Ultramorphological Sperm Characteristics in the Risk Assessment of Health Effects after Radiation Exposure among Salvage Workers in Chernobyl

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We present a pilot study of individuals (liquidators) who were engaged in clean-up operations after the disaster at the nuclear power plant at Chernobyl in Ukraine. In the 10 years since the disaster, adverse health effects among exposed individuals have not been clearly defined. There is widespread fear of damage to the reproductive system, with implications for fertility problems and adverse effects on offspring. Bearing this in mind, methods to evaluate the potential for production of fertile semen have been applied using quantitative ultramorphological (QUM) analysis. QUM analysis examines the organization and integrity of sperm organelles by electron microscopy, using both transmission electron microscopy and scanning electron microscopy. Significant differences were observed between clean-up workers and controls of similar age regarding certain ultramorphological parameters of the sperm head. The results of this pilot study suggest that QUM analysis of human sperm is a feasible approach for evaluating the fertility potential of individuals who were exposed to ionizing radiation from the Chernobyl nuclear power plant accident. — *Environ Health Perspect* 105(Suppl 6):1445–1449 (1997)

Key words: semen analysis, sperm ultramorphology, ionizing radiation, Chernobyl

Introduction

Ten years have elapsed since the disaster at the nuclear power plant at Chernobyl in Ukraine. Detailed descriptions of the event and some of its health implications have been reported (*1–3*). A unique demographic aspect of this event is the recent immigration to Israel of a great number of individuals who were employed as clean-up workers (liquidators) or who lived in the vicinity of the damaged power plant.

Although adverse health effects among such immigrants have not been well defined, there is apprehension about underreported or undetected damage. There is concern about possible damage to the reproductive system, with implications for fertility and adverse effects on offspring. Irradiation may have profound effects on the human reproductive system (*4,5*). Principal outcome variables previously studied in male populations exposed to ionizing radiation include abnormalities in spermatogenesis (*6*) and reproductive risk of neoplastic disease in offspring (*7*).

Examination of patients who have participated in controlled experiments or have received radiotherapy has provided some information about dose–response relationships between radiation exposure and suppression of spermatogenesis, and recovery potential (*8,9*). However, data obtained in connection with radiation accidents, including the nuclear reactor disaster in Chernobyl, have been difficult to interpret because of imprecise exposure assessment (*10*). Recent observations from evaluation by standard laboratory examination methods, however, suggest a high prevalence of adverse effects on spermatogenesis and sperm morphology among liquidators (*11*).

The types of molecular damage induced by ionizing radiation in general have been amply described in the literature (*12,13*). It has been demonstrated that the major injury to DNA is strand breakage. With increased dosages, multiple types of DNA damage occur (*14*). Particular emphasis has also been given to mitotic death of reproductive gametes (*15*).

In mammals, spermatogenesis is the only biological process in which meiosis (chromatin reduction division) occurs in the adult state. This process, especially at the pachytene stage, is very sensitive to xenobiotic influences including ionizing radiation.

Although the lowest dose of irradiation causing cellular (somatic) or genetic damage has not been clearly defined, it has been suggested that even low dosages of ionizing radiation may be etiologically connected with various types of malignant diseases and birth defects (*16,17*).

Differences in sensitivity to radiation among species may be great e.g., the effective doses required to produce a given effect in mice are 3 to 7 times higher than in man (*18,19*). Not only is the output of human sperm (number of spermatozoa per gram tissue) 4 times lower than in other mammals, but there are relatively more abnormal cells in the ejaculate of a normal male (*20,21*). Environmentally related toxic effects on spermatozoa appear much more pronounced in man (*22*). It has been suggested that, to some extent, the fertility potential of human sperm may indicate effects of enviromental exposures and that sperm morphology is

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Abbreviations used: QUM, quantitative ultramorphological; SEM, scanning electron microscopy; TEM, transmission electron microscopy.

an important parameter to consider regarding these exposures.

Current methods for evaluating reproductive toxicity of xenobiotics include clinical examination, evaluation of testicular hormones in blood, and semen quality analysis. A thorough semen analysis provides a wide spectrum of information reflecting the spermatogenic and steroidogenic functions of the testis and the functional state of the genital accessory glands. Thus, male fertility potential can be assessed with semen quality analysis by comparing the sperm characteristics with parameters established for fertile males in the examining laboratory, or with those accepted by the World Health Organization (*23*).

Sperm morphology is an important and stable parameter. However, it is difficult to evaluate because of the heterogeneity of human sperm and also because critical information can be obtained only by the examination of sperm organelles of extremely small dimension.

