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"Exposure to heavy metals in male residents in the Land of Fires: Epidemiological study on the impact of cadmium on testicular structure, and clinical risk factors of testicular cancer"

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INTRODUCTION

Primary testicular functions, namely spermatogenesis and steroidogenesis, require a finely tuned cooperation among different testicular cell populations, which is granted by proper endocrine, paracrine and autocrine signaling, and structural integrity [1, 2]; testicular morphostructural alterations, testicular parenchymal structure inhomogeneity, testicular calcifications and microlithiasis, testicular hypotrophy, varicocele and testicular solid lesions, are highly interconnected conditions, which might reflect a functional damage in such a tight cooperation and might determine, or predispose to, infertility and testicular cancer (TC). Testicular parenchymal structure inhomogeneity is associated with cryptorchidism, infertility, and an increased risk for TC; testicular calcifications, which consist of isolated or multiple micro or macro intra-testicular calcium deposits resulting from trauma, inflammation and/or other pathological processes, and a picture of microlithiasis, namely, the presence of at least 5 calcium deposits per ultrasound (US) field, are associated with infertility and represent a risk factor for TC, if bilateral or associated with additional risk factors; testicular hypotrophy, defined as a testicular volume <12 ml, is associated with infertility; varicocele, a venous reflux in the pampiniform plexus, is associated with an increased risk of ipsilateral testicular hypotrophy and potentially infertility [3]. TC is characterized by extremely heterogeneous etiopathogenesis, and histological and molecular characteristics. The most frequent category of testicular tumors, represented by germ cell tumors (GCT), is also the most frequent form of solid tumors in young patients within 15-34 years of age [4]; besides the mentioned associations, established risk factors for GCT also include family history for GCT, poor semen quality, and Klinefelter syndrome [4-6]. The second macro category of testicular tumors is represented by non-GCT (NGCT), commonly affecting males between the third and sixth decade of life, with poorly characterized risk factors [7, 8]. The global incidence of TC has increased worldwide over the past 2 decades [9, 10], showing marked geographical discrepancies and a higher incidence rate in industrialized countries, therefore suggesting a potential etiological role of environmental factors [11-13]. Exposure to several endocrine disruptors (ED) during fetal life has been gaining much consideration as a potential determinant of infertility and testicular dysgenesis [14] (), and has been recently hypothesized that adult risk factors for TC might include occupational exposures to polyvinylchloride, pesticides, non-ionizing radiation, heat and heavy metals, despite less evidence has been provided in this regard [15, 16].

Campania Region has been characterized by a waste management crisis since 1980, resulting in the largely documented illegal disposal and burying of urban, toxic and industrial waste, accompanied by the diffuse practice of waste burning, determining the spreading of several types of substances

acting as ED, such as polychlorinated biphenyls, hydrocarbons, heavy metals etc., and a consequent significant increase in local environmental pressure [18]. Moreover, Campania is the largest region of Southern Italy, and the region with the youngest population in Italy (); therefore, much interest has been raised in addressing the potential effects of the local high environmental impact (HI) on the incidence of reproductive disorders and TC, despite dedicated studies on men living in this geographical area are scarce or absent, to date. In particular, current scientific literature is devoid of large studies evaluating semen quality and fertility potential in Campania Region, except for a few studies showing 1) reduced sperm motility and increased sperm DNA fragmentation and telomeres length in males aged 18-50 years resident in the HI area so-called "Land of Fires", comprising 90 municipalities within the Province of Naples and Caserta [19-21]; 2) worse seminal parameters in men exposed to traffic pollutants [22, 23] and a negative correlation between seminal parameters and heavy metals concentration in the soil [24], in the metropolitan area of Naples. Lastly, a 2011 ecological study reported a significant increasing trend for TC incidence across waste exposure index categories, in an HI area interested by dumping waste sites comprising 35 municipalities belonging to the Province of Naples [25].

