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Environmental pollution due to cadmium: measure of semen quality as a marker of exposure and correlation with reproductive potential

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Summary

Purpose: Contradictory reports exist in the literature regarding an association of cadmium with parameters of semen quality. The aim of the study was to assess cadmium levels in both blood and seminal plasma and to analyze the relationships between cadmium concentrations and lifestyle and semen parameters. *Material and Methods:* Fifty healthy male volunteers were recruited to provide semen and blood samples. Each patient completed an extensive questionnaire regarding his occupation, residence, social status, diet, water source, smoking habits, and medical and surgical history. Semen analysis was carried out according to WHO guidelines. Detection of cadmium in both semen and blood samples was carried out by means of atomic absorption spectrophotometer. *Results:* Mean concentrations of cadmium were 8.18 ± 1.6 ng/ml in blood samples and 2.56 ± 0.9 ng/ml in semen samples. Cadmium blood levels were significantly higher in men from industrialized areas and in current smokers, but were not correlated with semen levels. A significant positive correlation was found between cadmium blood levels, number of immotile spermatozoa, and teratozoospermia index (TZI). Significant inverse relationships between cadmium blood concentration and type-a and type a + b motility were found. *Conclusions:* The present data show a significant correlation between blood cadmium concentrations, cigarette smoking, occupational exposure, and parameters of semen quality. Such a reduction in spermiogenetic function could be an early marker of a toxic effect by cadmium pollution.

Key words: Cadmium; Seminal plasma; Male infertility; Environmental pollution.

Introduction

Many studies carried out on infertile men have linked environmental factors and remarkable impairment of semen parameters (concentration, motility, and cell morphology). It has been suggested that testicles are among the most vulnerable organs to the action of environmental, chemical, and physical agents. Heavy metals are partially high-density chemical elements and are toxic even at low concentration. They are generally ubiquitous and represent a continuous and uncontrollable source of environmental pollution: penetrating human organism through food, air, and water, they sediment in various tissues or organs and are united to the cell structures which they sediment in, thus preventing the development of some vital functions [1-3].

Traces of some metals (mercury, cadmium, lead, and aluminium) were found in gonadal tissues, in oocytes, in sperm, in biological fluids, and even in embryos, at variable concentration levels, likely influenced by residence, occupation, and lifestyle [4, 5]. In gonadal tissues they may be responsible for ultrastructural alterations, both genetic and somatic, as well as for functional modifications affecting cell metabolism with interactive and lasting toxic effects [6-8].

Cadmium is a trace toxic mineral similar to zinc in its structure, with no biological function in human organism. It is found in the environment because it derives from eco-dispersion phenomena due to both productive activities and to public utility services – such as landfills for urban solid waste, incinerators, thermoelectrical stations – potentially polluting air, water, food, and also to cigarette smoke. Cadmium values between 20% and 50% are absorbed through respiratory system into blood (95% of it is found in erythrocytes, the rest in plasma): partly attached to a protein – metallothionein – cadmium finally reaches the testicles. Exposure to cadmium has been related to adverse effects on reproductive functions in mammals: in experimental animals, toxic effects on testicles led to necrosis, atrophy, and edema, presumably because of specific vascular damages [9-11]. Occupational exposure has been associated to a decrease in sperm quality and to an increase of spontaneous miscarriages, by reason of a hypothesized – direct or hormone-mediated – effect on testicular function [12-14].

To date, contradictory data exist in the literature regarding an association of cadmium and parameters of semen quality [15-18]. According to Amad *et al.* there is a nega-

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tive correlations between seminal lead and cadmium levels on semen parameters (sperm motility and vitality) [19]. Moreover, many studies showed a correlation between exposure to cadmium and semen features, and a strictly correlation with age, diet, and smoking [20]. The aim of this study was to evaluate the presence of cadmium in biological fluids (blood and semen) at toxic levels, and to examine the relationship between cadmium concentration and lifestyle, geographical area of residence, occupational exposure, age, and semen parameters.

Materials and Methods

Fifty healthy patients attending the Outpatient Infertility Center at Second University of Naples were recruited to provide semen samples. All the patients were between 24- and 42-years-old (average 32.5 ± 5.5 years) and childless, signed an informed consent form, and completed an ad hoc questionnaire. Every patient was requested to provide a semen sample after at least three (not more than five) days of abstinence from intercourse; a blood sample was taken from each patient.