Quantitative Ultramorphological Analysis

Methods have been developed in the Male Fertility Laboratory and the Reproductive Toxicology Unit in the Department of Life Sciences at Bar Ilan University whereby quantitative ultramorphological analysis (QUM) is applied in evaluating the potential of the human testis to produce fertile semen (*24–27*).

QUM analysis examines the organization and integrity of subcellular sperm organelles using both transmission electron microscopy (TEM) and scanning electron microscopy (SEM). With TEM and appropriate staining techniques, it is possible to view both cross-sections of the cell and two-dimensional images of ultrafine anatomical details. Forty-two specific sperm malformations are revealed by this technique (*24,25,28*). The SEM allows an overall view of sperm ultrastructure, especially of head and tail skeleton, and 65 specific malformations can be recorded. Thus, the combined use of TEM and SEM yields comprehensive results, supplying more details about pathological malformations in each subcellular organelle.

Based on the categories and frequencies of the various ultramorphological changes, the QUM analysis provides valuable information about sperm function potential, swim-up for intrauterine insemination, conception potential, and *in vitro* fertilization potential (*25*). Instead of relating to each morphological characteristic, as viewed by TEM and SEM, for each semen sample a proportional combination of categories is calculated that allows discrimination between fertile and infertile males.

Information obtained from QUM analysis and fertility status can be used as a biological indicator in evaluating causative factors of effects from environmental and occupational exposures. A detailed description of the QUM analytic system has been recently published (*29*).

To our knowledge, this is the first study on the effects of Chernobyl-related ionizing radiation on the ultramorphology of human sperm.

Materials and Methods

Populations

The study population consisted of 18 liquidators. Seven (39%) worked inside the power plant for a few minutes only and were evacuated to a no-radiation zone. The remaining 11 (61%) worked up to 8 months at a radius of 30 km from the reactor. Accumulated exposure was assessed by measurement with a dose record tag.

Since the accident, all individuals have lived in a nonradioactive area and remain under medical surveillance because of their radiation exposure at Chernobyl. None reported fertility problems before the accident. A local Ukrainian control group, consisting of 18 males, had not been exposed to radiation in any unusual manner.

Laboratory Methods

Routine semen analysis was performed on samples collected after 4 days of abstinence according to WHO guidelines (*23*) and a method described by Glezerman and Bartoov (*30*).

Quantitative Ultramorphological Analysis

Sperm cells were separated from seminal plasma and fixed in 2% formaldehyde–2% glutaraldehyde according to the method described by Bartoov et al. (*25*).

We performed TEM on sperm cells that were fixed according to the method described by Glavert (*31*) and processed the cells for observations with JEOL T2000 TEM at a magnification of approximately 8000 to 50,000. We examined 100 random cells to obtain the TEM morphogram. Scanning electron microscopy (SEM was performed on the cells subsequently processed according to the method described by Bartoov et al. (*25*), for observations with a JOEL JSM 35 SEM at a magnification of approximately 12,000. We examined 100 random cells to obtain the SEM morphogram.

The ultramorphological status of the following sperm subcellular organelles was assessed: acrosome, postacrosomal lamina, nucleus including karyoplasm, neck, axonema, mitochondrial sheath, and outer dense fibers including fibrous sheath.

The great variety of defects complicates the determination of the ultramorphological status of the sperm cells. Thus, the specific malformations have been categorized into five ultramorphological patterns.

Four categories of morphological abnormalities or states are identified in subcellular organelles: I) agenesis, II) incomplete genesis, III) malformation, and IV) degradation. The fifth category (V) represents normal intact sperm cell organelles with normal ultramorphological status.

Each of the specific malformations is quantitatively expressed by its incidence in the above cells. The frequency of a specific ultramorphological parameter observed by both the TEM and SEM examination is calculated as an average of the two observations. Tables 1 and 2 summarize the specific ultramorphological malformations of the sperm cell subcellular organelles that are considered in the QUM analysis.

Statistical analysis was performed using the SPSS-X package, including *t*-tests for separate variance and chi-square test. All values are expressed as means ± SE.

Results

No significant difference was observed between the control and the study groups with respect to sperm density, viability, or morphology observed by light microscopy, semen volume, or biochemical markers. However, these groups differed with respect to percentage of motile and progressively motile spermatozoa ($t = 3.3$, $p \leq 0.01$, and $t = 3.2$, $p \leq 0.01$) (Table 3).

Considering the ultramorphological status of the sperm cell subcellular organelles, both groups exhibited a statistically similar integrity of organelles, except for the intactness of the nucleus. The latter was significantly lower in the exposed males as compared with the controls ($t = 3.6$, $p \leq 0.01$) (Table 4). The only ultramorphological pattern associated with this difference was the incomplete genesis of the nucleus (Table 1). When specific ultramorphological malformations comprising this pathological pattern were analyzed, the frequency of amorphous head shape in the study group

Table 1. Definition of the utramorphological patterns of the sperm head subcellular organelles and neck region.

