Original Article

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Association of Cadmium, Chromium, Manganese and Lead with Hyperprolactinemia: A Pilot Study

Obstetrics and Gynaecology Section

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ABSTRACT

Introduction: Hyperprolactinemia is one of the most common hypothalamic-pituitary dysfunctions where serum prolactin levels increase beyond normal range. Studies have suggested association of heavy metals with prolactin levels.

Aim: To investigate the association of serum levels of heavy metals with prolactin levels in hyperprolactinemia patients.

Materials and Methods: A total of 102 hyperprolactinemia patients (>100 ng/mL serum prolactin levels) and 25 controls were included in the study. Hyperprolactinemia patients were classified into macroprolactinemia (n=22) and true hyperprolactinemia (n=80) based on post Poly Ethylene Glycol (PEG) recovery of prolactin of <25%. Serum Cadmium (Cd), Chromium (Cr), Manganese (Mn) and Lead (Pb) levels were analysed using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) method. Statistical analysis was done using SPSS version 21.0 and Stata version 14.2. Student's t-test and Pearson correlation were used. The $p<0.05$ was considered statistically significant.

Results: There was no significant correlation between serum levels of prolactin and heavy metals Cd ($r=0.067$, $p=0.457$), Cr ($r=-0.065$, $p=0.465$), Mn ($r=-0.076$, $p=0.393$) and Pb ($r=-0.148$, $p=0.097$). No significant difference was found in serum levels of heavy metals between macroprolactinemia and true hyperprolactinemia patients ($p=0.521$, 0.690, 0.564 and 0.488 for Cd, Cr, Mn and Pb, respectively). ROC analysis also did not reveal any significance in any of the four heavy metals studied.

Conclusion: The results suggest that probably there is no association of serum prolactin levels, macroprolactinemia or hyperprolactinemia with heavy metals.

Keywords: Heavy metals, Hypothalamic-pituitary dysfunction, Macroprolactinemia, Prolactin

INTRODUCTION

Heavy metals in the environment enter our body through various sources, get accumulated in brain and interfere in the normal functioning of hypothalamic-pituitary-gonadal axis [1]. Studies have shown role of dopaminergic system in heavy metal neurotoxicity [2,3]. Heavy metals also act as xenoestrogens, mimicking the action of oestrogen in body and activating cell responses normally mediated by oestrogen [4].

Prolactin is produced by lactotroph cells of anterior pituitary and performs more than 300 roles including reproduction, metabolism, immune response, homeostasis and angiogenesis [5]. Prolactin secretion is regulated by hypothalamic-pituitary-gonadal axis. Dopaminergic system is inhibitory to prolactin secretion [6]. Estradiol stimulates lactotroph cell proliferation in the anterior pituitary and increases secretion of prolactin [7]. Hyperprolactinemia is one of the most common hypothalamic-pituitary dysfunctions where serum prolactin levels increase beyond normal range [8]. Hyperprolactinemia is a major cause of galactorrhoea, irregular menses and infertility in young women and loss of libido and infertility in men [9]. The condition in hyperprolactinemia where large immune-complex macroprolactin molecules exist as major molecular form of prolactin in sera is called macroprolactinemia [10]. Besides the known causes of pituitary adenoma, drug induced and secondary causes like chronic kidney disease, 16-35% hyperprolactinemia cases remain idiopathic [11,12]. Several studies have investigated prolactin levels in environmental and occupational exposure to heavy metals and have found significant relations [13-17].

