Effect of Chemotherapy on Semen in Chronic Myeloid Leukemia (CML) Patients

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Abstract. Decreased fertility has been reported in patients suffering with various cancers. Use of chemotherapy and radiotherapy for treatment of cancer may further add to reduced fertility. It has been reported that the disease itself and the therapy may contribute to deficient spermatogenesis or sperm transport in male patients. Therefore the present study was planned to assess the effect of disease (Chronic Myeloid Leukemia) and chemotherapy on semen and reproductive functions. Semen analysis was carried out in 10 untreated patients (who were yet to start therapy) and in 14 CML patients undergoing chemotherapy. Preliminary results of the present study show that Chronic Myeloid Leukemia affects spermatogenesis in male patients. Treatment with chemotherapeutic drugs further affects spermatogenesis and sperm transport. To confirm the findings of the present study, a large number of untreated and treated cases need to be followed up.

Key words: CML, Chemotherapy, Fertility, Semen quality, Structural changes.

Introduction:

Chronic Myeloid Leukemia (CML) is a clonal myeloproliferative disorder. It results from neoplastic transformation of the primitive haematopoietic stem cell. CML accounts for 7-15% of all leukemia's in adults ranging from 1-1.5 cases per 1,00,000 population. (Morrison, 1994).

The Philadelphia (Ph) chromosome is found in the malignant cells in more than 90% of patients with CML. It is the result of breaks on chromosomes 9 and 22, with a reciprocal translocation of the distal genetic material, t (9; 22), (q34; q11). This translocation transposes the c-abl protooncogene from its normal location on chromosome 9 to a new position on chromosome 22, in proximity to the breakpoint cluster region (bcr). A new hybrid bcr-abl oncogene is formed. It produces an abnormal 8.5 kb on RNA that encodes for a 210 kD (P210) fusion protein. This protein has increased tyrosine kinase activity than its normal equivalent (P145) and has shown transforming capacity in the normal haematopoietic cells into CML cells.

Until 1980, hydroxyurea and busulfan were the two most effective anti-CML agents. They were superior to irradiation or other drugs, such as melphalan, 6 mercaptopurine and chlorambucil. They provided excellent disease control with minimal toxicity, but were inexpensive and administered orally. Cytogenetic response apart from hematological remission is rare. Interferon (IFN) as a biotherapy has given better prognosis leading to cytogenetic remission and prolonged survival. It is only the bone marrow transplantation (BMT) which has altered the advancement towards the final phase, though it can be applied to a limited group of patients. Thus, an optimal therapy for CML is still not final.

Many side effects of the above mentioned drugs in relation to sexual functions are known. Busulfan was associated with unpredictable prolonged myelosuppression, organ fibrosis (lungs, heart, and marrow) and Addison's like disease. The major toxic effects of hydroxyurea are leukopenia, megaloblastic anemia and thrombocytopenia. Rare side effects are stomatitis, alopecia and neurological manifestations like depression. Interferon-alpha is associated with early flu like side effects (fever, chills, anorexia, lack of appetite) in most patients, which are not dose limited and can be managed symptomatically. Late side effects are dose limiting in 10% to 25% of patients and include persistent fatigue, weight loss, neurotoxicity (depression), a triad of depression, fatigue and insomnia). Hypogonadism is reported with Busulfan therapy in CML patients. The Italian Cooperative Group of Study reported that survival advantage with cytogenetic response was seen with interferon therapy. (Italian Co-operative group on CML, 1994).
Wetzler et al (1995) reported that impotence in men is not infrequent with interferon therapy.

The development of newer and more active antineoplastic agents, increasing use of combination chemotherapy and improvements in supportive care have increased the survival and cure rates of many malignant diseases including acute leukemia, Hodgkin’s disease, Burkitt’s lymphoma and germinal testicular tumors. The antifertility effects are among the most important. The depressant effects of various agents on spermatogenesis have been reported. It is believed that azoospermia and severe oligospermia usually are seen after intensive chemotherapy. Thachil et al. (1981) showed that cancer itself seems to have an adverse effect on fertility before any form of treatment. CML generally occurs in the 4th decade of life. Sexual and reproductive functions, which are one of the most primary biological functions in man, may be affected. Therefore, we have studied the effects of disease and therapy (hydroxyurea and interferon) on male reproduction, using semen analysis. Testicular biopsies are not possible in these patients because of ethical reasons. For this study it was planned to carry out semen analysis in clinically diagnosed CML patients before and while on chemotherapy.