Ultramophological pattern	Acrosome	Postacrosomal lamina	Nucleus	Neck
I. Agenesis	Unelongated head + lack of acrosome	Unelongated posterior cap + lack of lamina	Pin head	ND
II. Incomplete genesis	Incomplete acrosome (principal or equatorial)	Incomplete lamina	Subelongated head (round, small oval), amorphous, subcondensed chromatin, cytoplasm around the nucleus	Abaxial
III. Malformation	Hyperplasia, vesiculated, or unaffixed acrosome	ND	Overelongated head (tapering, narrow), excess nuclear membranes, vacuoles in karyoplasm	Surrounded by cytoplasm
IV. Degradation	Elongated head + lack of lamina	Elongated posterior cap + lack of acrosome	Degraded karyoplasm	Disrupted
V. Intact	—	—	—	—

ND, not defined.

Table 2. Definition of the ultramorphological pattern of the sperm tail subcellular organelles.

Ultramorphological pattern	Outer dense fibers + fibrous sheath	Mitochondria	Axonema
I. Agenesis	ND	ND	Normal connecting piece + lack of tail or stump tail
II. Incomplete genesis	Normal tail plasmalemma and axonemal complex + partial FS or lack of one or more ODF element	Normal tail plasmalemma and axonemal complex + partial mitochondial helix	Normal FS and ODF + missing one or more element of axonemal complex, or tail kinked around the nucleus
III. Malformation	Normal tail plasmalemma and axonemal complex + disorganization, vacuoles, excess element, or breakage of the ODF and FS, coiled, kinked, or bent tails	Normal tail plasmalemma and axonemal complex + aggregation or disorder of mitochondrial helix	Normal FS and ODF + disorganization or excess microtubular elements of the axonemal complex
IV. Degradation	Chaotic arrangement of ODF or FS + normal axonemal complex	Degraded tail plasmalemma or axonemal complex + lack or partial mitrochondrial helix	Chaotic arrangements if the elements of axonemal complex
V. Intact	—	—	—

Abbreviations: FS, fibrous sheath; ND, not defined; ODF, outer dense fibers.

Table 3. Semen parameters (%) observed by light microscopy that differed significantly between the control and exposed populations.[a]

Semen parameters by light microscopy	Population	
	Control (n=18)	Exposed (n=18)
Motile spermatozoa	61.9 ± 2.5	44.1 ± 4.8
Progressive motile spermatozoa	25.0 ± 3.5	10.5 ± 2.8

[a]Values are means ± SE.

Table 4. Ultramorphological patterns (%) of the sperm nucleus of the control and exposed populations.[a]

Ultramorphological pattern	Population	
	Control (n=18)	Exposed (n=18)
Agenesis	2.7 ± 0.7	3.9 ± 1.1
Incomplete genesis	20.6 ± 0.7	7.5 ± 1.7
Malformation	21.6 ± 1.4	26.1 ± 1.9
Degradation	1.1 ± 0.4	1.1 ± 0.3
Intact	51.0 ± 2.5	31.8 ± 4.7*

[a]Values are mean ± SE. *Significant difference between the control and study groups ($p \leq 0.05$)

was significantly higher than in the control group (17.8 ± 2.4% vs 9.3 ± 1.4%, t= 3.0, $p \leq 0.01$) (Figure 1). A more detailed description of this study will be reported elsewhere (*32*).

Discussion

These preliminary results of routine semen analysis and QUM of spermatozoa of liquidators from the Chernobyl nuclear reactor reflect a decrease in motility and percent of progressive motility in the samples from the liquidators as compared with their controls. There were no other significant differences between the groups demonstrated by routine sperm analysis. However, a higher frequency of malformations of certain sperm cell organelles was found in the liquidator group, detectable only by the quantitative ultramorphological analytic method.

The observed abnormalities in head shape of spermatozoa are of interest, considering the target organelles previously shown to be subject to radiation injury (*12–14*).

We propose to use the QUM analytic method to identify ultramorphological malformations of subcellular target organelles of spermatozoa affected by exposure to ionizing radiation. This method may also be useful in defining a dose–response relationship between radiation dose and biological markers of effects.

Such information is important for determining safe exposure levels, for evaluating potential reversibility of radiation-induced damage, and for developing appropriate therapeutic methods.