Cadmium (Cd) is a heavy metal with role in signal transduction and inhibition of gene expression [18]. Cadmium, at nanomolar concentrations, displayed xenoestrogenic activities by affecting lactotroph proliferation and hormone release from anterior pituitary cells [4], while at micromolar concentrations, is cytotoxic and inhibits prolactin release [13]. Chromium (Cr) has a toxic effect on hypothalamus-pituitary-gonadal axis. Chromium (VI) accumulates in pituitary and causes a reduction in serum prolactin levels in vivo. Cr (VI) causes apoptosis of lactotroph cells in vitro by generation of reactive oxygen species [14]. Exposure to Manganese (Mn) was shown to increase prolactin levels [15]. However, study on prolactinoma patients found significantly lower Mn content in RBCs compared to controls, suggesting a relation between hyperprolactinemia and Mn levels via oestrogen regulation [16]. Lead (Pb) is one of the oldest environmental poisons having toxic effects due to its ability to substitute calcium and accumulation in brain and bone for long time [17]. The possible inhibitory effect of lead on dopaminergic pathway was observed in Pb exposed male workers having high prolactin levels [2].

The association of heavy metals with prolactin levels point towards a potential relation of heavy metals with hyperprolactinemia condition also. Therefore, the present study was aimed to find correlation of prolactin levels with heavy metals Cd, Cr, Mn and Pb in hyperprolactinemia patients and to compare levels of heavy metals between macroprolactinemia and true hyperprolactinemia as the latter are more commonly associated with the symptoms of hyperprolactinemia.

MATERIALS AND METHODS

This was a cross-sectional pilot study conducted between June 2014-February 2017. The study was approved by Institutional ethics committee (Ref. No.: IESC/T-63/21.01.2015, RT-39/01.04.2015). Written informed consent was taken from each participant before inclusion in the study. The study included 102 hyperprolactinemia patients with prolactin level >100 ng/mL in two occasions of >1 month interval aged 19-48 years recruited from Reproductive

Biology department of a tertiary care hospital. Women with physiological hyperprolactinemia were excluded from the study. A total of 25 age-matched healthy women recruited from the general community during the same period of time having normal prolactin level were included as controls. As a causation and to check the accuracy of the kit, 25 healthy women were taken as controls. Detailed clinical and medical history was taken as per pre-determined proforma. The minimum initial evaluation included complete medical history, physical examination, hormone measurements and CT/MRI for prolactinoma or other pituitary adenoma. Prolactin assays were done on highly specific Chemiluminescence Microparticle Immunoassay (CMIA) (7K76 G6-5314/R06 B7K760) using ARCHITECT PLUS i2000SR automated immunoassay system (Abbott Laboratories, USA).

About 2 mL peripheral blood was collected from each participant in plain vial and was allowed to coagulate at room temperature for 30 minutes. Serum samples were obtained by centrifuging blood sample at 5000 rpm for 5 minutes at room temperature and were stored at -80°C until further analysis. PEG precipitation was done with PEG 6000 (Catalogue# SC- 302016) to classify hyperprolactinemia patients into macroprolactinemia and true hyperprolactinemia based upon post PEG precipitation recovery of prolactin of <25% [12].

Serum levels of Cd, Cr, Mn and Pb were measured by ICP-AES (Model JY 2000, HORIBA JobinYvon, France) at Department of Pharmacology, AIIMS, New Delhi, India. The limits of detection for serum heavy metals were as follows: Cd, 0.35 µg/L; Cr, 0.5 µg/L; Mn, 0.3 µg/L; Pb, 5 µg/L [19]. Wavelengths for each heavy metal, selected from a pre-defined set using the ICP software version 5.2, were: Cd, 228.56 nm; Cr, 283.56 nm; Mn, 257.61 nm and Pb, 220.35 nm. One mL serum was mixed with 4 mL conc. HNO_3 and 1 mL H_2O_2 in digestion cylinders. The samples were digested as per the cycle: temperature 70°C, rinse time 5 minutes, hold time 2 minutes; temperature 100°C, rinse time 5 minutes, hold time 5 minutes; temperature 130°C, rinse time 5 minutes, hold time 5 minutes. The digested samples were allowed to cool at room temperature followed by dilution to 10 mL volume with double distilled milliQ. The diluted samples were run in triplicate for heavy metal estimation by ICP-AES along with the multi-element standard of concentration 100 parts per billion (ppb) which was run after every 15 samples.

