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IS THERE ANY ROLE OF METAL TOXICITY IN AMENORRHEA?

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ABSTRACT

Introduction: Metal toxicity has played an important role in many diseases. Apart from cancers, it is proven to be toxic in various system dysfunctions like teratogenicity, various tissue disorders and organ failures. Lead, mercury and cadmium are already proven reproductive toxicants. Since West Bengal is the most arsenic prone state in India, arsenic is the selected metal in our study, to find any association with amenorrhea.

Objectives: Objective of this study is to find out any possible correlation between arsenic toxicity and causation of amenorrhea in population of West Bengal.

Methodology: Ethical clearance from the institutional committee has been obtained for this study. Patients attending the genetics Out Patient Department of our hospital referred from different hospitals were screened for amenorrhea. Detailed clinical features and lifestyle history were recorded with expert opinion of clinician. In this study, 20 healthy females were selected as control. Peripheral venous blood and hair samples were taken with their proper consent for human leukocyte culture and arsenic estimation, the latter performed by flow injection hydride generation atomic absorption spectrometry. Karyotyping was done for chromosomal analysis.

Result: Among 50 amenorrhea patients of different chromosomal profile 14 patients showed elevated arsenic level above safe limit (0.8µg/g). Whereas only 2 control individual showed arsenic count above safe limit.

Conclusion: Other metals like lead, cadmium, mercury are proven toxicants for Human reproductive and endocrine systems and potential cause for infertility. In our study the amenorrhea patients showed significantly high arsenic count than control individual. Thus we can conclude that arsenic may have an association with amenorrhea.

Keywords: Amenorrhrea, Karyotyping, Metal Toxicity, Arsenic, West Bengal

INTRODUCTION

Amenorrhea is a condition characterized by absence of menstruation during puberty or later life. Amenorrhea is divided into two type it Primary Amenorrhea (PA) and Secondary Amenorrhea (SA). Primary Amenorrhea is defined as the absence of menstruation by the age of 16 in the presence of normal development of sexual character or by the age of 14 if secondary sexual characteristics have failed to develop. Secondary Amenorrhea is defined as absence of menses for consecutively for six months or for 3 cycles at intervals after normal menarche. Causes of amenorrhea are mainly pituitary/hypothalamic disorders, gonadal dysfunction and outflow tract abnormalities (Schorge *et al.*, 2008). These abnormalities are due to endocrine disorders, genetic, psychological or structural anomalies; now a day different environmental factors are also emerging as an important cause of this infertility and amenorrhea. Several cytogenetic studies reported that frequency of chromosomal abnormalities in PA varies greatly from 15.9% to 63.3% (Joseph *et al.*, 1982; Optiz *et al.*, 1983; Ten *et al.*,1990). But a great percentage of patients present no such conventional cause to explain their problem. Different studies have shown that Metal toxicity as environmental toxicants play an important role in a series of diseases from cancers to systemic dysfunctions like teratogenicity, various tissue disorders, and organ failures. Lead, mercury and cadmium are already proved to be reproductive toxicants. Arsenic (As) are a proven carcinogen and its toxicity found to be in acute or in chronic form affecting various human systems. Since West Bengal is highest arsenic affected state in India, arsenic is the selected metal for our study. This study is the first effort to find any association of As toxicity with amenorrhea.

MATERIALS AND METHODS

Sample collection

The study subjects included 50 patients with amenorrhea referred for chromosomal analysis to the Cytogenetic outdoor Vivekananda Institute of Medical Sciences, Kolkata of India from August 2016 to February 2017 and 20 controls. Age distribution, geographical origin of the controls was matched with the cases. The study was carried out in accordance with The Code of Ethics of the World Medical Association (World Medical Association, 1964/2013) for experiments in humans. The study has been reviewed and approved by the Ethics Committee of Vivekananda Institute of Medical Sciences, Ramakrishna Mission Seva Pratishthan, Kolkata. An informed consent was taken from each patient as per the norms of Institutional Ethics Committee before sample collection. The age group of the subjects ranged from 14 to 35 years with a mean of 20.26 ± 4.12 years. Pedigrees with details were drawn and in depth clinical evaluation and clinical information were obtained from all subjects by administering a detailed questionnaire. About 2 ml of peripheral venous blood sample in heparinised vial and hair sample was collected from each patient aseptically and each sample was given a unique identification number.

