DNA damage and chromosomal aberrations in various types of male factor infertility

Fotini Papachristou, Ph.D.,[a,b] *Maria Simopoulou, Ph.D.,*[b] *Stavros Touloupidis, Ph.D., M.D.,*[c] *Christos Tsalikidis, M.D.,*[b] *Nikolaos Sofikitis, Ph.D., M.D.,*[d] *and Theodore Lialiaris, Ph.D., M.D.*[a]

[a] Departments of Genetics, [b] Experimental Surgery, [c] Urology, Medical School, Democritus University of Thrace, Alexandroupolis; and [d] Department of Urology, Medical School, University of Ioannina, Ioannina, Greece

Objective: To understand and delineate the nature and level of DNA damage in association to semen parameters in infertile men.
Design: A prospective experimental study.
Setting: Alexandroupolis University General Hospital.
Patient(s): Eleven fertile and 27 infertile men with various types of infertility.
Intervention(s): DNA damage was induced by addition of mitomycin C and caffeine to lymphocyte cultures.
Main Outcome Measure(s): Sister chromatid exchange (SCE) levels were assessed in cultures providing a quantitative index of genotoxicity and chromosomal analysis was performed using G-banding and C-banding techniques.
Result(s): Karyotyping analysis indicated chromosomal fragility, trisomic lines, and marker chromosomes in some infertile men. Double minute chromosomes were noticed in 11 infertile men and were positively correlated with elevated SCE levels. Necrospermia and varicocele, irrespectively of the degree of severity, were positively correlated with elevated SCE levels.
Conclusion(s): Infertile men are prone to have DNA damage; the nature and level of DNA damage varies and is associated with semen parameters. The presence of double minute chromosomes alone is associated with increased double-stranded breaks and abnormal sperm concentration. This study could provide the basis to establish whether and through which process double minute chromosomes could be related to poor semen parameters and regulation of DNA repair. (Fertil Steril® 2008;90:1774–81. ©2008 by American Society for Reproductive Medicine.)

Key Words: Genetic instability, male infertility, chromosomal abnormalities, sister chromatid exchange, double minute chromosomes

The genetic basis of infertility varies including structural or numerical chromosomal abnormalities (1). Infertile individuals have demonstrated increased chromosomal fragility compared with fertile men (2). Chromosomal fragility is defined as the generation of mutations by physical, chemical, or biological factors in various sites, in one or more chromosomes, in one or more cells, in elevated frequencies compared with normal cells (3). Previous studies on genome integrity and infertility indicated an elevated rate of DNA nicks and double-stranded breaks (DSBs) in human sperm, potentially leading to failure of conception and miscarriage (4). The correlation between the incidence of chromosome aneuploidies in sperm and somatic cells has also been demonstrated, as well as a possible role of mitotic instability in unexplained infertility (5). Mutations in polymorphic sites, such as microsatellite repeat sequences in infertile men that present meiosis arrest or obstruction, have been correlated with malfunctions in nucleotide excision repair and mismatch repair mechanisms (6). Many mutated proteins, possessing a helicase activity or are otherwise involved in cell cycle regulation and DNA repair function, result in genetic disorders presenting genome instability (7, 8). Such a protein encoded by the *BLM* gene is responsible for Bloom's syndrome (9).

Certain clinical conditions, such as varicocele, cryptorchidism, have an effect on spermatogenesis. The type and level of infertility can be classified according to semen parameters (e.g., oligozoospermia, asthenozoospermia, teratozoospermia). Because spermatogenesis and, consequently, the quantity and quality of semen is affected by various factors (genetic, anatomical, environmental), it is probable that the nature and type of DNA damage may differ among these patients. Hughes et al. studied the damaging effect of roentgenograms and hydrogen peroxide in fertile and infertile normozoospermic and asthenozoospermic men (10). They concluded, in agreement with other studies, that asthenozoospermic men were more prone to these damaging parameters compared with normozoospermic infertile men, who in turn presented an increased level of cytogenetic damage compared with fertile men (10, 11). Other studies reported that infertile men with varicocele seem to present increased reactive oxygen species (ROS) levels in semen and in the spermatic vein and consequently elevated levels

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Reprint requests: Theodore Lialiaris, Ph.D., M.D., Department of Genetics, Medical School, Democritus University of Thrace, P.O. Box 93, Alexandroupolis 68 100, Greece (FAX: +30-25510-30522; E-mail: lialiari@med.duth.gr).

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of DNA damage, apoptosis, and, hence, malfunctions in spermatogenesis (12–18).

The objective of this study was: [1] to evaluate the nature and type of DNA damage present in infertile men arising spontaneously or elicited by genotoxic agents. Assessing SCE levels enabled this evaluation, [2] to investigate whether the presence of numerical or structural aberrations is associated to certain semen parameters (quality or quantity), using G- and C-banding techniques, and [3] to establish a possible association between chromosomal fragility and the specific semen parameters.