Material and Methods :

Patients : Thirty four diagnosed cases of Chronic Myeloid Leukemia (CML) and ten healthy married people as normal controls were considered for the present study. These included both treated and untreated patients belonging to various age groups. Treated patients were on hydroxyurea and in some at later stage were put on combined therapy with α-Interferon. These cases were referred from the Haematology Clinic, All India Institute of Medical Sciences, New Delhi. Haematological and clinical data were collected in a pre-designed proforma from the case sheets in consultation with the concerned clinicians. All cases were analysed cytogenetically for Philadelphia chromosome (results are reported elsewhere).

Semen Analysis : For evaluation of effect of chemotherapy in chronic myeloid leukemia (CML) patients the various parameters like volume, consistency, viability, total sperm count and sperm morphology were seen by using WHO laboratory manual (1992).

Sample collection and delivery : Samples were collected after a minimum duration of 48 hours and not longer than 7 days of sexual abstinence. The samples were obtained by masturbation and ejaculated into a clean, wide-mouthed glass container in a separate room next to the laboratory and delivered to the laboratory immediately. These were left at the room temperature for 30 minutes to one hour for liquefaction. After liquefaction the total seminal fluid volume was measured in a graduated cylinder. Consistency was estimated by gently pushing the semen through an injection needle (21G-diameter) and length of thread was observed. The samples were further processed for measurement of sperm motility, viability, total sperm count and morphology.

Motility : After liquefaction, samples were mixed thoroughly and a drop of specimen was delivered onto a clean glass slide and covered with the cover slip. The weight of the coverslip spreads the sample for optimal viewing. The freshly made and wet preparations of slides were left to stabilise for one minute.

The microscopic field was scanned carefully and motility of each spermatozoa was noted. The categories used for classifying sperm motility were designated as a, b, c, d and defined as follows.

(a) if the spermatozoa had a rapid and linear motility (good);
(b) if it had a slow or sluggish linear or non-linear movement (moderate or weak);
(c) if it had a non-progressive motility
(d) if the spermatozoa is immotile;

Usually 4 to 6 fields were screened and one hundred successive spermatozoa were classified to get the percentage motility.

Viability : A drop of semen was mixed with a drop of 0.5% eosin on a slide and covered with a coverslip. The slides were screened and a minimum of one hundred spermatozoa (unstained and stained) were counted under the high power of light microscope. Dead sperms appeared stained and
live ones were shiny or unstained. The sperm viability was expressed as percentages.

**Spermatozoa concentration**: The well mixed 50ml of liquefied semen was diluted with diluent (consisting of 50 gms. NaHCO3 of 35% v/v formalin and distilled water) in a small clean glass tube. The diluted specimen was mixed thoroughly and a drop was transferred to a standard haemocytometer and covered with a coverslip. Cells were allowed to sediment in a moist chamber and then counted under a light microscope at a magnification of 100X Counting was done as follows:

The central square of the grid of Neuber haemocytometer containing 25 large squares was counted for samples containing less than 10 spermatozoa per square. For samples containing 10-40 spermatozoa per square, 10 squares were assessed and for samples containing more than 40 spermatozoa per square, 5 squares were counted. A spermatozoa lying on the dividing line was counted only if it was on the upper or left side of the square.

The concentration of spermatozoa in the original semen sample in millions/ml was obtained by multiplying the number of spermatozoa counted with the conversion factors.

**Sperm morphology**:

*Light Microscopy*: a drop of semen was smeared onto a slide, air dried and fixed in equal parts of ether : ethanol (95% v/v). Smears were then stained using Giemsa stain.

Dried slides were scanned under oil immersion in 100X objective to assess morphological abnormalities of sperm head, mid-piece and tail. A minimum of 500-1000 sperms were screened, & photographed for morphological abnormalities.

*Scanning Electron Microscopy (SEM)*: The SEM study of spermatozoa was undertaken to evaluate any ultrastructural abnormalities in the surface morphology of spermatozoa obtained from patients treated with HU or HU and IFN-a as compared to normal group.

For SEM studies, one or two drops of semen after liquefaction were fixed in 1 ml of 2% gluteraldehyde for 10 minutes. The sample was centrifuged for 5 minutes at 1500 rpm and supernatant was discarded. The cell suspension was washed with 0.1 M phosphate butter, then in distilled water. The cells were washed with distilled water, centrifuged and supernatant discarded. The cells were resuspended in 1 ml distilled water and smeared on glass coverslip and air dried. Sputter coating with gold was done. The coated samples were observed and photographed under SEM Electron Microscope (Philips 501BA).

**Results**:

A total of thirty four patients were included in the study, of which 24 were treated with HU or a-IFN and 10 were untreated. Of the 24 treated patients, 2 were unmarried and rest of them had living and healthy children. Eight patients from this group became impotent after 3-5 months of therapy. Therefore, semen analysis was possible only in 16 patients at the time of first analysis. After 3-5 months of therapy, semen analysis was done only in 14 patients because one patient became impotent and one patient discontinued follow-up. It seems that chemotherapy has some effect on the potency in male CML patients.

