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Title:

Occupational exposure to endocrine disrupting chemicals and other parental risk factors in hypospadias and cryptorchidism development: a case-control study

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Summary

Aim of the Study

Endocrine disrupting chemicals (EDC) are exogenous agents that are capable of altering the endocrine system functions, including the regulation of developmental processes. The aim of this study was to investigate the association between endocrine disrupting chemicals (EDC) exposure and other parental factors in the aetiology of hypospadias and cryptorchidism.

Methods

A case-control study was conducted. Cases (210) were infants between 6 months and 14 years of age diagnosed with hypospadias or cryptorchidism attended in our hospital over a period of 18 months, and controls (210), infants within the same range of age and without any urological disorders who attended the outpatient clinic of the same hospital during the same. Their selection was independent of exposures. Data on parental occupational exposure to EDC and other sociodemographic variables were collected by face-to-face interviews and systematically for both cases and controls. Crude and adjusted odds ratios (OR) were estimated to control for confounding with their 95% CI by means of logistic regressions. Specifically, three final models of dichotomous outcome were constructed: one for cryptorchidism, one for hypospadias, and the third considering both malformations together. Hosmer-Lemeshow test was used to assess the goodness-of-fit of the models. Their discriminatory accuracy (DA) was ascertained by estimating their areas under the receiver-operating-characteristics (ROC) curves (AUC) along with their 95%CI.

Results

Associations were found between advanced maternal age (OR adjusted=1,82; 95%CI: 1,14-2,92), mother´s consumption of antiabortives (OR=5.40; 1.40-38.5) and other drugs (OR=2.02; 1.31-3.16) during pregnancy, maternal and paternal occupational exposure to EDC (4.08; 2.03-

8.96 and 3.90; 2.41-6.48), respectively, fathers smoking (OR=2.0; 1.33-2.99) and with urological disorders (OR=2.31; 1.15-4.90). Maternal and paternal high educational level could be protective of cryptorchidism (OR=0.47; 0.28-0.76) and OR=0.63; 0.42-0.93, respectively). The discriminatory accuracy of the models for the whole sample (AUC=0.75; 0.70-0.79), for cryptorchidism (0.76; 0.71-0.82), and for hipospadias (0.75; 0.69-0.81) was moderately high.

Conclusions

Advanced age, some parental occupational exposure to EDC, some drugs consumption, smoking and the father´s history of urological disorders may increase risk and predict the developments of these malformations. Studies with higher samples sizes are needed to assess associations between individual EDC occupational exposures and drugs and these malformations.

Introduction

The ability of some synthetic chemicals to interfere with the human hormonal system has been known since the 1940s when diethylstilbestrol (DES) started to be used to prevent spontaneous abortions [1]. However, the term “endocrine disruptor” was not defined until 1991 during the Wingspread Conference in Wisconsin [2] where a group of experts met to assess the causes of health problems observed in people and animals from developed countries including disorders of the reproductive system. An endocrine disrupting chemical (EDC) was then defined as an exogenous substance capable of altering the hormonal balance and causing adverse effects on an organism or its progeny.

Most of these exogenous chemicals have an estrogenic and anti-androgenic effect and may disturb the androgen/estrogen balance in the developing male fetus and affect external

genital differentiation in animals [3] and humans [4]. Most of them are lipophilic and may remain in the body fat for many years; they can reach the fetus across the placenta during pregnancy and the newborn during breastfeeding [5], and eventually affect the newborn health. Mankind produces the vast majority of these substances, which are present on the environment as industrial pollutants, pesticides, plastics and cosmetic products among others.

In 1993, R. Sharpe and N. Skakkebaek hypothesized that "the increasing incidence of reproductive abnormalities in the human male may be related to increased estrogen exposure in utero" [6]. Since then, several studies have attempted to demonstrate the role that environmental EDC may have in hypospadias and cryptorchidism etiology by trying to find associations between them [7–9]. The aim of the present study was to investigate the association and the discriminatory accuracy of alleged risk factors associated with both malformations development, including parental occupational and non-occupational exposures to EDC.