Setting up of lymphocyte cultures

Lymphocyte cultures were set according to Moorhead *et al*. Peripheral venous blood samples were aseptically transferred into sterile culture tubes with 4 ml of RPMI-1640 medium each (Gibco, Life technologies), supplemented with l-glutamine, 10% foetal bovine serum (Gibco, Life technologies), Penicillin–streptomycin solution and phytohaemagglutinin (Gibco, Life technologies). Parallel cultures were set for each sample. The culture tubes were marked accordingly and incubated in a CO_2 incubator for 72hrs. 50µl colchicine was added to each culture tube at the completion of 70hrs to arrest the cells at metaphase. After 72hrs of incubation, the cell suspensions were centrifuged for 10 min at 1000 rpm. The supernatant was discarded and the pellet was treated with hypotonic solution (0.075 M KCl) by gentle flushing. The centrifuge tubes were incubated again at 37°C for 20 min. The tubes were again centrifuged carefully at 1200 rpm for 10 mins. The supernatant was removed and 10 ml of freshly prepared pre-chilled Conroy's fixative was added to the pellet and mixed thoroughly. The tubes were allowed to stand overnight and washed with freshly prepared pre-chilled Conroy's fixative repeatedly for 3–4 times.

Estimation of arsenic

Arsenic estimation from the hair samples of the cases and controls was performed by flow injection hydride generation atomic absorption spectrometry method.

RESULT

During the study period 50 female patients with amenorrhea and 20 control individuals were studied for cytogenetic analysis and arsenic estimation. Age of the subjects ranged from 14 to 35 years with a mean of 20.26±4.12 years. The result chromosomal analysis

Table 1: The karyotype variations of the patients and their comparative USG findings

Cytogenetic variations		Karyotype	No .of cases (n)	USG Findings
Normal Karyotype		46,XX	33	Normal, Mullerain agenesis Hypoplastic uterus, Streak ovaries , pre-pubertal uterus
NUMERICAL ABNORMALITY	Mosomy X	45,XO	10	Small atrophied uterus, Streak gonads, Absent ovaries
	Turners mosaic	45,XO/46,XX	1	Hypoplastic uterus, Small ovaries
STRUCTURAL ABNORMALITIES	isochromosome	46,XX,i(Xq)	1	Streak ovaries, Infantile uterus
	Mosaic isochromosome	45 XO/46Xi(Xq)	3	Streak ovaries, Infantile uterus
Sex Reversal		46,XY	1	Streak gonads, rudimentary/absent uterus, Inguinal hernia Streak ovaries, Infantile uterus
Others		46, XX, 22pstk+	1	Normal uterus , ovaries and external genetalia

The karyotype results showed 66% patients (n=33) with normal chromosome composition and 34% (n=17) demonstrated abnormal chromosomal pattern. Most of the patients irrespective of their karyotype revealed abnormal USG findings. Hypoplastic or infantile uterus and streak ovaries were the most common USG finding for most of the patients. 14 out of 50 cases and 2 out of

20 control individuals showed elevated arsenic count above safe limit (0.25 µg/g; recommended) (Arnold *et al.*, 1990) in their hair samples; among these 14 cases with elevated As count 11 cases were having normal female karyotype (46, XX) two cases showed Turner karyotype with monosomy X (45, X) and one patient with stalk in chromosome 22 (46, XX, 22pstk+). All the control individuals were chosen randomly from arsenic exposed as well as non-exposed areas of West Bengal. With reference to the literature study result, based on the maximum permissible limit of arsenic concentration in groundwater being 50µg/L (recommended by World Health Organization) different districts of West Bengal have been clustered into three groups of arsenic affected areas. These are: highly affected (Out of 149 blocks in 8 districts and 100 wards of Kolkata, 107 blocks and 30 wards are affected), mildly affected (Out of 29 blocks in 5 districts, 4 blocks are affected) and unaffected (Out of 63 blocks in 5 districts, none is affected).Tabular comparison of highly affected districts to un affected districts are represented in Table 2, Table 3 and Table 4 .