The volume of semen was 0.5 ml to 2.5 ml at the time of 1st analysis and follow up. Out of 16 patients, 9 were azoospermic at the time of 1st analysis and one more patient became azoospermic at the time of 2nd analysis. Disease and chemotherapy seem to have depressant action on spermatogenesis. The spermatozoa were immotile in 2 patients at the time of first analysis. After continuing chemotherapy the motility decreased even further. The spermatozoa were not viable (70-80%) in 5 patients at the time of first analysis and in 4 patients at the time of 2nd analysis. The total sperm count was low (0.25 mln - 12 mln) at first analysis and showed further decrease in count (0.25mln - 4 mln) after continuing chemotherapy for 3-5 months.

In 10 untreated patients, 5 were unmarried and 5 had healthy living children. Out of 10 untreated patients, 2 were impotent.

Therefore, semen analysis was possible only in 8 patients before therapy. The findings were
compared with the five individuals form the general population. The preliminary results on these 8 patients showed that there was significant (p < 0.05) reduction in the motility, total sperm count, viability and volume in CML patients when compared to that of normal individuals. These patients are being followed up after chemotherapy. Studies on larger number of patients for a longer period (follow up) are necessary to confirm the effect of chemotherapy on semen in CML patients.

Spermatozoa morphology:

Sperm morphology was studied in CML patients undergoing therapy and in normal control.

(a) Light Microscopy:

In normal group, most of the spermatozoa had an oval shaped head with regular outline and acrosomal cap was covering more than 1/3 of the head surface. The midpiece was cylindrical, less than 1/3 of the width of the head, straight and regular in outline. The tail was slender, uncoiled and presented a regular outline. Twenty to 25% of spermatozoa showed abnormalities of the head, midpiece and tail, while in CML patients about 40-50% spermatozoa were deformed. There was no obvious difference in the morphology of spermatozoa from normal control semen and of untreated patient samples. The abnormalities of head and tail were seen as pin head, small oval head, tapered head, pyriform head and amorphous head and coiled tail.

(b) Scanning Electron microscopy:

The ultrastructural study of the surface morphology of spermatozoa was done in semen sample in normal group & untreated patient (Fig. 1a-f).

The normal morphology of sperms were confirmed as an oval shaped head with regular outline and acrosomal cap covering more than 1/3 of the head surface. The midpiece was slender, less than 1/3 of the width of the head, straight and regular in outline. The tail was straight, uncoiled and presented a regular outline. In CML patients, the surface morphology of spermatozoa was found to be quite deformed in many sperms. The deformities of head were pin head, pyriform head, small oval head, duplicate and amorphous head. The acrosomal cap was covering less than 1/3 of the head surface. The midpiece was swollen near the head, which was tapered towards the tail and broken. The outline was not regular. The tail was cylindrical with irregular outline, coiled, broken and fragmented in majority of the spermatozoa. Some of the observations specially the surface and fragmentation were not very clear under light microscopic examination. SEM studies while confirming a light microscopic observation had shown irregular surface and fragmentation. The fragmentation may be the result of damage rendered by chemotherapeutic drugs which weakened the spermatozoa.

Discussion:

The results of surgery, radiotherapy, chemotherapy & bone marrow transplantation for malignant disease traditionally have been calculated on the basis of survival rates alone. However, other outcome criteria emerge if therapy is curative. Surviving patients who are in the reproductive age group may wish to have children after an interval free of disease. Therefore, we undertook a prospective study of semen analysis from newly diagnosed patients with CML to establish the impact of therapy on semen quality.

It is reported that a good number of patients with cancer have decreased potentials fertility. Sensitive drugs and irradiation may lead to infertility. Infertility after therapy must not simply be attributed to treatment itself since it also may be related to pre-existing deficient spermatogenesis or sperm transport because of disease.

In the present study most of the patients had live, healthy children before the diagnosis of disease. At diagnosis before treatment, 2 out of 10 patients were found to be impotent. Majority of the patients showed decrease in sperm count (0.25 to 12 million).

These data clearly suggests that CML disease condition also affects spermatogenesis. Following the treatment deficient spermatogenesis and sperm transport are very pronounced. The present study had the advantage that most of the patients were
married and had normal living children. *Thachil et al* (1981) reported that leukemia, Hodgkin’s disease, non-Hodgkin’s lymphoma and testicular tumours constitute the most prevalent malignancies in men in the reproductive age groups. Most of these patients are not married at the time of diagnosis therefore it is not possible to have reliable fertility data.

A few instances of impotence have been reported, which may be psychogenic (Chapman *et al*., 1979). In the present study, impotence was observed in 2 patients before treatment of CML.