Materials and methods

Study design

We conducted a case-control study at the *Miguel Servet* University Hospital of Zaragoza, reference´s center in Pediatric Urology for population of the Spanish Autonomous Communities of Aragon, La Rioja and the province of Soria, where the proportion of population employed in industrial and agricultural activities is considerable. All cases and controls were Caucasian.

As cases, we included children aged between 6 months and 14 years diagnosed with cryptorchidism and/or hypospadias in the outpatient of Pediatric Urology for a period of 18 months, from July 2015 to January 2017. Cases of cryptorchidism were defined as infants who

presented one or both non-descended testicles into the scrotum and who underwent an orchidopexy surgery. All cases were at least 6 months old to exclude the cases with a delayed descent of the testis. Patients with acquired undescended testis were excluded. Cases of hypospadias were defined as those with displacement of the urethral opening anywhere along the shaft in the ventral position of the penis, including proximal and distal forms. All patients with endocrine or genetic cause of hypospadias and/or cryptorchidism (2 cases of DSD and 1 case of congenital adrenal hyperplasia) were excluded as cases.

In the control group, we included children with the same range of age who attended to Pediatric Surgery and General Pediatrics outpatient clinics during the same period at the same center, after a clinical examination confirming a normal configuration of external genitalia and no history of inguinal/genital surgery or endocrine disease. Their selection was independent of exposures. No hospitalized patients were included.

Data collection

After approval from our Institutional Review Board and upon informed consent of the family, data were collected through an epidemiological questionnaire that was performed by face-to-face interview with both parents in the consulting room. All of those invited agreed to participate. Only 9.6% of the cases did not complete the whole questionnaire, so we had to draw the missing data from the electronical medical records. The epidemiological questionnaire included mainly three groups of variables.

Patient´s data: gestational age, birth weight, presence of others malformations at birth and family history of hypospadias and/or cryptorchidism. Mother´s data: age at delivery, educational level (low: no completed school and no job training; medium: completed high

school with job training; high: completed high school and university studies), profession prior and during conception period (those entailing direct exposure to substances already described in the previous literature as EDC), occupational exposure to EDC and obstetric history (use of contraceptives, tobacco and alcohol consumption, use of drugs during pregnancy, if it was a natural pregnancy or in vitro fertilization (IVF), previous abortions, parity and gynecological diseases). Father´s data: age at patient´s birth, educational level, tobacco and alcohol consumption, profession prior and during conception period, occupational exposure to EDC and urological history.

Regarding parental profession, we defined four professional categories "of risk" because of their specific exposure to EDC: agriculture (e.g. pesticides, chlorinated insecticides, dicarboximide fungicides), industry (e.g. bisphenol A, polychlorinated biphenyls, dioxins) cleaners and hairdressers (e.g. phthalates and parabens).

Data analysis

For descriptive statistics, measures of central tendency (mean ± standard deviation) were estimated for all quantitative variables and on qualitative variables, percentages with their 95% confidence intervals (CI) were calculated. To compare data obtained in each group, we used parametric and non-parametric statistical tests for continuous variables (T test and U-Mann-Whitney test, respectively) and Chi-square and Fisher's exact tests when necessary for categorical data. A p-value of < 0.05 was defined as statistically significant.

Then for the analysis of alleged risk factors, we estimated both crude and adjusted odds ratios (OR) to control for confounding with their 95% CI by means of logistic regressions. Specifically, three final models of dichotomous outcome were constructed: one for cryptorchidism, one for hypospadias, and the third considering both malformations together. We did so mainly for two reasons: 1) to take into consideration the hypothesis considered in the literature that both defects share common etiopathogenic mechanism [3,4,6] and 2) to

ascertain if the results concerning the associations changed when analyzing them separately, thus trying to add more insights in an attempt to contribute to clarify this issue. Hosmer-Lemeshow test was used to assess the goodness-of-fit of the models.

Average measures of association between putative risk factors and outcomes are unsuitable for ascertaining their performance. Even strong associations (OR or RR > 10) have a low capacity of both risks factors and biomarkers to discriminate between cases and noncases in the population. Exposures with very high disease risks have a low population prevalence and account for a small fraction of cases. Therefore, average measures of association ought to be interpreted in tandem with measures of discriminatory accuracy [7,8]. For these reasons, their discriminatory accuracy (DA) was ascertained by estimating their areas under the receiver-operating-characteristics (ROC) curves (AUC or C statistic) along with their 95%CI. The statistical analyses were performed using SPSS v.20 software.