STATISTICAL ANALYSIS

Statistical analysis was performed using IBM Statistical Package for the Social Sciences (SPSS) software, version 21.0. Results were expressed as mean±SD (range) and median (IQR). Student's t-test was performed to compare mean between macroprolactinemia and true hyperprolactinemia patients. Pearson correlation coefficient was used to find correlation between heavy metals and prolactin. The $p<0.05$ was considered statistically significant. ROC curve was performed on Stata version 14.2.

RESULTS

Total 102 hyperprolactinemia patients were included of which 98 (96.08%) were females and 4 (3.92%) were males. Mean age of hyperprolactinemia patients was 30.61±6.6 (range 19-48) years. Prolactin levels varied greatly among hyperprolactinemia patients, ranging from 100 to 8484 ng/mL, with median prolactin level 159.17 ng/mL (128.43, 245.36). The demographic, aetiologic and clinical profile of hyperprolactinemia patients are presented in [Table/Fig-1-3], respectively.

The mean age of 25 healthy control females was 27±4.7 (range 20-39) years and mean prolactin levels 11.8±4.2 ng/mL. The serum levels of Cd, Cr, Mn and Pb in controls were 14.3±0.8 ppb; 189.3±20.2 ppb; 33.3±4.3 ppb and 20.8±8.1 ppb, respectively.

	Hyperprolactinemia patients (N=102)
Age (years), mean±SD	30.6±6.6
Males, n (%)	4 (3.9)
Females, n (%)	98 (96.1)
Married, n (%)	86 (84.3)
No. of smokers, n (%)	2 (2.0)
No. of alcoholics, n (%)	4 (3.9)

[Table/Fig-1]: Demographic profile of hyperprolactinemia patients.

	Hyperprolactinemia patients (N=102)
Pituitary adenoma, n (%)	19 (18.6)
Drug induced, n (%)	36 (35.3)
Idiopathic, n (%)	36 (35.3)
Other secondary causes, n (%)	11 (10.8)
Macroprolactinemia, n (%)	22 (21.6)
True hyperprolactinemia, n (%)	80 (78.4)

[Table/Fig-2]: Aetiological details of hyperprolactinemia patients.

	Hyperprolactinemia patients (N=102)
Respiratory disease/Asthma, n (%)	0 (0)
Hypertension, n (%)	8 (7.8)
Diabetes, n (%)	1 (1.0)
Renal disease, n (%)	10 (9.8)
Adrenal disorder, n (%)	0 (0)
Thyroid disorder*, n (%)	20 (19.6)
Bone/Joint pain, n (%)	55 (53.9)
Body mass index, mean±SD	23.9±4.7
Prolactin (ng/mL), median (IQR)	159.17 (128.43, 245.36)
TSH (mU/L), median (IQR)	2.58 (1.6, 3.8)

[Table/Fig-3]: Clinical profile of hyperprolactinemia patients.
*14 patients euthyroid with thyroid medication; 4 patients hypothyroid without thyroid medication; 1 patient hypothyroid with thyroid medication; 1 patient euthyroid with goitre

The present study found no significant correlation between serum levels of Cd, Cr, Mn and Pb with prolactin levels in hyperprolactinemia patients and controls [Table/Fig-4].

Variable	Prolactin
Cadmium	
Pearson correlation	0.067
Sig. (2-tailed)	0.457
N	127
Chromium	
Pearson correlation	-0.065
Sig. (2-tailed)	0.465
N	127
Manganese	
Pearson correlation	-0.076
Sig. (2-tailed)	0.393
N	127
Lead	
Pearson correlation	-0.148
Sig. (2-tailed)	0.097
N	127

[Table/Fig-4]: Pearson correlation of serum levels of heavy metals with prolactin levels.