Each semen sample was preliminarily analyzed to register chemical and physical parameters (such as volume, pH, viscosity, colour, liquefaction), sperm concentration (registered in millions of spermatozoa per milliliter), morphology (morphology of 100 cells – divided into forms with head, mid-piece, and tail defects and normal forms – was evaluated per each slide); teratozoospermia index (TZI) was obtained dividing the total number of abnormalities (head, mid-piece, and tail abnormalities) added together by the number of abnormal spermatozoa, while the sperm deformity index (SDI) was calculated dividing the number of defects by the number of counted cells. These indices are predictors of fertilization potential of spermatozoa, both in vivo and in vitro. TZI value varies from 1.00 (every abnormal spermatozoon shows one anomaly only) and 3.00 (every abnormal spermatozoon shows head, midpiece and tail abnormalities). Previous studies have shown that a TZI >1.6 is associated to a low fertilization rate in infertile couples who have not received medical treatment; it has also been proven that SDI 1.6 is the highest value to be compatible with in vitro fertilization. The evaluation of sperm motility was performed considering the number of: fast straight motile cells, slow straight motile cells, in situ motile cells, and immotile cells.

Cadmium evaluation was performed on sperm and blood samples by graphite furnace atomic absorption spectroscopy an highly efficient technique designed to perform the qualitative and quantitative analysis of heavy metals in a wide variety of samples. The sample, properly treated for matrix separation and for atomization, was then dried, incinerated, and atomized at a temperature which allows the atomic absorption. The atoms of the element under observation absorb energy only at their own wavelength and according to their number. During cadmium analysis, drying, incinerating, and atomizing temperatures were respectively 120°C, 500°C, and 2,200°C, in conditions of transportation gas (Argon) flow interruption. A 228.8 mm wavelength was adopted.

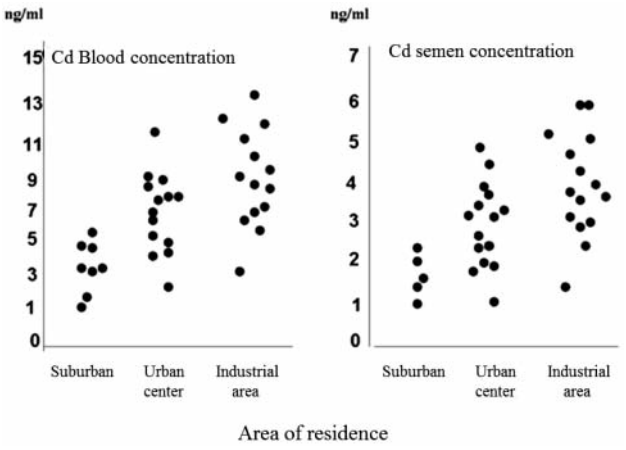

Figure 1. — Blood and semen concentration of cadmium (cd) in relation to the area of residence.

Data are presented as averages ± DS. The statistical analysis was performed using Student's *t*-test and Pearson for parametric data. Groups comparing analysis and correlation analysis were carried out using, respectively, Mann-Whitney test and Spearman rank-test, for non-parametric data. *P* values < 0.05 were considered as statistically significant.

Results

Out of the selected 50 metals exposed patients observed, 28 were current smokers, the other 22 were non-smokers from at least three years. Twenty-four patients lived in areas close to chemical or metal industries, but none of them had a proven occupational exposure to heavy metals. Mean values of cadmium concentration in blood and in semen were 8.18 ± 1.6 ng/ml and 2.56 ± 0.9 ng/ml. Cadmium blood levels were significantly higher in men from industrialized areas (Figure 1) and in current smokers, but were not correlated with semen levels, though the authors observed a positive correlation trend ($p = 0.251$ and $p = 0.079$) (Table 1). An inverse correlation trend was found between the highest metal values and sperm quality, especially in motility, which was inversely proportional to blood cadmium levels, but not correlated to sperm cadmium levels (Table

Table 1. — *Correlation of blood and semen concentrations of cadmium (Cd) with semen parameters.*

	Cd blood	Cd semen	Sperm concentration	Motility Type a	Motility Type d	Motility Type a + b	F. N.	TZI	SDI
Cd blood concentration		*p*= 0.251	No	*p*= −0.45	*p*= 0.47	*p*=−0.42	*p*= −0.62	*p*= 0.13	*p*= 0.293
		p= 0.079	correlation	*p*= 0.001	*p*= 0.001	*p*= 0.003	*p*= <0.001	*p*= 0.36	*p*= 0.04
Cd semen concentrations	*p*= 0.251		No	*p*= −0.15	*p*= 0.13	*p*= −0.13	*p*= −0.206	*p*= 0.49	*p*= 0.48
	p= 0.079		correlation	*p*= 0.31	*p*= 0.3	*p*= 0.36	*p*= 0.15	*p*= 0.001	*p*= <0.00