Results

A total of 420 patients were studied, 210 cases and 210 controls with a mean age of 3.37±2.64 years (range 0.5-12). Cases included 107 patients with hypospadias and 103 with cryptorchidism; eight cases presented both malformations (3.8%). Table 1 shows the prevalence of exposure to the alleged risk factors considered in the study for cases, controls, and the general population. The only difference found in the fraction of exposed between controls and the general population was mother´s age. We did not find general population estimates of mother´s and father´s occupational EDC exposure and antiabortives.

Regarding perinatal factors, no statistically significant differences were found between birth weight (BW) medians of cases and controls (3,067 ± 0,653kg versus 3,110 ± 0,596kg respectively, p = 0.47) in the whole sample, but BW median was significantly lower in the

hypospadias group compared with the controls (2,929 ± 0,682kg versus 3,360 ± 0,590kg respectively, p = 0.01). Later, when this variable was dichotomized in low birth weight (LBW) and no-LBW (defined as < 2500 grams), a significant increase of the crude OR was observed in hypospadias group (OR = 2.24; 95% CI: 1.20-4.17). No differences between cases and controls were found (38 ± 2, 42 and 37, 93 ± 1, 91 weeks respectively, p = 0.75) regarding medians of gestational age (GA). When this variable was considered categorical, the percentage of preterm births (defined as a GA < 37 weeks) was significantly higher in cases than in controls both for the whole sample (19 versus 10%, p = 0.02) and for the hypospadias group (19.6% versus 10.5%, p = 0.02). An increased OR for prematurity was also observed in hypospadias group (OR = 2.21; 95% CI: 1.16-4.21).

The most frequent associated malformation in cases was inguinal hernia with an incidence of 1.2%. No statistically significantly differences were found for the presence of other malformations at birth between cases and controls (9.5% versus 8% respectively; p = 0.50). The difference between the frequency of family history of hypospadias and/or cryptorchidism between cases (23, 10.9%) and controls (6, 2.9%) was also statistically significant (p = 0.001). In cryptorchidism group, 11.6% of subjects had family history of cryptorchidism, 4 of them in siblings, 3 in parents, 4 in paternal uncles and 1 in a paternal cousin; and in hypospadias group six patients had family history of hypospadias, 3 of them in siblings and 3 in their parents.

Tables 2, 3 and 4, show the adjusted ORs of the alleged parental risk factors for the pooled sample, and for cryptorchidism and hypospadias separately, estimated with the final logistic regression models chosen on the basis of their goodness of fit and discriminant accuracy. According to the adjusted ORs and their 95%CI, when considered together cryptorchidism and hypospadias were associated with advanced maternal age, maternal and

paternal EDC exposure, smoking fathers, antiabortives and other drugs consumption during pregnancy. No conclusion can be drawn for either advanced paternal age or father´s urological history since their 95%CI include 1.0. They were kept in the final models because their inclusion increased both their goodness-of-fit and discriminant accuracy.

Alleged risk factors associated with cryptorchidism were advanced maternal age, paternal and maternal EDC exposure, paternal smoking, and antiabortives. Those associated with hypospadias were advanced maternal age, paternal EDC exposure, father smoking, and other drugs consumption.

No conclusion can be drawn from the association between father´s urological history with cryptorchidism and hypospadias when considered separately. Since their 95%CI include 1.0 their association with these malformations could be both direct or inverse despite more values of the 95%CI are consistent with a direct association. The same applies to maternal education level but only with regard to its exclusive association with cryptorchidism, and to in vitro fertilization with hypospadias. Paternal education level appears to have an association only with cryptorchidism, which is the only clear inverse association of all found. Therefore, advanced maternal age, paternal EDC exposure, and smoking are the only alleged risk factors associated with both cryptorchidism and hypospadias. When stratifying the analyses by fathers´ and mothers´ exposure to EDC for cryptorchidism and hypospadias separately, no differences were found compared with the pooled analyses.

