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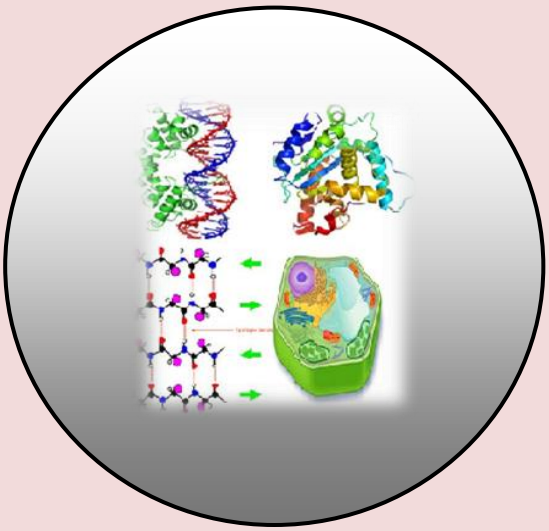

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Estimation and Correlation of the Blood Levels of Heavy Metals in Exstrophy-Epispadias Patients: A Pilot Study

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ABSTRACT

Many studies have been conducted to find association between heavy metals and congenital anomalies such as Cleft lip-palate, neural-tube-defects, esophageal atresia and tracheo-esopheageal fistula, cardiac anomalies, skeletal anomalies and hypospadias. However, association of heavy metals with exstrophy-epispadias has not been reported. Here, we report a case-control study on the role of heavy metals in exstrophy-epispadias. After obtaining ethical clearance from institutional ethical committee and informed written consent, 2.0ml blood samples were collected from 20 primary exstrophy-epispadias patients and their mothers and 20 age-matched controls, admitted for other indications and without any congenital anomalies, and their mothers. Obtained blood samples were digested in Microwave Reaction system and were evaluated for the levels of heavy metals by Inductively-Coupled-Plasma-Optical-Emission-Spectrometer (ICP-OES). Statistical analysis was performed using Chi-square test, Mann-Whitney U test and Spearman-Correlation-Coefficient on the SPSS-version 16.0. The p-value<0.05 was considered significant. Only mean blood levels of Cr and Hg were found raised in patients and mothers of study group when compared with subjects and mothers of control group. However, difference was statistically insignificant. Moreover, we found no correlation of Cr and Hg levels between mothers and children of patient group. In conclusion, it may be stated that exposure to heavy metals may have a role, though not significant in the etiology of exstrophy-epispadias. Further, multi-institutional studies with larger sample size are needed to confirm the association of heavy metals with exstrophy-epispadias.

Keywords: Bladder Exstrophy, Correlation of Heavy Metals, Heavy Metals and Risk Factors for Exstrophy Bladder.

INTRODUCTION

Bladder exstrophy is a rare and complex urogenital birth defect with an incidence of 1 in 10,000 to 1 in 50,000 live births (Gearhart and Mathews, 2010). Its exact etiology is still not clear, however, some previous studies have reported the possible role of epidemiological, genetic and hormonal factors in the etiology of bladder exstrophy (Gearhart and Mathews, 2010). Although till date, no specific environmental risk factor that predisposes to bladder exstrophy has been identified.

Over the last few decades, heavy metals such as arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), and mercury (Hg) have increasingly became significant worldwide environmental pollutants because of increasing industrialization, urbanization and increasing agricultural production due to frequent use of pesticides. These heavy metals are potentially toxic having proven harmful effect on both human beings and animals. Increased exposure of pregnant women and their fetuses to these harmful toxic metals may be responsible for increase in incidence of many birth defects. Therefore, there has been growing interest in investigating the role of heavy metals as possible environmental risk factors in the etiology of various birth defects. Various studies involving animal models and humans have reported the involvement of heavy metals in various congenital anomalies like Cleft lip-palate, Neural-tube-defects, esophageal atresia and tracheo-esopheageal fistula, various cardiac anomalies, skeletal anomalies and hypospadias (Donald, 2010, Thulstrup and Bonde, 2006, Melek et al., 2014, Sharma et al., 2014, Bailey et al., 2006, Al-Sabbak et al., 2012, Hill et al., 2009, Brende et al., 2005). However, there is hardly any report on the association of heavy metals with bladder exstrophy. We conducted the present study to evaluate the status of heavy metals concentrations in occurrence of patients suffering from Exstrophy-epispadias complex.