We believe that this information will increase our understanding of radiation-induced damage and its various stages. QUM analysis may be an effective tool for identifying biological response variables for application in studies of genetic and molecular epidemiology directed toward assessing health risks of radiation exposure.

Consistent evidence for adverse reproductive outcome after Chernobyl-related radiation exposure has not been previously reported from studies performed in Ukraine, Belarus, and Western European countries. However, data are not yet available on reproductive outcomes of liquidators, or of women who were pregnant at the time of the accident (*33*). Valuable information on the effects of radiation may be achieved by long-term follow-up, particularly of workers and populations living near Chernobyl. This, in fact, is the most promising way of obtaining the quantitative information that is essential for assessing health risks associated with the nuclear reactor accident in Chernobyl (*33–35*).

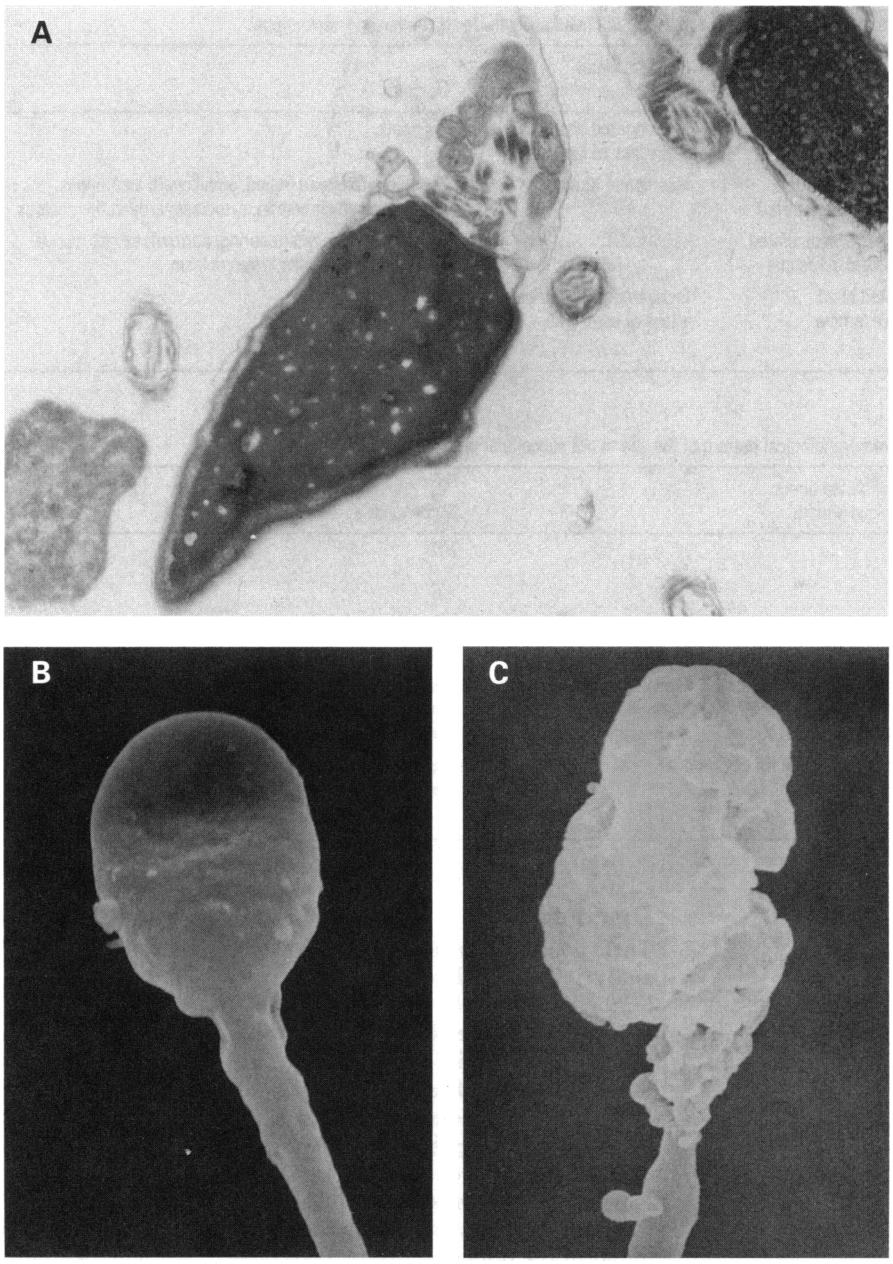

Figure 1. (*A*) TEM micrograph of normal spermatozoa. (*B*) SEM micrograph of normal spermatozoa. (*C*) SEM micrograph demonstrating amorphous head shape of spermatozoa. Magnification × 10,000.

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