The SCE methodology can be used to quantitate genetic stability and thus evaluate chromosomal fragility. For eukaryotic cells to overcome the potentially mutagenic and cytotoxic effects of DSBs generated spontaneously or by alkylating agents, the postreplicational DNA repair mechanism of DSBs is triggered. Particularly in the holomogous recombination repair pathway, SCEs play a crucial role (19). Dysfunctions of this mechanism may lead to chromosomal fragility, characterized by chromosomal deletions and rearrangements.

To accomplish our objective, two agents were used: the antineoplastic and genotoxic agent mitomycin C (MMC) and caffeine (CAF), a well-known inhibitor of prereplicational DNA repair mechanisms and single-stranded break (SSB) postreplicational repair (20). Antineoplastic drugs induce SCE frequencies. Such a drug that highly increases SCE levels is MMC (21). Caffeine can inhibit certain DNA repair mechanisms and therefore it also elicits an increase in SCEs, which can be attributed to the genotoxic properties of alkyliotic factors and x-ray radiation. It has been reported that simultaneous treatment with CAF and MMC in vitro caused chromosomal scissions as well as a significant increase in SCE levels (22). Hence, these two agents were used to investigate genetic instability and evaluate chromosomal fragility.

MATERIALS AND METHODS

Heparinized blood samples were obtained from 11 fertile and 27infertile men. All volunteers were 20–38 years old, smokers, and not under any medical treatment. Infertile men fulfilled the following criteria: [1] all were diagnosed with a primary type of infertility; [2] no history of infertility in their family; [3] no hormonal dysfunctions, sexually transmitted diseases (STDs) or other parameters that may impair spermatogenesis; and [4] poor semen parameters (Table 1). Patients diagnosed with varicocele were also included in the study. All participants were inpatients at the Department of Urology of Alexandroupolis University Hospital.

Peripheral blood lymphocytes (0.4 mL) were cultured in chromosome medium B (5 mL) (Biochrom KG, Berlin, Germany) for 72 hours at 37°C. Cells were treated with MMC (12 ng/mL culture medium) and CAF (120 μg/mL culture medium) (22, 23) along with 5-bromodeoxyuridine (BrdU) at a final concentration of 4 μg/mL. Cultures were kept in the dark to prevent or minimize photolysis of BrdU. Colcemide was added to all cultures at 70 hours for an additional incubation of approximately 2 hours. Cell harvesting followed. Chromosome preparations were stained using modified fluorescence plus Giemsa technique (24).

Scoring was performed in a blind fashion. Sister chromatid exchanges, which is a quantitative index of genetic instability, was evaluated for each subject with either treatment. Mean SCE levels were evaluated only in suitable second division metaphases.

The G-banding and C-banding karyotyping analysis was carried out using standard protocols (25). Fifty metaphases were karyotypically analyzed for each individual in both banding techniques. When an abnormality was identified, 100 metaphases were analyzed to avoid a misdiagnosis.

To compare various treatments, logarithmic transformation of SCE values was performed using one-way analysis of variance (ANOVA) and the Duncan test, for pair-wise comparisons. The linear regression coefficient (r) was also estimated where appropriate. A probability $P<.05$ was considered as statistically significant (24).

RESULTS

Karyotyping analysis revealed no numerical or structural abnormalities in fertile men. The levels of SCE in control cultures were 6.86 $\pm$ 0.29 SCE/metaphase (Table 2). The CAF elicited a 56.41% increase and MMC, a 58.02% increase in comparison to untreated cultures. The combined administration of MMC and CAF gave a statistically significant increase of SCE frequencies, reaching a twofold increase in comparison to controls (Table 2).

Chromosomal abnormalities were identified in 26.9% of the infertile men, a higher than expected percentage (Table 3). Numerical abnormalities encountered in samples presenting mosaic lines, were identified in 4 of 27 patients studied. The extra genetic material varied in size, and a centromere was identified in all of the samples. Two of the extra chromosomes observed were identified as chromosomes 2 and 3, whereas the other two were classified as marker chromosomes of unknown origin. Karyotyping analysis revealed that two patients presented with chromosomal fragility, more specifically located at the distal parts of the long arms of chromosomes 1 and 4 (Table 3). The fragile site at chromosome 1 was identified in the q31–32 region and was noticed in 13.6% of analyzed metaphases, whereas an acentric fragment along with a deletion in this region was identified in 13.6% of analyzed metaphases in the same patient. A deletion in 4(q31.1–32) along with an acentric fragment was noticed in 50% of analyzed metaphases. Some infertile men were also found to carry 21ps+, 1cenh+, 15stk+ polymorphisms, and a carrier of inv(9) was also identified. According to their sperm diagram the sole azoospermic and one of the oligoasthenozoospermic patients