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Human urinary phthalate metabolites level and main semen parameters, sperm chromatin structure, sperm aneuploidy and reproductive hormones

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ABSTRACT

The aim of the study was to assess the association of phthalate metabolites levels in urine with semen parameters (sperm concentration, motility, morphology, CASA parameters), sperm chromatin structure, sperm aneuploidy and reproductive hormones. The study population consisted of 269 men who were attending an infertility clinic and had normal semen concentration (20–300 mln/ml) or slight oligozoospermia (15–20 mln/ml). Participants were interviewed and provided a semen sample. The phthalate metabolites were analysed in the urine using a procedure based on the LC–MS/MS method. Urinary phthalate metabolites levels were significantly associated with a decrease in sperm motility (5OH MEHP, MEHP, MINP), CASA parameters (MBP), testosterone level (MEHP) and an increase sperm DNA damage (MBP) and sperm aneuploidy (MBzP, MBP, MEHP, MEP). In view of the importance of human reproductive health and the widespread usage of phthalates, it is important to further investigate these correlations.

1. Introduction

Phthalates are a class of industrial chemicals that are dialkyl- or alkylarylesters of 1,2-benzenedicarboxylic acid and have been used for a variety of purposes [1]. Their industrial applications are related to the length of their ester chain. They are divided into two distinct groups, with very different applications, toxicological properties and classification. High molecular weight phthalates (e.g. di(2-ethylhexyl)phthalate (DEHP)) with alkyl chain lengths from 8 to 13 carbons are widely used as general-purpose plasticisers in polymers, primarily in polyvinyl chloride (PVC) resins [1], to make rigid PVC more flexible and useful, such as for wiring and cables. These phthalates are also used in a variety of consumer products, flooring and wall coverings, in food contact application and medical devices (bags for blood, parenteral nutrition, tubings and catheters) [2]. Low molecular weight phthalates with an alkyl chain of 2–7 carbons (e.g. diethyl phthalate (DEP), dibutyl phthalate (DBP)) are used in personal-care products, some cosmetics/fragrances, lacquers, varnishes, as solvents and as plasticisers in cellulose acetate [2–4]. As a result of their extensive use and their moderate resistance to degradation, phthalates are distributed widely in the environment [5]. Humans are exposed via multiple pathways, such as through food, water (oral), air (inhalation), and consumer products (dermal) [6]. Phthalates are rapidly metabolised to their monoesters in humans, and some of them can be further oxidised and conjugated with glucuronide before excretion in urine or feces [7–9]. Urinary phthalate metabolites have been used extensively as biomarkers of human exposure. Because phthalates are ubiquitous in daily life, the potential consequences of human exposure to phthalates have raised concerns in the general population.

Some phthalates are reproductive and developmental toxicants in laboratory animals (DBP, DEHP, diisobutyl (DIBP)) and are classified in the EU as reproductive toxicants; whereas others are not (DEP, diisononyl (DINP), diisodecyl (DIDP)) [10–12]. DEHP induced anti-androgenic action and abnormalities of the male reproductive system in prenatally exposed animals likely affecting the normal development of the testes [10,12–15]. Hypospadias and cryptorchidism, testicular injury leading to lowered sperm counts

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and reduced anogenital distance (AGD) as well as a decrease in testosterone biosynthesis involving damage to the Leydig cells [16,17] have also been observed. In particular, phthalates may have endocrine-disrupting chemical properties that may affect the reproductive and developmental processes in humans. Consequently, recent studies have investigated the potential impact and toxicity of exposure to phthalates on aberrant male reproductive development [18]. Several recent epidemiological studies have addressed the male reproductive toxicity of phthalates [19–26]. Most of the studies suggest that the urinary phthalate metabolites levels (at least one of the metabolites) may affect semen quality. Moreover, it has been documented that phthalate metabolites levels are associated with increased DNA damage, a lower sperm concentration and density as well as decreased motility and morphology. In addition, the urinary phthalate metabolites levels also appeared to be negatively associated with the testosterone level [27,28], follicle-stimulating hormone FSH [19] and luteinising hormone (LH) [23].

Although several studies have explored the association between phthalate exposure and male reproductive function, none have carefully assessed semen quality parameters, sperm chromatin structure, sperm aneuploidy and level of reproductive hormones in one study. The aim of the present study is to assess the association of the urine phthalate metabolites levels with semen quality parameters (sperm concentration, motility, CASA parameters, morphology), sperm chromatin structure, sperm aneuploidy and the level of reproductive hormones (FSH, estradiol, testosterone) in adult men. According to our knowledge, this is the first such study that examines the relationship between phthalate metabolites levels and numerical chromosomal aberrations in sperm.