Among the environmental pollutants deriving from the illegal disposal, burying and burning of waste within Campania Region, the heavy metals cadmium (Cd) is a known human carcinogen, consistently shown to affect male reproductive function and fertility at multiple levels, by exerting both endocrine and non-endocrine actions, which include testicular vascular damage, blood-testis barrier disruption, inflammation, cytotoxicity on Sertoli and Leydig cells, oxidative stress, apoptosis, epigenetic actions, and disturbance of the hypothalamus-pituitary gonadal axis. Although most of the underlying mechanisms have been fully elucidated particularly in experimental in vitro and in vivo animal models, the majority of observational studies in humans also support a role for Cd as a male reproductive toxicant, even though findings are more controversial due to heterogeneity of study design, patterns of exposure, and co-exposure to multiple pollutants [17]. Moreover, Cd has been shown to contribute to testicular tumor development by both endocrine-dependent and endocrine-independent mechanisms, the former being initiated by Cd-induced testicular vascular damage with a reduction of testosterone and a compensatory increase of luteinizing hormone (LH), resulting in overstimulation of remnant Leydig cells and onset of Leydigiomas, and the latter being mediated by early occurring Cd-induced aberrant gene expression and genotoxic effects within the testis, resulting in proliferation of cells with genetic instability. Although fully established in animal models, the potential role of Cd in the etiology of TC has been less investigated by observational studies in humans so far, and do not allow to draw firm definitive conclusions. Lastly, few additional evidences specifically highlighted a potential role for Cd in the

etiology of testicular morphostructural alterations, including testicular parenchymal structure inhomogeneity, testicular hypotrophy, and varicocele; nevertheless, available data are mainly derived from animal models, whereas observational studies in humans are scarce.

To the best of our knowledge, no studies described testicular morphostructural characteristics, nor the potential impact of local environmental factors, particularly, Cd exposure, on such aspects, in men living in HI areas within Campania Region. The aim of the current study was to evaluate for the first time the prevalence of testicular morphostructural alterations, including testicular parenchymal structure inhomogeneity, testicular calcifications and microlithiasis, testicular hypotrophy, varicocele and testicular solid lesions, in a large cohort of adult men living in three municipalities (Acerra, Afragola, Giugliano in Campania) belonging to the HI area "Land of Fires" of Campania Region, and to determine the potential relationship between these outcomes and cumulative exposure to Cd, objectively evaluated by inductively coupled plasma mass spectrometry (ICP-MS) quantitation in whole semen samples.

STUDY DESIGN

The current research study is a single-center, observational, cohort study, with a cross-sectional design; evaluation of participants was performed at a single time-point, at study entry. A preliminary descriptive analysis of clinical and US testicular morphostructural characteristics was performed, and prevalence of testicular parenchymal structure inhomogeneity, testicular calcifications and microlithiasis, testicular hypotrophy, varicocele, testicular solid lesions was determined in the total cohort. Whole semen Cd (sCd) level was determined by ICP-MS, and was correlated to lifestyle-related, clinical, andrological and US parameters. In a subanalysis of data, sCd level was set as dichotomous variable and participants of the total cohort were firstly grouped as being below (undetectable) or above (detectable) sCd level of detection (LoD) cutoff point, and secondly grouped as being below or above median sCd level, in order to detect differences in continuous variables and in the prevalence of testicular morphostructural alterations, according to sCd.

PARTICIPANTS AND METHODS

Participants recruitment

Participants recruitment was performed in line with the principles of the Declaration of Helsinki, and the research study was carried out upon approval of the Ethical Committee of "Federico II" University of Naples (Prot. N°158/19). Study cohort included Caucasian males of reproductive age resident in Campania Region, recruited within "Exposoma e Plurifocalità nella Prevenzione Oncologica" research project funded by Campania Region - POR-FESR 2014-2020. An awareness campaign for TC prevention in HI areas of Campania Region, identified on the basis of the Campania Region Environmental Protection Agency (ARPAC) reports (ARPAC D.L. 136/2013) as having the highest concentration of illegal disposal sites of toxic waste, and being characterized by frequent uncontrolled waste incineration, was promoted by Dipartimento di Medicina Clinica e Chirurgia, Sezione di Endocrinologia, Diabetologia Andrologia e Nutrizione, Unità di Andrologia e Medicina della Riproduzione e della Sessualità Maschile e Femminile (FERTISEXCARES), Università Federico II di Napoli, Naples, Italy. The awareness campaign was promoted by publication of the initiative on the official website of the FERTISEXCARES center, by social media networks, and by locally distributed flyers; participants were also consecutively enrolled by actively contacting them by phone, based on telephone directories, and with the support of general practitioners and local pharmacists.