No results could be obtained concerning the DA of each of the individual alleged risk factors given their small sample sizes. Nevertheless, when these factors are considered together, their DA, as indicated by the AUC of each model, is moderately high because the lower limit of the 95%CI of the AUC of the three models (for the whole sample, for

cryptorchidism and for hypospadias) is 0.70. The final models fit the data quite well (Tables 2, 3 and 4).

Discussion

Several factors have been considered as possible risk factors of cryptorchidism and hypospadias in the scientific literature. Regarding perinatal factors, LBW and prematurity have been classically associated with cryptorchidism and hypospadias [9–12]. However, we only could confirm this observation in hypospadias group in the bivariate analyses, not in the final multivariate models. These two variables are strongly dependent on each other and associated with suboptimal first-trimester intrauterine growth. Their association with hypospadias may be related to early placental dysfunction causing insufficient amounts of androgen production affecting male genital development [16]. Accordingly, hypospadias has been associated with both low weight of the placenta [17] and preeclampsia later during pregnancy [18].

Regarding familiar history of cryptorchidism, our results would support the previous familiar predisposition described in cryptorchidism development and not only in first-degree relatives [16,17]. In hypospadias group, the familiar prevalence observed (5.6%) was similar or slightly lower than the 7% incidence described in a nationwide cohort study conducted by Schnack et al. [21]. Moreover, a significant increase in risk was observed in relation to the presence of family history of cryptorchidism and/or hypospadias in all the groups, but only in the bivariate analysis. These results would support the genetic predisposition previously described in the literature for both malformations and clearly highlight the importance of genetic factors in their pathogenesis.

Concerning maternal factors, mother´s age appears to be associated with both cryptorchidism and hypospadias, what is consistent with other studies, and has been attributed to the concentration of endogenous estrogens and the possible accumulation of

persistent chemicals [15,19–21]. We also found an association between antiabortives and other drugs consumption during pregnancy in all the groups. As antiabortives, we include drugs used both to prevent an abortion threat (medroxyprogesterone acetate) and to suppress preterm labor (ritodrine and nifedipine). Our results are consistent with those of other studies suggesting a potential etiological effect of some drugs on cryptorchidism and hypospadias development [22,23]. As for other drugs consumption, the most frequently drugs used were hormones (46.5%), followed by analgesics and antipyretics (19.5%), which have been found to increase the risk of cryptorchidism and hypospadias in other studies [24,25]. Moreover, mothers receiving hormone replacement therapy during pregnancy (mainly insulin and levothyroxine) had *Diabetes Mellitus* or hypothyroidism, what has also been related to an increase in cryptorchidism and hypospadias [20,26,27]. We could not assess the associations between each drug and hypospadias and cryptorchidism given the small sample sizes.

Regarding paternal factors, we also found a significant increased risk in smoking fathers. Paternal smoking had been previously associated with the occurrence of multiple birth defects [30] and specifically with hypospadias by Pierik et.al [14]; our results suggest that it could be associated with hypospadias and also with cryptorchidism development. Maternal smoking exposure have been reported as a risk factor for both malformations, and paternal smoking could have an effect through passive exposure of the mother. On the other hand, and similarly to EDC exposure, tobacco could affect offspring by epigenetic effects on germ cells (e.g. DNA repair, chromatin structure, apoptosis), whereas exposure of the oocyte or embryo to contaminated seminal fluid could also play a role [31].

As for environmental factors, both maternal and paternal EDC occupational exposures were found to be associated with an increased risk for both malformation in the pooled sample and in the cryptorchidism group. For mothers, the percentage of exposed was significantly higher in case than in controls in hairdressers and for fathers in industry and

agriculture categories. These results are consistent with the endocrine disruption hypothesis previously described by Sharpe and Skakkeabeak [6] and with data previously described in literature. In 2003, Vrijheid et al. found an increased risk of hypospadias in children whose mother were hairdressers [32]. Similarly, in a case-control study including 408 patients with hypospadias, the percentage of parents exposed to toxics during work was significantly higher in cases than in controls and within the cases, the most frequent maternal profession was hairdresser [8]. Moreover, in a Danish cohort study conducted, hairdressing and agriculture were the parental professions most frequently described among the parents of patients with cryptorchidism and hypospadias [33], and others found an increased risk of hypospadias and cryptorchidism in children of farming parents [14]. Regarding industry, some studies reported a higher incidence of hypospadias and cryptorchidism in children of parents who lived close to industrial zones [31-33].

