6 cycles]

*MOPP was not given to any patient in the prospective group, hence no pre-treatment and post-treatment values were known.

LH and sperm counts in two groups, it was insignificant. The percentage of patients who achieved normospermia at follow up did not vary significantly. As per the drug regimens used COPP, has been documented to be most gonadotoxic. Procarbazine containing regimes result in prolonged azoospermia in the vast majority of patients [2,6, 10-17] .But much lesser degrees of long term gonadal toxicity are apparent with the newer forms of chemotherapy.[1, 3, 6] In our study patients who received COPP, 100% patients had normospermia before treatment. More than 90% continued to have azoospermia at a follow up of around five years, which is similar to other studies[10,11] (table 3). FSH levels had a gradual decline, but continued to remain four fold higher than normal. COP was the safest regimen used. FSH had a two fold rise, but returned to normal at follow-up with sperm count showing an increase. Roeser et al has demonstrated azoospermia in 30% patients at a follow -up of 34 months.[18] Cyclophosphamide in a dose of 4 to 7 gm/m2 produced azoospermia in all men, but recovery occurred in 100% after a follow up of 31 months.[12] Lopez et al in their study showed cumulative cyclophosphamide dose and basal follicle stimulating hormone (FSH) levels were identified as independent factors associated with azoospermia or severe oligo-azoospermia.[19] Pryzant et al demonstrated 100% azoospermia following chemotherapy

Table 4. Effects of chemotherapy on patients below and above the age of 30 years

	Age < 30 years (mean 22 years) (n=33)				Age > 30 years (mean 36.9 years) (n=27)			
Value	Pre-Treatment Mean (Range)	Post - treatment Mean (Range)	FU Mean (Range)	*p*	Pre-treatment Mean (Range)	Post-treatment Mean (Range)	FU Mean (Range)	*p*
FSH (IU/L)	6.36 (2.41-22.4)	24.51 (2.56-49.22)	9.35 (2.0-38.96)	<0.05	5.09 (2.21-(12.7)	38.85 (4.68-99.6)	20.31 (5.12-78.12)	<0.05
LH (IU/L)	7.11 (1.2-12.4)	5.80 (1.24-11.0)	6.19 (1.21-25.88)	>0.05	6.40 (3.0-10.1)	6.35 (3.1-17.0)	7.00 (3.12-23.35)	>0.05
Sperm count X 10^6/ml	50.33 (0-156)	13.97 (0-86)	27.00 (0-96)	<0.05	54.86 (0-107)	7.80 (0-49)	12.25 (0-84)	<0.05

F.U: Follow-up, years

Table 5: Effects of chemotherapy on testicular activity as per follow-up period

Values	FU > 5 years (Mean 80.26 months) (n=15)	FU < 5 years (Mean 33.07 months) (n=15)	p value
FSH (IU/L)	5.80 (2.0-11.81)	21.64 (2.59-78.12)	<0.01
LH (IU/L)	5.71 (1.21-25.88)	7.33 (3.12-23.35)	>0.10
Sperm count x106/ml	27.53 (0-84)	14.73 (0-96)	>0.10

FU: Follow-up

with CHOP combination, 66.6% of whom showed recovery to normal counts at a follow up of 7 years.[20] The recovery rate in our study was 25% at follow-up, where as FSH levels showed a decline to 12.3 IU/L at follow-up from a level of 41.52 IU/L at the completion of treatment (Table 3).

Only 5 patients had received MOPP regimen in the retrospective group, hence comparison could not be made as compared to pre-treatment values. 100% had achieved normospermia and normal FSH and LH levels at follow-up, comparable with other studies [18, 21]. Sherins et al postulated that recovery of spermatogenesis even after extensive chemotherapy is possible, more likely to occur after more than 2 years of follow up. [22] Patients having the largest reductions in their sperm concentration after treatment required the longest recovery period for spermatogenesis.

This is evident from a study conducted by Bahadur et al in patients with lymphoma and leukemia over a 26 year period.[4] Chapman et al found that recovery in spermatogenesis was unlikely after five years.[23] In our study also, difference in mean FSH levels in patients of <5 year or >5year follow-up was significant; recovery of normal sperm counts in 66.6% of patients at >5 year follow up compared to 20% normospermia in patients having <5 year follow-up ($p<0.01$)(Table 5). The mean pre-treatment FSH was 5.72 IU/L, which after completion had five fold rise and then declined to 14.83 IU/L, associated with a similar variation in sperm count as depicted in table 1. The percentage of normospermic patients also showed significant variation from pre-treatment values of 82.5% to 16.50% post-treatment and 42.9% at follow-up ($p<0.01$). The severity of the reduction in sperm concentration following treatment is unpredictable; likely to be more severe in patients treated with radiotherapy and alkylating agents. [24]

To conclude the effect of cyclophosphamide and procarbazine containing chemotherapy is significant compared to the patients who received combinations without procarbazine. Recovery of testicular functions was less likely with the addition of procarbazine to chemotherapy regimens even after a prolonged period (5 years).

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