Inclusion and exclusion criteria

Inclusion criterion was residence for at least 10 years in one of three municipalities (Acerra, Afragola, Giugliano in Campania) belonging to the HI area "Land of Fires" of Campania Region. Exclusion criteria included: 1) psychological and/or psychiatric disorders; 2) endocrine or other systemic diseases; 3) reported history of or concurrent non-therapeutic use of psychotropic substances and/or recreational drugs; 4) reported history of or concurrent alcoholism, or suspicion of alcohol abuse. No "a priori" selection based on the presence or absence of infertility and/or andrological nor metabolic disorders was applied as a criterion for participants enrolment. A written informed consent for the participation to the study was obtained upon enrolment of participants attending to FERTISEXCARES center; a written informed consent from a legal representative was obtained in case of underage participants. Upon enrolment, a progressive code number was assigned to each participant, in the respect of anonymity.

Clinical procedures and data collection

Participants attending to FERTISEXCARES were interviewed on medical history and medications and underwent a complete physical examination, which included measurement of clinical and anthropometric parameters: body weight, height, body mass index (BMI), waist circumference (WC) and hip circumference (HC) were recorded. Body weight was measured to the nearest 0.1 kg by using an electronic scale and height to the nearest 1 mm by using a stadiometer. BMI was calculated as body weight (kg) divided by the square of height (m). WC and HC were measured with an anthropometric tape – the mean of two measurements was used. A complete andrological examination was performed, including evaluation of ano-genital distance (AGD), testicular volume by Prader orchidometer and testicular consistency, and occurrence of any superficial lesions or clinical varicocele. Participants were interviewed face-to-face by specifically trained personnel using a structured questionnaire to collect data concerning lifestyle-related parameters, particularly, smoking habit and occupational history with self-reported occupational exposure to toxic chemicals. Nonsmoker was defined as a person who had never smoked, or who had quit smoking more than 2 years before the interview; current smoker was defined as a person who had smoked at least 100 cigarettes in his lifetime and who still smoked cigarettes or had quit smoking less or equal than 2 years before the interview. Smoking habit was quantitatively evaluated based on self-reported information by using pack-year parameter (1 pack-year = 365.24 packs of cigarettes or 7305 cigarettes; number of pack-years = packs smoked *per* day X years as a smoker)[26].

Ultrasound analysis

Scrotal US was performed in longitudinal, transverse, and oblique scans, according to the European Academy of Andrology (EAA) US guidelines (31), with patient in a supine and standing position, by using a 10 MHz high-frequency linear probe (6-15 MHz) with both grey scale and color-doppler. Testicular parenchymal structure echogenicity and homogeneity were evaluated with a 3-points scale. The presence of testicular calcifications was reported: microcalcifications were defined as deposits with a maximum diameter of up to 3 mm; testicular microlithiasis was defined as the presence of at least 5 microcalcifications/US field. Testicular diameters were collected, including anteroposterior and lateromedial diameters in transverse scan and longitudinal diameter in longitudinal scan, and testicular volume was determined by the ellipsoid formula; testicular hypotrophy was defined as testicular volume <12 ml. The presence of varicocele was assessed with patient in both supine and standing position, according to Valsalva classification. Testicular solid lesions were reported in 3 diameters, with the assessment of vascularization. Testicular or epididymal cysts, hydrocele, and measures of epididymal structures were also recorded.

Trace elements analysis

Quantitative determination of Cd was performed in whole semen by ICP-MS (Aurora M90; Bruker). Briefly, 500 µl of whole semen sample were transferred into glass tubes and 1 ml of ≥ 69%, (v/v) nitric acid (HNO_3) (TraceSELECT®) was added. Sealed tubes were subjected to sample acid oxidative digestions using an automated microwave digestion system (DISCOVER SP-D; CEM) and the following protocol: from room temperature (RT) to 160°C ramp time 3'; constant temperature 160°C hold time 2'; from 160°C to 80°C cooling time 2'; from 80°C to RT with no auxiliary cooling control. Once at RT, 2% (v/v) HNO_3 was added to the mix to final volume 10 ml. Cd elemental concentration was determined by comparison to certified standard solutions calibration curve; final Cd concentration was reported as µg/l. The LoD and limit of quantification (LoQ) were calculated as the blank signal plus three or ten times its standard deviation, respectively. LoD for Cd was 0.2 µg/l. Recovery was calculated to be within the range 70-120%.