2. Materials and methods

2.1. Study population

The study population initially consisted of 344 men who were attending an infertility clinic for diagnostic purposes and who had normal semen concentration of 20–300 mln/ml or slight oligozoospermia (semen concentration of 15–20 mln/ml) (WHO 1999) [29], from the study entitled "Environmental factors and male infertility", which is the part of the "Epidemiology of reproductive hazards in Poland – multicentre study in Poland" project supported by the National Center for Research and Development in Poland, grant no. PBZ-MEiN-/8/2//2006. The Nofer Institute of Occupational Medicine Bioethical Committee Board approved the study (Resolution No. 9/2007 (04.06.2007)) and written informed consent was obtained from all subjects before their participation. Assessment of phthalate metabolites in urine was performed on samples from 269 men, so the presented analysis is based on this sample size.

Caucasian men from Lodz, Poland who were under 45 years of age (range: 22.0–42.2 years) participated in the study. All participants were interviewed and provided a semen sample during their office visit. The interview included questions about demographics, socio-economic status, medical history related to past diseases which may have had an impact on semen quality, lifestyle factors (consumption of coffee, alcohol, tobacco) and occupational exposure.

In addition, a detailed smoking history was collected. The smoking status was verified by measuring cotinine level in the saliva in a laboratory at the Nofer Institute of Occupational Medicine. The saliva cotinine level was measured using high performance liquid chromatography coupled with tandem mass spectrometry/positive electrospray ionisation (LC-ESI + MS/MS) and the isotope dilution method. This procedure has been validated under ISO 17025 criteria and accredited by the Polish Center of Accreditation (Certificate AB215). Men were identified as smokers when their saliva cotinine level was higher than 10 ng/ml.

2.2. Semen and reproductive hormone analysis

A semen sample was produced on site by masturbation and collected into a sterile container. The volume of the sample was 3.5 ml ± 1.5 ml (1.7–12.3 ml). The length of abstinence prior to sampling was documented. After collection, the sample was liquefied at 37 °C for 20 min before analysis and maintained at 37 °C during the assessment. The semen quality parameters – liquefaction time, agglutination volume, pH, sperm concentration and motility – were determined according to the WHO 1999 guidelines (World Health Organization, 1999) [29].

Sperm counts and percentage motility were determined with the use of computer-aided semen analysis (CASA) (Hamilton-Thorne Version 10HTM-IVOS) using 2 Chamber Leja slides (Leja, The Netherlands). A 5 μl well-mixed, homogeneous sample was applied to each chamber of the Leja slide. The Leja slide was placed on a warm CASA stage and analysed. At least 200 sperm cells were counted for motility assessment. Two known concentrations of Accu-beads (Hamilton-Thorne Inc., Beverly, MA, USA) were used each day for quality control and to confirm the accuracy of the CASA counts. A manual count of the same sample was also performed for the sperm concentration. For the manual count, the sperm concentration was obtained by averaging the total number of sperm in both chambers on the Leja slide. Three CASA parameters, straight-line velocity (VSL), curvilinear velocity (VCL), and linearity (LIN), were used as measures of sperm progression, sperm vigor, and swimming pattern, respectively [30].

Sperm morphology was quantified using strict Kruger criteria [31]. The semen smears were air-dried, fixed and stained according to Papanicolaou. A total of 200 sperm per sample were analysed.

Human plasma was collected in lithium heparin EDTA to determine levels of testosterone, FSH and estradiol among the study participants. Measurement of FSH, testosterone and estradiol was performed using a Chemiluminescent Microparticle Immunoassay (ARCHITECT System; Abbott, Longford, Ireland). The results are expressed as IU/L for FSH, pg/ml for estradiol and ng/ml for testosterone. The lower limits of detection for FSH, estradiol and testosterone were 0.05 IU/l, 17.9 pg/ml and 0.08 ng/ml respectively [32].

Male reference values for these assays were: 2.49–8.36 ng/ml for testosterone, 1.7–12 IU/l for FSH and <62 pg/ml for estradiol [33].

2.2.1. The sperm chromatin structure analysis

Assessment of the sperm chromatin structure assay (SCSA) was performed using flow cytometry [34]. Briefly, after partial denaturation of the DNA (pH = 1.5), the samples were stained with metachromatic fluorochrome: acridine orange (Ex/Em = 488/525 and 615 nm). Fluorescence in green (515–530 nm) and red (>630 nm) bands was measured using a flow cytometer (DAKO Galaxym DAKO, Denmark). The fluorescence bands corresponded to intact double-stranded DNA (green fluorescence) and fragmented, single-stranded (red fluorescence) sperm DNA. Approximately 15,000–25,000 spermatozoa were acquired for each sample at a flow rate of 400–500 events/s. An artificial parameter, alpha *t* (alpha *t* = red/(green + red) fluorescence), was created for the calculations. Cells with an abnormal chromatin structure (i.e. fragmented DNA) showed a distinct shift of the alpha *t* parameter value. The DNA Fragmentation Index (DFI) was calculated according to the formula: DFI = (cells with a shift of the alpha-*t* parameter/all cells) × 100 each analysed sperm cell and was shown on a histogram. Cells with an abnormal chromatin structure showed a distinct shift of the alpha *t* parameter value [35].