It is believed that chemotherapy with certain drugs produces germinal aplasia with resultant oligospermia and azoospermia. Recently, reduced sperm count has also been reported by Arai *et al* (1997) and Botchnan *et al* (1997) in testicular cancer. The exact events of spermatogenesis sensitive to specific agents are being investigated in animal systems. In the human alkylating agents are the most significant in inducing sterility.

Gonadal dysfunction in patients receiving chemotherapy for cancer results in clinically marked decrease in testicular volume, oligospermia, or azoospermia and infertility. It is also associated with marked elevations of serum follicle-stimulating hormone (FSH) levels (Van Thiel *et al*., 1972). This finding suggests that the seminiferous tubule may be a site for feedback inhibition of FSH secretion (Schilsky *et al*., 1980). The occurrence of infertility in men receiving single alkylating agent therapy is clearly dose related. Effects of chemotherapy on ovarian function can be assessed through development of amenorrhea, menopausal symptoms, & estrogen deficiency symptoms. Evaluation of the effects of chemotherapy on ovarian function is hampered by the relative inaccessibility of the ovary to biopsy.

Side effects of long term chemotherapy such as infertility must be considered unavoidable. There is not much of data available on newly introduced chemotherapeutic drugs such as an a-Interferon and even on hydroxyurea. In the present study most of the patients were on hydroxyurea and some received a-Interferon at some stages. It is not possible to comment on effects of a-Interferon on fertility as a single drug at present.

A similar pattern of germ cell loss followed by slow recovery has been observed in patients who have undergone chemotherapy for various diseases. Single-agent chemotherapy, as for example the administration of cyclophosphamide for nephrotic syndrome, is more likely to be followed by recovery of fertility, whereas multiagent chemotherapy, such as the use of MOPP (mustine, vincristine, procarbazine and prednisolone) in the treatment of lymphomas and leukaemias, or the use of VBP (vinblastine, bleomycin and cis-platin) in the control of testicular malignancies, is often accompanied by complete germ cell loss as well as leydig cell dysfunction with a low incidence of recovery.

A particularly grave situation is that of acute lymphocytic leukemia of childhood, which has a tendency to infiltrate the testes. Since the testicular involvement may be detected during bone marrow remission and is often the first sign of a relapse of the disease, the clinical suspicion is that the leukemic cells in the testes are capable of re-seeding the bone marrow, leading to a systemic recrudescence of the disease with time. For this reason, bilateral testicular biopsies are now routinely performed in most centers before systemic chemotherapy is stopped, if testicular leukemic infiltrates are found, vigorous attempts are usually made to eradicate them. To achieve this goal, direct X-irradiation of the gonads with dosages as high as 2000 to 2500 rads is used, along with intensified multiagent chemotherapy. With such measures, not only the germ cells, but also the leydig cells are damaged beyond recovery, the androgen replacement therapy is often necessary to bring about pubertal development. Clinically, the patients have elevated FSH and LH. The plasma testosterone is low and gives a less than normal response to HCG stimulation or not at all. In patients who survive into the postpubertal period, azoospermia is common.

Since many young patients with lymphomas, leukemias, and testicular cancers are now enjoying long-term survival following high-dose radiation and combination chemotherapy, increasing attention is focussed on the extent of damage to the gonads and the potential for future fertility following such
modes of treatment. Because many of the agents used are not only toxic to the germ cells, but are also mutagenic and teratogenic, the problem is considerable (Schilsky et al., 1980).

Routine semen analysis was performed using WHO standards for semen analysis. The sperm count, motility and morphology do not correlate absolutely with fertility, even well-performed semen analysis cannot be sufficiently diagnostic in many instances. The reports are available that semen samples with counts as low as 6 million/ml have been associated with pregnancy while those as high as 330 million/ml were found with fertility impairment. Morphology appears to be the best predictor of fertility potential among the routine semen parameters.

Semen samples in the present study were examined under scanning electron microscopy in order to examine the surface of the sperms under high magnification. The observations have confirmed some of the findings of light microscopic examination.

Preliminary results of the present study and the available literature show that cancer itself seems to have adverse effect on fertility before any form of treatement. On treatment, the quality of semen in majority of the patients was poor and resulted in oligo and azoospermia. Hence sperm banking may be a helpful alternative for future reproduction in these patients.

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References :
Figure 1
Ultrastructural photomicrographs showing morphology of Spermatozoa 1a and 1b are from normal individual and 1c to 1f are from CML patients showing normal sperm (N), deformed head (DH), double head (DOH) and swollen midpiece (SM). Magnification of 1a and 1c is 1800X, 1d is 3500X and of 1b, 1e and 1f is 7000X.