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ARSENIC TOXICITY- A POSSIBLE SUSCEPTIBLE FACTOR FOR OCCURRENCE OF DOWN SYNDROME

Priyanka Biswas[1]*, Amit Kumar Chakraborty[1], Gargi Podder[1] and Madhusnata De[1]

[1]Department of Genetics, Vivekananda Institute of Medical Sciences, Ramakrishna Mission Seva Pratishthan, Kolkata. 99, Sarat Bose Road, Kolkata -700026, India.

*** Corresponding Author: Priyanka Biswas**
Department of Genetics, Vivekananda Institute of Medical Sciences, Ramakrishna Mission Seva Pratishthan, Kolkata. 99, Sarat Bose Road, Kolkata -700026, India.

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ABSTRACT
Down Syndrome (DS) is the most common live-born birth defect in humans and serious autosomal chromosome aberration compatible with post-natal survival. Recent studies have suggested that DS birth is attributable to different environmental factors. A possible association between the etiology of DS and potential role of heavy metal, especially arsenic has been found, but there is limited understanding of these complicated association. The aim of the present study was to examine levels of arsenic in hair of DS child and also investigate the relationship between DS and other risk factors including socio-economic status, maternal age, and literacy of mothers of DS cases.A case-control study of 84 Down syndrome (DS) cases and their parents in West Bengal, India was conducted. 12 healthy controls were also included in the study. All procedures were done with the informed consent of participants' parents. DS children participated in the study were confirmed after performing karyotyping. For arsenic estimation, hair samples were obtained from DS cases, controls and their parents. It was analyzed by atomic absorption spectrophotometer. The current study showed that the parents of DS child had significantly high levels of arsenic in hair samples, either in body burden level or toxic level of arsenic. Most of the DS child had high concentration of arsenic, whereas the parents of healthy child had normal level of arsenic.High levels of arsenic (body burden level and toxic level) present in the body of parents may be an etiological factor for development of DS in the offspring.

KEYWORDS: Down Syndrome, Arsenic, Karyotyping, Body burden, Toxic.

1. INTRODUCTION
Down Syndrome (DS) is the most common live-born birth defect in humans and serious autosomal chromosome aberration compatible with post-natal survival. DS was popularly known after the name of John Langdon Down who described the syndrome for the first time in 1866 (Down, 1866). DS is caused by having three copies of the genes on chromosome 21, rather than the usual two. The DS child has distinctive physical features including facial appearances including flattened nose, protruding tongue and upward slanting eyes (Mongolian slant). The hands are short and broad with short fingers and often have a single palmar crease and poor muscle tone. Most DS children have mental retardation in the mild to moderate range and delayed development of motor skills. In addition, DS individuals have higher risks of several diseases including congenital heart defects, duodenal stenosis or atresia, imperforate anus, Hirschsprung disease, muscle hypotonia, immune system disorders, increases risk of childhood leukemia and early onset of Alzheimer's disease (Epstein et al. 1991). DS individuals have both physical and intellectual disabilities. As adults, their mental abilities are typically similar to those of an 8 or 9 year old.

The incidence of DS is 1 out of 800 to 1000 live births in all ethnic groups. The increase in the incidence of DS cannot fully be explained by genetic defects and it is thought that environmental events may act as an additive either through interaction with some genes or directly. There are well-recognized risk factors such as parental age and a positive family history for this disorder. Advanced maternal age has been identified as a key risk factor. The other risk factors leading to the prevalence of congenital anomalies that could mediate the impact of socio - economic status include nutritional factors, lifestyle, environmental and occupational exposures, access to and use of health services and ethnic origin. The possibility of an etiological role for environmental factors such as toxic heavy metal exposure has risen after a sharp increase in DS.

Heavy metals are elements exhibiting metallic properties, which may be toxic and thus, are considered as worldwide environmental health hazards (Akyuzlu et al.,

2014). Humans can be exposed to heavy metals through water, industrial paint, chemical products, building materials, fertilizers, silver dental fillings, fish that is high in mercury, mercury-containing preservatives in vaccines and many more. Among heavy metals, lead, arsenic, mercury and cadmium can cause birth and neurological defects, abnormal fetal developmental behavioral abnormalities and immune dysfunctions. Although these abnormalities are similar to symptoms of the DS, it is not known how these metals affect the development of DS. Heavy metals can be inherited by the fetus during pregnancy (Fido et al., 2005) and a growing fetus is more vulnerable to toxicants due to both the incomplete blood - brain barrier and the decreased capacity for drug detoxification (Rodier et al., 2000). Furthermore, the lack or excess of essential minerals (trace elements) may heighten the toxicity of metals (Fido et al., 2005). Among all these heavy metals, the point of focus of this study is on arsenic.

Arsenic is a well known toxin and is considered a human carcinogen based on epidemiological evidences. Arsenic exists principally in two valence states: As^{3+} (arsenite) and As^{5+} (arsenate). Arsenite is considered to be more potent and genotoxic than arsenate. Arsenic is released in the environment by natural means such as solubilization from geologic formations into water supplies. Evidences have been found indicating that arsenic acts indirectly with other agents to ultimately enhance the specific genotoxic effects that may lead to carcinogenesis. Arsenic has long been known to cause chromosomal damage, induced by a DNA cross linking agent 1,3-butadiene diepoxide (Yager et al, 1993). But most investigators have been unable to induce direct gene mutation.The specific co-clastogenic effect of arsenic has been mediated by inhibiting DNA repair. The repair inhibition may be a basic mechanism for the comutagenicity and presumably the cocarcinogenicity of arsenic. Arsenic exposure leads to a specific enhancement of effects related to genotoxicity that may be important in development of cancer.

