

Effect of chemotherapy on sperm DNA fragmentation

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DOI: 10.22034/PMJ.2020.46377

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Submitted: 2020/05/20

Accepted: 2020/07/12

Keywords:

Sperm DNA fragmentation
Chemotherapy
Infertility
Cancer

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Abstract

Chemotherapy and radiotherapy adversely affect spermatogenesis, a consequence that is particularly relevant to young men who have yet to establish families. The harmful effects of chemotherapy on spermatogenesis are variable, depending on the type and dosage of chemotherapeutic agents used. The current study assessed the effect of cancer therapy on sperm DNA fragmentation by Comet assay. Sperm DNA fragmentation was evaluated in cured cancer patients. The results showed a significant relationship between chemotherapy and double-stranded and single-stranded sperm DNA fragmentation.

INTRODUCTION

Spermatozoa are highly specialized cells tasked with delivering an intact paternal genome to an oocyte and supporting the successful development of an embryo (1). Both genetic and epigenetic alterations can impair these tasks. In the last two decades, research has been focused on sperm DNA fragmentation, as the presence of DNA breaks represent the most frequent genetic anomaly found in ejaculated human spermatozoa (2). In particular, the discovery that high levels of sperm DNA fragmentation (SDF) can be found in the spermatozoa of sub/infertile men has raised concerns regarding the reproductive functions of these men and, most importantly, the health of the offspring. Accumulating evidence indicates that several lifestyle factors influence SDF levels (3). In addition to lifestyle and radiation, some diseases are known to increase SDF. Chemotherapy and radiotherapy may detrimentally affect gonadal function and spermatogenesis, resulting in impaired fertility (4). Such treatment may leave men azoospermic or with varying degrees of spermatogenic compromise. High levels of sperm DNA fragmentation have been implicated in delayed assisted conception, higher miscarriage rates, increased pregnancy loss, and adverse effects on the short- and long-term health of children born from assisted reproductive technologies (5). Currently, there is insufficient evidence regarding the impact of malignancy on sperm DNA, but there are concerns that those with cancer may have a higher level

of sperm DNA damage. Chemotherapy and radiotherapy adversely affect spermatogenesis, a consequence that is particularly relevant to young men who have yet to establish families (6). The harmful effects of chemotherapy on spermatogenesis are variable, depending on the type and dosage of chemotherapeutic agents used (7). Moreover, it is not possible to predict with certainty whether spermatogenesis will return to normal after therapy.

The current study assessed the effect of cancer therapy on sperm DNA fragmentation by Comet assay. Sperm DNA fragmentation was evaluated in cured cancer patients. The treatment process of all subjects included chemotherapy and radiotherapy.

METHODS AND MATERIALS

Semen samples from 10 males were obtained in collaboration with reproduction center. Each person provided two samples: a frozen semen sample (semen cryopreservation) taken from patients before the treatment process and a sample taken from the patient after the treatment and recovery process. In this study, comet assay was used to examine sperm DNA fragmentation. In this technique sperm cells are mixed with agarose and layered on a microscopic slide. Detergents and high salt concentrations are used to lyse their cell membranes and remove nuclear proteins (protamines and histones), which relaxes the DNA into a supercoiled nucleoid structure. The

nucleoids are subjected to an electrophoretic field resulting in the migration of the broken strands of DNA through the agarose. Cells with DNA damage will resemble comets when visualized using a fluorescent dye, with the comet's tail length and fluorescent intensity proportional to the degree of DNA fragmentation. The non-fragmented DNA remains in the nucleus or comet's head. Alkaline and neutral comet assay procedures, staining, and classification of fragmented or non-fragmented sperm were performed on all semen samples. Intra-individual differences were measured in five samples, and the variability mean was less than 5% of SDF for both alkaline and neutral comet assays. Alkaline and neutral comet assays were performed simultaneously on two different slides. First, an aliquot of the total semen was thawed and washed three times in PBS. Then, sperm cells were diluted to a concentration of 10×10^6 spermatozoa/ml, and 25 μ l was mixed with 50 μ l of low melting point agarose 1% in distilled water. Rapidly, 15 μ l of the mixture was placed on two different pre-treated slides for gel adhesion (1% low melting point agarose), covered with coverslips, and allowed to jellify on a cold plate at 4 °C for 5 minutes. Next, coverslips were carefully removed; slides were submerged for 30 minutes in two lysing solutions and washed for 10 minutes in TBE. For the neutral comet assay, electrophoresis was performed in TBE buffer at 20 V (1 V/cm) for 12 minutes and 30 seconds; then slides were washed in 0.9% NaCl for 2 minutes. For

the alkaline comet assay, the slide was incubated in denaturing solution (0.03 M NaOH, 1 M NaCl) for 2 minutes and 30 seconds at 4 °C; electrophoresis was then performed in 0.03 M NaOH buffer at 20 V (1 V/cm) for 4 minutes. After that, both neutral and alkaline slides were incubated in the neutralizing solution (0.4 M Tris-HCl, pH 7.5) for 5 minutes and in TBE for 2 minutes. Finally, the slides were dehydrated in an ethanol series of 70%, 90%, and 100% for 2 minutes each. Statistical analysis of SDF data was performed using SPSS software. The Mann-Whitney U test was used to compare samples, and the confidence interval was set at 95%.