Contrarily, an inverse association was found between fathers and mothers educational level and cryptorchidism. This could be explain because of the direct relation that educational level have with the professional category, as parents with a high-educational level will not probably work as hairdressers, farmers or industry workers and will have less probabilities of being exposed to EDC in their working place.

This study has some potential limitations. The low sample sizes do not allow to look for individual associations between both malformations and individual drug´s consumption, EDC exposures to specific toxics and professions. The fact that controls were drawn from the hospitals could introduce a selection bias, including Bergson´s bias. However, excluding mother´s age, the percentage of controls exposed to the alleged risk factors was similar to that of the general population thus impeding the introduction of these biases. The prevalence of control mothers > 35 years being lower than in the general population could overestimate the magnitude of its association with the development of hypospadias and cryptorchidism. The

magnitude of some associations could also be overestimated due to differential recall between parents of cases and controls concerning their exposures. Unbiased population estimates of the prevalence of mother´s and father´s EDC occupational exposure and the consumption of antiabortives are lacking, precluding drawing conclusions about selection bias regarding these risk factors. The unambiguous diagnoses of both malformations, and the systematic way in which information was drawn from cases and controls rule out both misclassification bias information bias.

Conclusions

Hypospadias and cryptorchidism are both multifactorial pathologies in which genetic and environmental factors are implicated. According with our results**,** advanced maternal age, maternal and paternal EDC occupational exposure, antiabortives and other drugs consumption could be risk factors for both malformations development, LBW and prematurity could be risk factors for hypospadias and the parental high-educational level could have a protecting effect for cryptorchidism. Further studies with a wider variety of population controls and higher statistical power are needed particularly, to focus on the associations between individual EDC occupational exposure and drugs and these two malformations, and to confirm our findings.

Conflict of interest statement

There are no conflicts of interests

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None

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Summary table Adjusted ORs for alleged parental risk factors in the whole sample

Parental variables	OR	IC 95% OR	
		Inferior limit	Upper limit
Advanced maternal age	1,82	1,14	2,92
Maternal EDC exposure	3,48	1,57	7,67
Antiabortives	5,57	1,08	28,75
Other drugs consumption	2,22	1,36	3,62
Advanced paternal age	1,36	0,81	2,26
Paternal EDC exposure	4,20	2,48	7,11
Smoking fathers	2,07	1,32	3,26
Father´s urological history	2,10	0,95	4,62
Constant	0,26		

Goodness-of-fit: Hosmer-Lemeshow chi-square = 1.10; p = 0.99.

Table 1 Prevalence of exposure to alleged risk factors in cases and controls

Alleged risk factors	Cases (%) (n=210)	Controls (%) (n=210)	P value	Prevalence in general population (%)
Mother´s age				
< 25 years	6.19	6.19		15.4
25-35 years	50.96	63,34		35.8
> 35 years	42.85	30.47	0.033	49.7
Mother´s educational level				
basic	34.76	28.10		28
medium	35.23	26.19		22
high	31.01	45.71	0.004	40
Mother´s occupational EDC exposure				
No	82.9	95.2		
Yes	17.1	4.8	< 0.001	Unknown
Mother´s professional category				
Agriculture	1.9	1.0		
Cleaners	2.9	3.8		
Hairdressers	6.2	0	0.016	Unknown
Industry	4.8	4.3		
Mother´s tobacco consumption				
No	83.3	85.2		
< 10 cigarettes/day	15.2	12.4	0.556	
> 10 cigarettes/day	1.4	2.4		
Mother´s alcohol consumption				
No	98.6	99		
1-7 glasses/week	1.4	1.0	0.652	
> 8 glasses/week	0	0		
Contraceptives				
No	64.8	63.3		
Yes	35.2	36.7	0.419	
Antiabortives				
No	94.8	99		
Yes	5.2	1	0.024	Unknown
Other drugs consumption during pregnancy				
No	65.7	79.5		
Yes	34.3	20.5	0.002	15-20
In vitro fertilization				
No	7.6	3.8		
Yes	92.4	96.2	0.141	
Previous abortions				
No	98.1	98.6		
Yes	1.9	1.4	0.703	
Parity				
Primiparous	49	43.8		
Multiparous	51	56.2	0.328	
Gynecological diseases				
No	88.1	87.1		
Yes	11.9	12.9	0.882	
Father´s age				