MATERIALS AND METHODS

Study Setting

The study was conducted in the department of Pediatric Surgery in collaboration with department of Biochemistry in a university teaching institution from June-2015 to December-2016.

Study design

This was a case-control study comprising of two groups; i) group-I (the study group) included 20 patients of primary exstrophy-epispadias complex who were admitted for the repair and without any other associated congenital anomaly, and their mothers, ii) group-II (the control group) included 20 age matched controls who were admitted for other indications such as peritonitis, abscess, intestinal obstruction, or trauma, but without any congenital anomaly, ruled out by clinical history, clinical examinations and essential radio-pathological evaluations, and their mothers.

Ethical clearance from institutional ethical committee was taken before start of study. The informed and written consent was taken from parents of both groups after explaining them about the details of study and the benefits of this study to the parents in future pregnancy, if planned and also to the society.

Inclusion and exclusion criteria

Group I included primary exstrophy epispadias without any other associated congenital anomaly, and their mothers.

Those children with failed exstrophy-epispadias repair or associated with other congenital anomalies were excluded from study. Group II included age matched controls admitted for other indications but without any congenital anomaly. In both groups, those patients whose parents had not given consent to participate in the study, were excluded from the study.

Collection of Blood samples

After taking informed consent, 2.00 ml of blood samples were collected from the patients and their mothers in both groups under strict aseptic precautions. Blood sample was collected in sterile EDTA (Ethylene di-amine tetra acetic acid) vial for estimation of lead (Pb), mercury (Hg), arsenic (As), cadmium (Cd) and, chromium (Cr).

Digestion of blood sample and Preparation of sample for estimation of heavy metals

All collected blood samples were sent to department of Biochemistry, for estimation of heavy metals by ICP-OES (Inductively-Coupled-Plasma-Optical-Emission-Spectrometer). All the blood samples were rapidly digested in a Multi-wave Reaction System (Multi-wave 3000, Anton Paar, Perkin Elmer, USA) microwave oven using standard pressure vessels (pre-cleaned by rinsing with ultra-pure nitric acid prior to use) and the Rotor 16HF100 (100 ml PFA vessels, 40 bar) and Pressure, Temperature (P/T) sensor. Digestion was done with the reagents 2.0mL of HNO_3, 1.0 ml of H_2O_2 and 1.0 ml of H_2O in microwave digestion systems according to the digestion program as described by Ansari et al 2015. The rotor was removed from the microwave oven and allowed to cool to room temperature. The vessels were carefully opened in a fume cupboard and the inner walls rinsed with DI water. The final volume of each sample was made up to 20 mL with 1 mL hydrogen peroxide and water.

The resulting clear solution was taken carefully in separate, properly satirised and dry test tubes sand allowed to be cooled to room temperature. Finally, the solution was diluted to 20.0 ml with Milli-Q water. The resulting clear solutions were ready for analysis of metals of interest. The metals were analysed by ICP-OES (Optima 8000, Perkin Elmer, USA) with operational conditions as described by Ansari et al [2015].

Statistical Analysis

The results are presented in mean ± SE and percentages. The Chi-square test was used to compare the categorical / dichotomous variables between cases and controls. The odds ratio with its 95% confidence interval (CI) was calculated. The continuous variables were tested for normalcy by using Kolmogorov test. The Mann-Whitney U test was used to compare the continuous variables between cases and controls. The Spearman correlation coefficient was calculated to find the correlation between two continuous variables. The p-value<0.05 was considered significant. All the analysis was carried out on SPSS 16.0 version (Chicago, Inc., USA).

RESULTS

Only Cr and Hg, out of five heavy metals (As, Cd, Cr, Pb, and Hg), were present in the blood samples of children and their mothers of both groups (Table 1). Although, detection of Cr and Hg in blood samples of children and mothers were more in group-I than in group–II, the differences were statistically insignificant. Children of group-I (exstrophy cases) had higher mean blood levels of Cr and Hg as compared to group-II (controls) (Table 2); however, the differences were not statistically significant.

Similarly, mothers of children of group I (mothers of exstrophy cases) had increased mean blood levels of Cr and Hg in comparison to group II mothers (control group) and these differences were also statistically insignificant (Table 3). Spearman correlation coefficient to correlate the mean blood levels of Cr and Hg of mothers and children of group I revealed no correlation between mean maternal blood Cr and Hg levels and mean children blood Cr and Hg levels in both groups (Table 4).