Results

Of the 20 semen samples collected from donors, comprising 10 samples cryopreserved before chemotherapy and 10 samples collected after chemotherapy, 10 samples (100%) taken before chemotherapy presented a profile with low values for both ssSDF and dsSDF, and 8 samples (80%) after chemotherapy presented a profile with high ssSDF and dsSDF values. Results and statistical comparisons of the data obtained by using both alkaline and neutral comet assays are shown in [Table 1](#) and [Figure 1](#). The results showed a significant relationship between chemotherapy and double-stranded and single-stranded sperm DNA fragmentation.

Table 1. Comparisons of data using both alkaline and neutral comet assays

Sample number	Alkaline Comet (%SDF)	Neutral Comet (%SDF)
Before chemotherapy (n=10)	1/10 10%	2/10 20%
After chemotherapy (n=10)	8/10 80%	9/10 90%

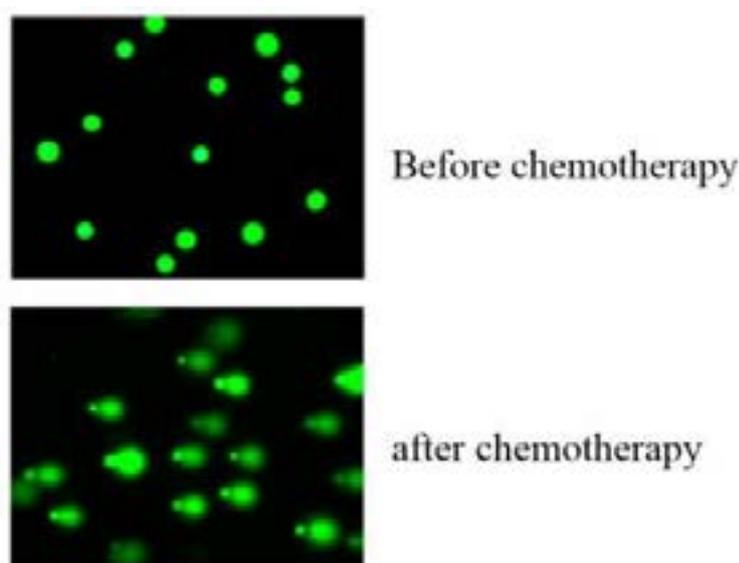


Fig. 1. Comet assay before and after chemotherapy

DISCUSSION

Cancer treatments are well-known to adversely affect male fertility. Reduction of sperm output arises from the cytotoxic effects of chemo- or radiotherapy upon the spermatogenic epithelium (8). However, if the epithelium survives, there is a hazard to reproduction as the treatments are also mutagenic. Some studies have shown that sperm DNA fragmentation (SDF) is increased in male semen samples after chemotherapy (9). High levels of sperm DNA fragmentation have been implicated in delayed assisted conception, higher miscarriage rates, increased pregnancy loss, and adverse effects on the short- and long-term health of children born from assisted reproductive technologies (10). A Dutch study in 2010 identified 764 male cancer patients presenting for semen cryopreservation before chemotherapy and radiotherapy. Almost two-thirds of all semen samples were abnormal, and men with testicular germ-cell cancer ($n = 292$) had significantly lower sperm concentrations (11). Currently, there is insufficient evidence regarding the impact of malignancy on sperm DNA. Moreover, there is limited and conflicting evidence on the effect of cancer on sperm DNA fragmentation rates. Smit et al. studied a population of 127 patients diagnosed with various malignancies. Only patients with non-Hodgkin lymphoma ($n = 15$) had increased DNA fragmentation of 25.1% (95% CI 8.7%–66.7%). Another study showed that sperm DNA fragmentation was significantly higher in cancer patients than in healthy fertile control subjects, but only in patients with testicular germ-cell cancer or Hodgkin lymphoma (12). Stahl et al. investigated DNA fragmentation in 74 patients with testicular cancer and 2,778 military conscripts who served as a control population (16). Interestingly, DNA fragmentation increased to 18% in those who had radiotherapy treatment and decreased to 9.1% in those who had chemotherapy (13). Those who had higher doses of chemotherapy had an even greater reduction in DNA fragmentation to 7.3%. In the current study, the structural changes of DNA in cancer patients

before and after chemotherapy were evaluated. The results showed a significant relationship between chemotherapy and double-stranded and single-stranded sperm DNA fragmentation.

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