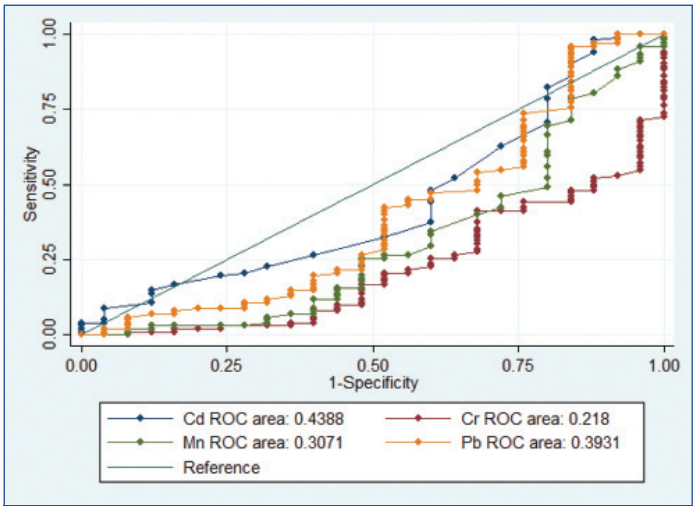
Out of the 102 hyperprolactinemia patients 22 were macroprolactinemia and 80 were true hyperprolactinemia patients based upon the presence of macroprolactin fraction of >75% in total prolactin as estimated by post PEG precipitation recovery of

prolactin of <25%. Comparison of serum levels of heavy metals between macroprolactinemia and true hyperprolactinemia found no significant difference in any heavy metal studied between the two groups [Table/Fig-5].

Variable	Macroprolactinemia (N=22)	True Hyperprolactinemia (N=80)	p-value
Prolactin (ng/mL)			
Median (IQR)	137.6 (115.43, 189.88)	164.02 (130.91, 257.58)	0.0543
(Range)	(100-566.3)	(100-8484)	
Cadmium (ppb)			
Mean±SD	14.16±1.01	14.30±0.84	0.521
(Range)	(12.7-17.7)	(12.9-18.1)	
Chromium (ppb)			
Mean±SD	171.42±12.41	169.44±22.35	0.690
(Range)	(149.9-208.0)	(9.6-220.4)	
Manganese (ppb)			
Mean±SD	31.28±1.81	30.95±2.56	0.564
(Range)	(27.9-36.9)	(13.2-36.3)	
Lead (ppb)			
Mean±SD	19.76±5.30	18.85±5.47	0.488
(Range)	(12.2-31.3)	(6.7-30.6)	

[Table/Fig-5]: Comparison of serum levels of heavy metals Cd, Cr, Mn and Pb between macroprolactinemia and true hyperprolactinemia.
p<0.05 considered significant

Cd had AUC 0.4388 with CI: 0.305-0.572. Cr had AUC 0.218 with CI: 0.122-0.314. Mn had AUC 0.307 with CI: 0.177-0.437. Pb had AUC 0.393 with CI: 0.252-0.534. None of the four heavy metals showed any significance in ROC analysis [Table/Fig-6].



[Table/Fig-6]: ROC curve analysis of Cd, Cr, Mn and Pb between hyperprolactinemia patients and controls.

DISCUSSION

Many studies have compared levels of heavy metals with prolactin levels in different target groups and have suggested effect of heavy metals on prolactin levels but none of the studies have compared levels of heavy metals in pathological hyperprolactinemia patients to probe whether heavy metals actually have role in pathologically raised prolactin levels [13-17]. The present study is unique as it analysed heavy metal levels in hyperprolactinemia patients to check association of heavy metals with hyperprolactinemia.

The present study found no significant correlation between serum levels of Cd, Cr, Mn and Pb with prolactin levels in hyperprolactinemia patients and controls. No statistically significant difference was found in any of the four heavy metals studied between macroprolactinemia and true hyperprolactinemia patients either. ROC analysis, also, did not reveal any significance in Cd, Cr, Mn or Pb in hyperprolactinemia patients and controls.