DISCUSSION

Bladder exstrophy is a rare and complex urogenital birth defect characterised by the absence of anterior bladder wall and exposed posterior bladder wall through the defect of lower abdominal wall, and laid open urethral plate.

Similar to other congenital genitourinary anomalies, bladder exstrophy has no established risk factors and a multifactorial mechanism caused by a complex interaction between various hormonal, genetics and environmental factors have been reported to play a role.

We had designed this case control study to find the possible association of heavy metal exposure with the occurrence of bladder exstrophy, a rare urogenital birth defect, with reference to analysis of heavy metal levels in blood of boys and their mothers and compare it with that of age matched control cases. In our study, we observed higher mean blood levels of Cr and Hg in children and their mothers in group I when compared with blood Cr and Hg levels in children and mothers in group II (table); however, the difference were found to be statistically not significant ($p=0.42$). Odds ratio for the exposure of Cr was found to be more than 1 both in children with bladder exstrophy-epipsapdias complex (1.88) and in their mothers (1.58) that suggested that there may be some association of exposure of Cr with occurrence of bladder exstrophy. The Odds ratio for the exposure of Hg suggests that the mercury exposure has no association with occurrence of BEEC. On correlating the blood levels of Cr in children with bladder exstrophy-epipsapdias complex and their mothers in group I, we found a very weak association (Correlation coefficient=0.03). The evidence of linking the role of prenatal exposure of Cr with the skeletal and cartilage defects in growing fetus has mainly derived from experimental studies on animals (Bailey et al., 2006).

With worldwide increase in environmental pollution and contamination, numerous studies, both experimental and epidemiological, have been carried out to explore the involvement of different environmental contaminants or pollutants including toxic heavy metals (like Arsenic, Cadmium, Chromium, lead, and Mercury), persistent and volatile organics, and pesticides as possible risk factors for various birth defects (Vinceti et al., 2008, Wigle et al., 2007, Bound et al., 1997, Shalat et al., 1996, Sever et al., 1995). Recently, researchers have tried to corroborate or refute the role of heavy metals in various birth defects. It has been reported that exposure to heavy metals may alter the in-utero development of human fetus and it has adverse health consequences for the offspring, including a short gestation period, reduced birth weight, increased risk of metabolic, cardiac and psychiatric disease, and overall reduced lifespan (Llop et al., 2010, Landrigan et al., 2004, Perera et al., 1998, Seckl et al., 1998, Virtanen and Adamsson, 2012). There are certain reports that heavy metals may act as endocrine disruptors thus they may have a role in occurrence of congenital urogenital anomalies (Sharma et al., 2014).

Nassar, et al (2010) reported that maternal exposure to heavy metals such as mercury, lead and cadmium were significant risk factors for hypospadias, and mothers exposed to these heavy metals were two and a half times more likely to have a son diagnosed with hypospadias (Nassar et al., 2010). In another study based on analysis of serum level of heavy metals in boys with hypospadias and their mothers, Sharma T et al reported higher blood levels of cadmium and lead in mothers as well as in children and a positive association between maternal and child blood levels of cadmium and lead in hypospadias and suggested that higher blood levels of cadmium and lead may be associated with the increased risk of hypospadias (Sharma et al., 2014).

Association of prenatal exposure of Cr with occurrence of bladder exstrophy-epipsapdias complex in offsprings can be suggested by the endocrine disrupting potential of Cr (VI). It has also been suggested that soluble Cr (VI) is rapidly transported inside the cell by the nonspecific anion channel of the cytomembrane (Yang et al., 2013) and it leads to oxidative stress with the formation of Cr-DNA adducts and DNA–protein or DNA-Cr-DNA crosslinks resulting in multiple cellular dysfunctions that include DNA adduct formation, deregulated cell cycle and, apoptosis (Hamilton et al., 1998, Blankenship et al., 1994). However, none of the studies involving human subjects have shown any positive association between Cr and occurrence of birth defects. Our study is the first study that has shown a very weak association of Cr with bladder exstrophy-epipsapdias complex.