Statistical analysis

Statistical analysis was performed with GraphPad Prism and SPSS Statistics softwares. Distribution of continuous variables was assessed by D'Agostino & Pearson normality test. Descriptive analysis included calculating the mean ± standard deviation (SD) for continuous variables with normal distribution, whereas non-normally distributed continuous variables were reported as median with interquartile range (25°-75° centiles). Categorical variables were reported as absolute numbers and percentages. Between-groups comparison of continuous variables was analyzed by unpaired Student's t-test/ANOVA for normally distributed variables or Mann-Whitney/Kruskal-Wallis test for variables following non-normal distribution. Between-groups comparison of categorical variables was analyzed by Chi-squared test and Fisher's exact test, when appropriate. Spearman correlation test was applied to determine the linear relationship between non parametric continuous variables, by correcting for potential confounders. Moreover, Receiver Operating Characteristic (ROC) curves analysis was performed to identify threshold value. Statistical significance was set at $p < 0.05$ for all comparisons.

RESULTS

Total cohort lifestyle-related, clinical and ultrasound parameters

Four hundred sixty-five men aged 14-50 years (29.5 ±7.23) with a BMI of 25.99 ± 4.35 Kg/m^2, a mean duration of residence in HI areas of Campania Region of 25.61 ± 9.17 years and a mean time spent in HI areas of 16.79 ± 5.86 hours/24 hours were recruited within the current single-center, observational, cross-sectional cohort study. Among recruited participants, 29/465 (6.2%) refused to be interviewed concerning lifestyle-related parameters. Study cohort included 195/436 (44.7%) current smokers with a mean number of cigarettes smoked/day of 6.67 ± 8.65, accounting for 7.55 ± 8.73 pack years; occupational exposure to toxic chemicals was self-reported by 52/422 (12.3%) participants. Total cohort clinical and lifestyle-related parameters are shown in Table 1. Prevalence of US testicular morphostructural alterations, unilateral or bilateral, were: testicular parenchymal structure inhomogeneity 86/452 (19%), testicular microlithiasis 11/440 (2.5%), testicular hypotrophy 66/451 (14.6%), varicocele 160/452 (35.4%), III-V grade clinically relevant varicocele 62/452 (13,8%), testicular solid lesions > 5 mm 1/465 (0.2%). Total cohort andrological parameters and prevalence of clinical and US testicular morphostructural alterations are shown in Table 2.

Lifestyle-related, clinical, and ultrasound parameters according to whole semen cadmium levels

sCd level was determined by ICP-MS in 385 samples and was often below the LoD, namely, in 257/385 (66.8%) whole semen samples; median sCd level was 0.76 μg/l (0.32-1.84 μg/l, 10°-90° centiles), Therefore, sCd level was selected as a criterion for further analysis and treated as a dichotomous variable; in a first subanalysis of data, participants were grouped as being above (detectable) or below (undetectable) LoD cutoff point for sCd, in order to detect differences in lifestyle-related and andrological parameters, and in the prevalence of clinical and US testicular morphostructural alterations. No significant difference was found between the two subgroups concerning age (30.34 ± 7.36 years vs 29.5 ± 7.02 years), BMI (25.97 ± 3.8 Kg/m^2 vs 25.9 ± 4.13 Kg/m^2), mean duration of residence in HI areas of Campania Region (25.43 ± 9.69 years vs 26.12 ± 9.15 years) and mean time spent in HI areas (17.13 ± 5.99 hours/24 hours vs 16.49 ± 5.74 hours/24 hours), nor in the mean number of cigarettes smoked/day (6.67 ± 8.65 vs 6.97 ± 9.03) and pack years (8.26 ± 7.70 vs 7.88 ± 9.29), despite a significantly lower prevalence of smokers was found in the subgroup of participants with detectable, compared to undetectable sCd level [43/119 (36.1%)