The present study findings are supported by the study of Leite EMA et al., who did not find any significant correlation between blood Pb and serum prolactin levels in children with low environmental exposure to Pb, although the target group in the study varied from the present study [20].

The present findings are contradictory to many other studies presenting correlation between prolactin and heavy metals. Study on non-occupational group of men showed inverse correlation of Cd, Mn and Pb while positive correlation of chromium with prolactin [21]. Chromium levels have been inversely related to prolactin levels [14]. Study on rats showed that hexavalent chromium caused adverse effects on anterior pituitary and resulted in reduced serum prolactin levels [14]. In-vitro studies have shown that Cr (VI) decreases prolactin levels by producing oxidative stress which causes apoptosis of prolactin secreting lactotroph cells [22,23]. Occupational exposure to Mn has been shown to be positively correlated to prolactin levels [24]. The whole blood Mn concentration was associated with serum prolactin concentrations as shown by higher prolactin in welders compared to referents [24]. Study in rats presented that Mn exposure causes increase in prolactin by decreasing dopamine and Pit-1 which are regulators of both prolactin and dopamine [25]. Study on children had similar inverse relation between Pb and prolactin [26]. On the other hand, there are studies which showed significantly higher plasma prolactin values in Pb exposed workers [27]. Lucchini R et al., found high prolactin levels in male workers with occupational exposure to Pb [2].

The contrasting results in present study compared to others may be due to many factors. None of the earlier studies had hyperprolactinemia patients as the target group like the present study. Also, the same metal can have different effects on prolactin levels based upon the exposure dose [28], and unlike present study, most of the studies are done on occupational exposure of heavy metals where the exposure dose was very high compared to non-occupational environmental exposure.

Further, the inconsistency in association of prolactin levels with heavy metals in different studies may suggest potentially differing sites and mechanisms of actions of heavy metals [21]. Heavy metals can affect prolactin at different levels: directly at the cellular level [29], at dopaminergic level-by affecting dopamine receptor sensitivity and number in tubero-infundibular region, or by inhibiting release of dopamine in synaptosomal region [30,31]. In addition to these, prolactin secretion is also regulated by other neurotransmitters including Gamma-Aminobutyric Acid (GABA), glycine and glutamate [29], which might also be targeted by heavy metals affecting prolactin levels [32]. Effect of heavy metals on prolactin levels can also occur via xenoestrogenic property of heavy metals as oestrogen is known to up-regulate expression of prolactin gene; synthesis, storage and release of prolactin as well as proliferation of lactotroph cells in pituitary [7].

True hyperprolactinemia patients are usually associated with clinical symptoms of hyperprolactinemia. So, the study also compared heavy metals between true hyperprolactinemia and macroprolactinemia patients, which are big immune-complexes of prolactin and are not always associated with clinical symptoms of hyperprolactinemia. No significant difference was observed in heavy metals between macroprolactinemia and true hyperprolactinemia patients suggesting no differential role of heavy metals in true hyperprolactinemia or macroprolactinemia. We did not find any other study comparing heavy metals between macroprolactinemia and true hyperprolactinemia groups.

This suggests that although studies have reported positive (or inverse) association of heavy metals with prolactin levels in occupational and non-occupational groups, but hyperprolactinemia condition may not be associated with heavy metal levels.

Limitation(s)

One potential limitation of the study was that the study population was heterogenous with respect to the cause of hyperprolactinemia. This might have generated confounding factors contributing to the present findings.

CONCLUSION(S)

No significant correlation was observed between serum levels of prolactin and heavy metals in hyperprolactinemia patients. Also, levels of heavy metals did not vary significantly between macroprolactinemia and true hyperprolactinemia patients. Thus, probably there was no association of prolactin levels, macroprolactinemia or hyperprolactinemia with heavy metal levels of Cd, Cr, Mn and Pb. However, these findings should be validated in a large cohort of hyperprolactinemia patients.

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