Table 2: Percentage of arsenic affected blocks of highly affected districts of West Bengal

Districts	Blocks Affected (%)
Nadia	100
Maldah	92.8
North 24 Parganas	95.4
Murshidabad	92.3
South 24 Parganas	64.7
Howrah	58.3
Kolkata	30
Hoogly	88.88
Burdwan	50

Table 3: Percentage of arsenic affected blocks of highly affected districts of West Bengal

Districts	Blocks Affected (%)
Darjeling	8
Jalpaiguri	0
Kuchbihar	8.33
Dinajpur (N)	22.22
Dinajpur (S)	12.5

Table 4: Percentage of arsenic unaffected blocks of unaffected districts of West Bengal

Districts	Blocks Affected (%)
Bankura	0
Birbhum	0
Purulia	0
Midnapur (East)	0
Midnapur (West)	0

DISCUSSION

The causes of primary amenorrhea are mainly congenital. The possible causes are considered to be primary ovarian disorders, disorder in central nervous system, abnormalities of outflow tract, primary gonadal disorders and disorders in pituitary (Merin *et al.*, 2012) Different acquired causes like chronic illness, stress, malnutrition, intense exercise also contribute in occurrence of this problem. Whereas secondary amenorrhea is mainly happen due to acquired causes. Other environmental factors like radiation, certain effects of drugs, metal toxicity also play a significant role in causing either primary or secondary amenorrhea depending on the time of exposure in patient's lifetime.

Metal toxicity has been proved to be an important cause for female infertility these toxic metals affect the Hypothalamic-pituitary-ovarian axis buy altering their functions (Tiejian, 2003). The effects of heavy metals on female reproductive function was also reported by Sharara et al., 1998. They proposed the mechanism by which the chemicals affect ovarian function either by hormonal or immune disruption, altered cellular proliferation or inappropriate cell death (Sharara, Seifer, Flaws, 1998).

Studies reported that Mercury (Hg) acts as a major endocrine disrupter affecting male and female fertility altering integral functions of hypothalamic-pituitary-thyroid axis and the hypothalamic-pituitary-adrenal axis (Darbe, 2006). Lead is also proved toxicant to disrupt pubertal development (Sherry *et al.*, 2003).

India , Bangladesh and China are the worse as affected nations and in India, Uttar Pradesh, Bihar, Jharkhand and West bengaline the Ganga plain are hugely affected (Xia

& Liu, 2004; Chakraborty *et al.*, 2004). West Bengal and Bangladesh region, mainly Ganga Brahmaputra delta plain is highest Arsenic affected region in the world. Based on the degree of arsenic contamination districts of West Bengal is divided into three zones. Highly affected (As concentration in ground water sample is > 50µg/L) districts include 107 blocks and 30 wards affected out of 149 blocks in 8 districts and 100 wards of Kolkata. Mildly affected districts include 4 blocks affected out of 29 blocks in 5 districts. Unaffected districts include none affected out of 63 blocks in 5 districts (Chakraborti *et al.*, 2009). The huge amount of accumulated as in the farm soil of this region pose a great negative burden especially health burden on nearby residents. The association between as residing in the environment and that accumulated inside the human body can be established through their high concentrations in blood, urine and hair.

Arsenic toxicity is well established in the causation of skin, lung and bladder cancer (Saha *et al.*, 1999). Apart from that as toxicity include enzyme inhibition, endocrine and different epigenetic effects. Few human studies report a moderately increased risk of impaired foetal growth and foetal mortality.

In our study we found a significant number of cases show arsenic count in their hair sample above the safe limit (0.8µg/g) in compare to the controls. Most of the cases have normal female karyotype (46, XX). For these cases no other conventional factors of was hat much evident to explain the condition of amenorrhea. In animal studies it has been found that High Arsenic level may suppress the sensitivity of gonadotroph cells to GnRH (Gonadotropin it Releasing Hormone) and can also suppress gonadotropin secretion by elevating plasma levels of glucocorticoids in rats (Sarkar *et al.*, 2003; Kreiger *et al.*, 1982). Therefore perceiving the result of our study we can hypothesized that there might be an association with amenorrhea and elevated level of arsenic in the system. However a larger sample size, detailed invasive study and experimental studies on animal model are farther needed to establish this association.

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