vs 117/243 (48.1%); p=0.0328]. The prevalence of self-reported occupational exposure to toxic chemicals did not differ between the subgroup of participants with detectable, compared to undetectable sCd level [13/116 (11.2%) vs 36/231 (15.6%)]. Clinical and lifestyle-related parameters in subgroups of participants with detectable and undetectable sCd level are shown in Table 3. A significant difference was found in the subgroup of participants with detectable, compared to undetectable sCd level in mean (right and left) testicular volume (16.56 ± 4.68 ml vs 17.66 ± 4.34 ml; p=0.0153), but not in AGD (7,8 cm ± 1.4 cm vs 7,8 ± 1,8 cm). Participants with detectable sCd level had a significantly higher prevalence of unilateral or bilateral testicular hypotrophy [27/128 (21.1%) vs 25/250 (10%); p= 0.0059] and varicocele [59/128 (46.1%) vs 74/249 (29.7%); p= 0.0008], compared to those with undetectable sCd level, along with a tendency to a not significantly higher prevalence of unilateral or bilateral testicular parenchymal structure inhomogeneity [32/124 (25.8%) vs 42/251 (16.7%)]. No significant difference was found between the two subgroups concerning the prevalence of III-V grade clinically significative varicocele [23/126 (18%) vs 30/257 (11.7%)], unilateral or bilateral testicular microlithiasis [5/128 (3.9%) vs 13/251 (5.1%)] and testicular solid lesions > 5 mm [0/128 (0%) vs 1/256 (0.4%)]. Andrological parameters and prevalence of clinical and US testicular morphostructural alterations in subgroups of participants with detectable and undetectable sCd level are shown in Table 4. In a secondary subanalysis of data, participants were grouped as being above (N=49) or below (N=79) median sCd value or having undetectable sCd level, and the mean testicular volume significantly differed in the 3 independent groups (14.88 ± 3.79 ml vs 17.22 ± 5.03 ml vs 17.66 ± 4.34 ml; p<0.001).

Correlation analysis between whole semen cadmium levels and lifestyle-related, clinical and ultrasound parameters

Spearman correlation analysis demonstrated that sCd level was negatively correlated with mean (right and left) testicular volume (r= -0.185; p<0.001)(Figure 1) with AGD (r=-223; p=0.017), whereas it does not correlate with age, BMI, pack years and years of residence in HI areas. In linear regression analysis performed with mean (right and left) testicular volume as a dependent variable and sCd, smoking habit, age and BMI as independent variables, sCd was identified as the best predictor of mean testicular volume (ß =-0.274; p=0.001). The negative correlation persisted when correcting the analysis for the prevalence of III-V grade clinically significant varicocele (r=-0.236; p=0.009).

Therefore, ROC curves analysis was performed to identify the sCd threshold value potentially predicting testicular hypotrophy; the results of the analysis demonstrated that a sCd level > 0.76

μg/l correctly identified testicular hypotrophy with a 60% sensitivity and 70% specificity (AUC 0.679).

DISCUSSION

The current research study on clinically healthy young men of reproductive age, non-occupationally exposed to toxic chemicals, is aimed to evaluate the testicular morphostructural feature in association with Cd exposure. Campania Region has been characterized by a waste management crisis since 1980, resulting in the largely documented illegal disposal and burying of urban, toxic and industrial waste, accompanied by the diffuse practice of waste burning, which determined a significant increase in local environmental pressure, and this has motivated to evaluate the pollution in the young males in Campania. It is known that Cd induces severe structural damage to testicular vascular endothelium, resulting in potential testicular necrosis[27], this ability to determine damage to the vascular endothelium could be one of the explanations for why we find in this work a strong association with varicocele, having participants with detectable seminal cadmium having a higher prevalence of this vascular alteration. Among non-occupationally exposed population, tobacco smoke represents the main source of Cd since tobacco leaves accumulate large amounts of metal [17], however, in our case studies there were no significant differences in exposure to tobacco smoke between the two groups of participants. The recent scientific evidence available suggests that the Cd, having overcome the blood-testicular barrier, plays its harmful role in the testicle with an interplay with other substances or trace elements such as zinc (Zn) and selenium (Se) [17]. Protective effects of Zn and Se from Cd-induced testicular damage, infertility and TC were steadily proven by a number of experimental studies in animals [17]; mimicry and interaction between Cd and Zn and Se, and competition for transporters, enzymes, and molecules involved in important essential ion-mediated biological processes, could partially account for the different response or susceptibility thresholds to Cd [17]. In particular, two mechanisms of Cd accumulation within the testis are worth mention: ionic mimicry at the transporters belonging to the ZIP family of Zn transporters, which might favor Cd uptake within the testis in case of Cd excess or Zn deficiency, and prevention of Cd-induced testicular toxicity by immobilization of Cd in Cd-Se protein complexes [17]. These evidences suggest that the maintenance of adequate concentrations of trace essential ions and their dietary supplementation could contribute to protect testicular function from