Limitation of our study was relatively small sample size as it is a rare congenital anomaly with incidence of 1 in 10,000 to 1 in 50000 live births (Gearhart and Mathews, 2012). Thus, to find any significant association between exposure of heavy metals and risk of bladder exstrophy-epipsapdias complex, studies with larger sample size will be required keeping in view other factors such as genetic susceptibility to particular heavy metal, dietary habits, occupation and other environmental factors. This will require collaboration of various institutes for conducting of multi-institutional studies. If any significant correlation between serum heavy metals and exstrophy-epispadias complex is established, then it will be helpful in prevention, management and prognosis of exstrophy-epispadias complex.

Table 1. Comparison of estimation of heavy metals in blood of children and mothers of both groups.

Name of Heavy Metal		Children				Mothers			
		Group I	Group II	Odds Ratio[1]	P value	Group I	Group II	Odds Ratio[1]	P Value
Arsenic (As)	Present	0	0	-	-	0	0	-	-
	Absent	20	20			20	20		
Cadmium (Cd)	Present	0	0	-	-	0	0	-	-
	Absent	20	20			20	20		
Chromium (Cr)	Present	5	3	1.88 (0.38-9.27)	0.42	3	2	1.58 (0.23-10.70)	0.63
	Absent	15	17			17	18		
Lead (Pb)	Present	0	0	-	-	0	0	-	-
	Absent	20	20			20	20		
Mercury (Hg)	Present	7	7	1.00 (0.27-3.66)	1.00	8	6	1.55 (0.42-5.76)	0.50
	Absent	13	13			12	14		

1 – Chi Square Test

Table 1 showing that only Cr and Hg were present in the blood samples of both children and their mothers of both groups (p value 0.42 and 1.00 for Cr and Hg respectively in children, p value 0.63 and 0.50 for Cr and Hg respectively in mothers).

Table 2. Comparison of Blood levels of Chromium and Mercury between children of both groups.

Groups	Chromium (Cr)			Mercury (Hg)		
	No. of children	Mean ± SE (mcg/dl)	Range (mcg/dl)	No. of children	Mean ± SE (mcg/dl)	Range (mcg/dl)
Group I	5	23.40 ± 16.19	1-87	7	251.71 ± 62.19	30-525
Group II	3	18.00 ± 2.08	15-22	7	238.71 ± 90.68	14-412
P value[1]	0.42			0.90		

1- Mann-Whitney U test

Table 2 showing that mean blood level of Cr and Hg are higher in children of group I than those in group II children. (p value 0.42 and 0.90 for Cr and Hg respectively).

Table 3. Comparison of Blood levels of Chromium and Mercury between mothers of both groups.

Groups	Chromium (Cr)			Mercury (Hg)		
	No. of mothers	Mean ± SE (mcg/dl)	Range (mcg/dl)	No. of mothers	Mean ± SE (mcg/dl)	Range (mcg/dl)
Group I	3	20.50 ± 3.50	17-24	8	270.83 ±	9-1122
Group II	2	18.33 ± 0.17	2-37	6	251.88 ± 135.24	18-667
P value[1]	0.80			0.66		

1- Mann-Whitney U test

Table 3 showing that mean blood level of Cr and Hg are higher in mothers of group I than those in group II mothers (p value 0.80 and 0.66 for Cr and Hg respectively)

Table 4. Correlation of Chromium and Mercury levels between mothers and children in both groups.

Groups	Name of Heavy Metal	Correlation coefficient[1]	p-value
Group I	Chromium (Cr)	0.03	0.95
	Mercury (Hg)	0.09	0.78
Group II	Chromium (Cr)	0.00	-
	Mercury (Hg)	0.17	0.63

1- Spearmann correlation Coefficient test

Table 4 showing no correlation between the mean maternal blood Cr and Hg levels and mean children blood Cr and Hg levels in both groups (p value 0.95 and 0.78forCr and Hg respectively in Group I).

CONCLUSION

From the results of the present study, it may be concluded that exposure to heavy metals is not significantly associated with the occurrence of exstrophy-epispadias, although the role of these metals cannot be completely ruled out in the etiology of exstrophy-epispadias,

as our study showed a very weak association of Cr with the occurrence of exstrophy. However, as the sample size of study was small, therefore, a multi-institutional study with larger sample size is needed to further explore the association of heavy metals with exstrophy-epispadias.

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Compliance with Ethical Standards

The study has been done after ethical approval from Institutional ethical committee and all ethical standards has been followed while conducting this study.

Declaration

Conflict of Interest- All authors declare that they have no conflict of interest

Financial support- All authors declare that no funding or financial support was received for the study

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