Table 2. — *Semen parameters in smokers versus non-smokers (mean ± DS).*

	Smokers (28)	*p*	Non-smokers (22)
Sperm concentration*	15.9± 22.5	0.08	28.1 27.5
Motility type a	17.1 ± 9.6	0.02	25 ± 13.2
Motility type b	13.9 ± 3.9	0.09	15.9 ± 4.3
Motility type c	18.6 ± 3.6	0.53	19.5 ± 6.3
Motility type d	50.3 ±14.7	0.036	38.6 ± 23.4
Motility type a+b	31.1 ± 13.1	0.01	41.8 ± 17.3
Normal forms	30.7 ± 7.7	0.02	37.7 ± 7
Cadmium blood concentration	9.1 ± 1.3	0.001	7 ± 1
Cadmium semen concentration	2.8 ± 0.8	0.01	2.2 ± 0.8
TZI**	1.3 ± 0.1	0.01	1.2 ± 0.08
SDI***	1 ± 0.08	0.4	1.02 ± 0.08

* million/ml; ** teratozoospermia index ; *** sperm deformity index.

2). A large variation some of seminal parameters was observed among the patients: sample sperm concentration was the most variable parameter in donors who had 26.4 ± 35.1 x 10^6 spermatozoa/ml (range 1-100 million spermatozoa/ml). As for motility, the authors reported the percentages of mean numbers of fast- and straight-moving spermatozoa (a), slow straight-moving spermatozoa (b), mobile in situ spermatozoa (c) and spermatozoa with no movement (d).

Discussion

Semen can be used as an indicator of exposure and effect because structural and functional alterations specifically and precociously (especially with regards to spermatogenetic function) occur in the gonads. Such alterations are secondary to toxics of various nature, even when exposure corresponds to very low chronic doses. The results of the present study registered both significant positive correlations between total presence of cadmium in blood, number of immotile spermatozoa, and TZI, as well as relevant inverse correlations between total cadmium concentration, a-type and a+b motility, and number of morphological normal spermatozoa. On this basis, it can be inferred that sperm damages occur previously because of an adverse effect of cadmium on germinative epithelium.

The use of semen parameters as indicators of toxic effect of environmental contaminants seems to be not fully reliable because seminal characteristics should be evaluated together with clinical and hormonal data. A further difficulty is the high inter- and intra-individual variability of sperm count among different subjects at ordinary conditions, as well as in the same subject, in relation to several factors, such as sexual habits, seasonal changes, and lifestyle. Quantitative and qualitative alterations in semen can be produced by various conditions, not depending on individual features: this may cause further problems in interpretation. Furthermore, biological factors such as infections, ionizing radiations, and heat may have a great influence, especially when sperm canals are concerned. On the other hand, semen analysis is a simple and sensitive test which can be used as an early indicator of germinative epithelium damage due to exposure to environmental toxics, even at low concentrations of contaminants.

Data from literature regarding the relationship between cadmium and sperm quality are contradictory. Some authors [11, 21, 22] did not register any association between cadmium concentrations and sperm concentration and motility in a general population, as well as in battery workers; others observed an inverse relationship between cadmium concentration and semen volume [13] and motility [23-24], inferring adverse effects on prostatic function Some studies showed a significant negative correlation between cadmium concentration in semen and sperm concentration in oligoasthenospermic subjects in the general population. Pant N. *et al.* showed that lead and cadmium induce DNA damage, particularly influencing testosterone level [21].

Our data show a correlation between blood cadmium concentration, some risk factors (cigarette smoke, occupation, age, area of residence) and sperm function parameters: noteworthy was a decrease in spermatozoa number and vitality observed mostly in smokers and in industrial areas residents. Nonetheless, a relationship between cadmium concentration in blood and in semen was not observed, while a relationship was shown between semen cadmium concentration, TZI, and SDI. It can be hypothesized that cadmium penetrating into cells through voltage-dependent calcium channels does not accumulate because of competing with zinc (normally present in sperm) at intracellular bond site [25-26]. It can also be hypothesized that low concentrations of toxic may cause worsen morphologic characteristics of abnormal spermatozoa, as suggested by positive correlations between seminal cadmium levels and TZI-SDI. Such observation could find wide application in assisted reproduction. In conclusion, the impairment of seminal parameters in subjects exposed to cadmium may represent a precocious indicator of the toxic effect of cadmium exposition.

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