Cd toxicity, although the potential protective role of these essential trace elements against TC, per se, is less clear. Nevertheless, a recent case-control study demonstrated that serum Zn levels were decreased in patients with germ cell TC, compared to healthy men, therefore providing rationale for further studies [28]. Further studies that simultaneously evaluate the exposure and seminal concentrations of all these substances are needed to better understand this cross-talk. Another recent Italian study has shown similar results or that the seminal concentrations of Perfluoroalkyl compounds (PFCs), pollutants and known ED, were associated with a reduced testicular volume [29].

In conclusion, this study shows consistently for the first time in the Campania population of young males, how there is a close relationship, independent of age, BMI, voluptuous habits such as smoking, between Cd exposure levels and testicular abnormalities, such testicular hypotrophy and varicocele, further strengthening the concept of gonadal toxicity exercised by the Cd. The main limitation of this study is the cross-sectional design of the study makes as undefinable the causal relationship between morphostructural abnormalities and semen Cd levels, however, our study group set out to carry out samples in a control population of geographic areas with less environmental impact and it would also be interesting to evaluate the hormonal and seminal correlates of these populations to evaluate any associations with Cd or other heavy metals or trace elements. Therefore, additional well-designed observational studies and experimental research in humans, are fundamental to confirm the effects of Cd on testis.

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FIGURES AND TABLES

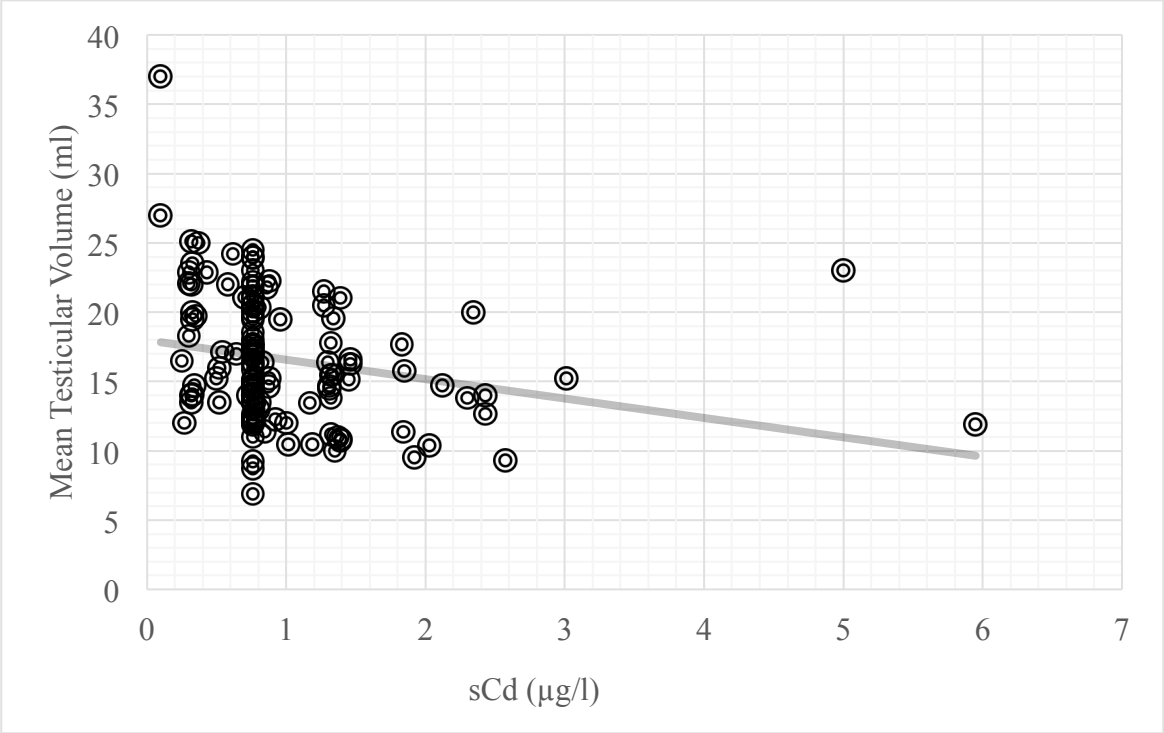

Figure 1. Distribution of whole semen cadmium (sCd) levels plotted against mean (right and left) testicular volume ($r = -0.185$; $p<0.001$).