< 40 years	70	76.7		74.11
> 40 years	30	23.3	0.151	25.9
Father´s educational level				
basic	47.6	34.8		
medium	35.7	26.2		
high	16.7	39	< 0.001	
Father´s occupational EDC exposure				
No	63.3	87.1		
Yes	36.7	12.9	< 0.001	Unknown
Father´s professional category				
Agriculture	9	2.9	0.002	2.7
Cleaners	0.5	0.5		
Hairdressers	0.5	0		
Industry	15.7	7.1	0.002	7.8
Father´s tobacco consumption				
No	56.2	71.9		
< 10 cigarettes/day	18.6	10.5		
> 10 cigarettes/day	25.2	17.6	0.003	25
Father´s alcohol consumption				
No	43.3	51.9		
1-7 glasses/week	53.8	43.3		
> 8 glasses/week	2.9	4.8	0.082	22,6
Urological diseases				
No	87.6	94.3		
Yes	12.4	5.7	0.027	6.1

Table 2 Adjusted ORs for alleged parental risk factors in cryptorchidism group

Parental variables	OR	95% CI	
		Inferior limit	Upper limit
Advanced maternal age	1,95	1,04	3,64
Maternal EDC exposure	5,46	2,32	12,83
Maternal educational level	0,66	0,35	1,23
Antiabortives	7,54	1,29	44,21
Advanced paternal age	1,26	0,66	2,40
Paternal EDC exposure	2,79	1,47	5,30
Paternal educational level	0,50	0,27	0,93
Smoking fathers	1,71	0,99	2,98
Father´s urological history	1,79	0,66	4,89
Constant	1,01		

Goodness-of-fit: Hosmer-Lemeshow chi-square = 4.88; p = 0.77.

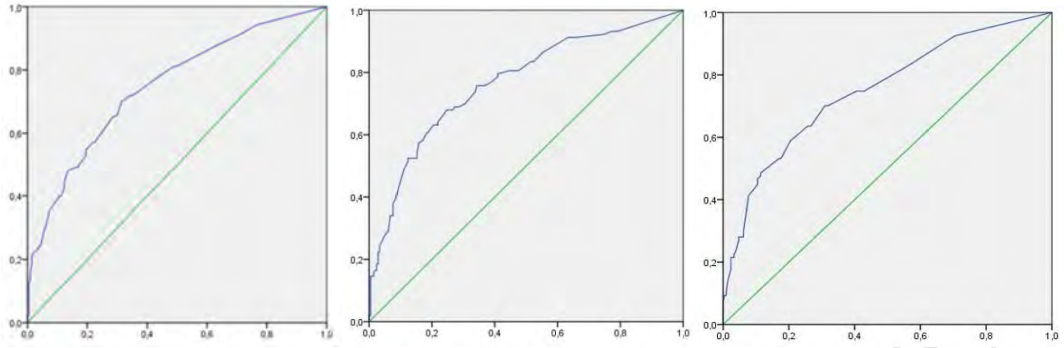
Table 3 Adjusted ORs for alleged parental risk factors in hipospadias group

Parental variables	OR	IC 95% OR	
		Inferior limit	Upper limit
Advanced maternal age	2,36	1,37	4,06
Other drugs consumption	2,60	1,47	4,62
In vitro fertilization	0,37	0,13	1,04
Paternal EDC exposure	5,07	2,76	9,31
Smoking fathers	2,12	1,22	3,70
Father´s urological history	2,39	0,98	5,84
Constant	0,90		

Goodness-of-fit: Hosmer-Lomeshow chi-square = 3.15, p = 0.78.

Figure 1 ROC curves showing the discriminant accuracy of the three predictive models

Whole sample | Cryptrochidism group | Hypospadias group



AUC =0.75; 95%CI:0.70-0.79. AUC = 0.76; 95%CI: 0.71-0.82. AUC = 0.75; 95%CI: 0.69-0.81.