	Total cohort (N=465)
Age (years)	29.5 ± 7.23
BMI (Kg/m^2)	25.99 ± 4.35
Mean residence in HI areas (years)	25.61 ± 9.17
Mean time spent in HI areas (hours/24 hours)	16.79 ± 5.86
Smokers	195 (44.7)[a]
N° Cigarettes/Day	6.67 ± 8.65[a]
Pack years	7.55 ± 8.73[a]
Occupational exposure to toxic chemicals	52 (12.3)[b]

Table 1. Total cohort clinical and lifestyle-related parameters. Values expressed as mean ± SD or number of cases/total and (percentage). Abbreviations: BMI, body mass index; HI, high environmental impact. [a]Determined in 436 participants; [b]determined in 422 participants.

	Total cohort (N=465)
Ano-Genital Distance (mm)	7.87 ± 1.61
Mean Testicular Volume (ml)	17.25 ± 4.55
Anamnestic Cryptorchidism	2 (0.5%)[a]
Testicular parenchymal structure Inhomogeneity*	86 (19%)[b]
Testicular Microlithiasis*	11 (2.5%)[c]
Testicular Hypotrophy*	66 (14.6%)[d]
Varicocele*	160 (35.4%)[b]
III-V grade clinically relevant varicocele*	62 (13.8%)[b]
Hydrocele*	147 (34.8%)[e]
Testicular Solid Lesions > 5 mm*	1 (0.2%)

Table 2. Total cohort andrological parameters and prevalence of clinical and ultrasound testicular morphostructural alterations. Values expressed as mean ± SD or number of cases/total and (percentage). *Unilateral or bilateral. [a]Determined in 418 participants; [b]determined in 452 participants; [c]determined in 440 participants; [d]determined in 451 participants; [e]determined in 423 participants.

	Detectable sCd (N=128)	Undetectable sCd (N=257)	p
Age (years)	30.34 ± 7.36	29.5 ± 7.02	NS
BMI (Kg/m^2)	25.97 ± 3.8	25.9 ± 4.13	NS
Mean residence in HI areas (years)	25.43 ± 9.69	26.12 ± 9.15	NS
Mean time spent in HI areas (hours/24 hours)	17.13 ± 5.99	16.49 ± 5.74	NS
Smokers	43 (36.1)[a]	117 (48.1)[b]	*p=0.0328*
N° Cigarettes/Day	6.67 ± 8.65	6.97 ± 9.03	NS
Pack years	8.26 ± 7.70	7.88 ± 9.29	NS
Occupational exposure to toxic chemicals	13 (11.2)[c]	36 (15.6)[d]	NS

Table 3. Clinical and lifestyle-related parameters in subgroups of participants with detectable and undetectable whole semen cadmium (sCd) level. Values expressed as mean ± SD or number of cases/total and (percentage). Abbreviations: BMI, body mass index; HI, high environmental impact. [a]Determined in 119 participants; [b]determined in 243 participants; [c]determined in 116 participants; [d]determined in 231 participants.

	Detectable sCd (N=128)	Undetectable sCd (N=257)	p
Ano-Genital Distance (cm)	7,8 ± 1.4	7,8 ± 1.8	*NS*
Mean Testicular Volume (ml)	16.56 ± 4.68	17.66 ± 4.34	*p=0.0153*
Testicular parenchymal structure Inhomogeneity*	32/124 (25 .8%)	42/251 (16.7%)	*NS*
Testicular Microlithiasis*	5/128 (3.9%)	13/251 (5.1 %)	*NS*
Testicular Hypotrophy*	27/128 (21.1%)	25/250 (10%)	*p=0.0059*
Varicocele*	59/128 (46.1%)	74/249 (29.7%)	*p= 0.0008*
III-V grade clinically relevant varicocele*	23/128 (18%)	30/257 (11.7%)	NS
Testicular Solid Lesions > 5 mm*	0/128 (0%)	1/256 (0.4%)	*NS*

Table 4. Andrological parameters and prevalence of clinical and ultrasound testicular morphostructural alterations in subgroups of participants with detectable and undetectable whole semen cadmium (sCd) level. Values expressed as mean ± SD or number of cases/total and (percentage). *Unilateral or bilateral.