This paper reports on the findings from a study designed to present the relationship between DS and level of arsenic and other risk factors including socio-economic status and maternal age of DS cases.

2. MATERIALS AND METHODS

2.1 Study setting and participants

84 DS cases were screened among 323 abnormal cases in the cytogenetics unit of the Genetics Department of Ramakrishna Mission Seva Pratishthan, Kolkata. 84 cases were confirmed DS after performing karyotyping of 323 cases. 12 healthy babies and their parents were also included in our study. The healthy babies and their parents were from arsenic affected areas. All procedures were done with the informed consent of participants' parents. Genetic Counseling was done with the help of counselors and psychiatrist of Ramakrishna Mission Seva Pratishthan Hospital.

2.2 Cytogenetic analysis of the patients

Blood cultures were done for analyzing chromosome aberrations as per routine procedures (Moorhead et al., 1960, Sharma and Talukder, 1974). Karyotyping was done on index patients. The banding technique was applied whenever necessary.

Leucocytes rich plasma (0.5ml) was added to 5 ml culture media supplemented with PHA M (0.04ml/ml of culture media) and 20% fetal bovine serum and incubated at 37ºC for 72 hrs. Colchicine was added at 70 h of culture. After 2 hrs of incubation, culture was centrifuged at 1000rpm for 10 min, treated with pre-warmed KCl (0.075M) for 15 min. Then it was fixed in acetic acid: methanol (1: 3) for 10 mins. Fixatives were removed by centrifugation and two more changes of fixative were performed. Fixed cell suspension was laid on clean grease-free glass slide and air-dried and stained with aqueous Giemsa. 100 metaphase plates were scored randomly for analyzing chromosomal aberrations.

2.3 Hair sampling

A tuft of hair of both DS and normal child was cut off from the scalp in the occipital region with a surgical stainless steel scissor and stored in polyethylene bags at room temperature. Hair samples were also obtained from their parents. Before analysis, samples were digested with 5ml of concentrated nitric acid and 3ml of concentrated sulfuric acid.

2.4 Metal analysis

Flow injection-hybrid generation atomic absorption spectrometry (FI-HG-AAS) at 327nm was used for estimation of arsenic in the collected biosamples. A Perkin-Elmer model 3100 AAS with a Hewlett-Packard-Vectra 386/25N computer with GEM software, Perkin-Elmer EDL System-2 and arsenic lamp (lamp current 400mA) were used for this purpose.

3. RESULTS

The present study intended to stratify the families into poor, average and good category according to their monthly income. The families with monthly income of upto Rs 5000/- were included in poor category and families with monthly income above Rs 20000/- were categorized as good. The income in between poor and good were categorized as average. In our study, among 84 DS cases most of the children were from low and average socioeconomic condition. In case of normal babies, 9 families were from poor socioeconomic background, as shown in Table I.

Table I. Socioeconomic status of studied family

Types	No. of families	Poor	Average	Good
DS babies	84	43	40	1
Normal babies	12	9	3	0

poor- upto Rs5000/- per month., average- above Rs5000/- to below Rs20000/- per month., good- above Rs20000/- per month.

It is a well known fact that increasing maternal age is a high risk factor for giving birth to DS babies. Our study projects indistinguishable result. The maximum numbers of cases were born to mothers aged above 30 years, whereas ages of mothers of healthy babies were between 21 - 25 years, as stated in Table II.

Table II. Age of mothers

Types	No. of mothers	20 years and below	21-25 years	26-30 years	Above 30 years
DS babies	84	4	21	26	33
Normal babies	12	2	8	1	0

DS patients visiting the out patients' department were from various districts of West Bengal, India. It had been observed that most number of cases were from Kolkata, South 24 Parganas, Midnapore and Nadia whereas Howrah, North 24 Parganas, Malda, Murshidabad, Hooghly and Burdwan had fewer number of cases, as shown in Table III. The arsenic level of the patients revealed that the maximum arsenic load was found in the patients coming from Kolkata, South 24 Parganas, Nadia and Midnapore. Healthy babies and their parents were from Atghara (North 24 Parganas) and Jainagar (South 24 Parganas).

Table III. Families of DS cases from different areas

No. of families	South 24 Parganas	North 24 Parganas	Midnapore	Howrah	Kolkata	Burdwan	Nadia	Hooghly	Murshidabad	Malda
84	15	7	19	7	14	2	9	3	3	5

The arsenic level found in the parents was 759.45±472.88µg/kg (mean±standard deviation). The DS children belonging to these parents had arsenic level of 1328.17±1145.68 (mean±standard deviation). The estimated normal level of arsenic is 80-250 µg/Kg, toxic level of arsenic is >1000 µg/Kg and body burden level of arsenic is 250-1000 µg/Kg. Thus from the present study, it is evident that the parents had body burden level of arsenic and the DS cases had toxic level of arsenic, as shown in Table IV.

Our study also reveals that the arsenic level found in healthy child was 711.67±356.32 µg/kg (mean±standard deviation) i.e. body burden level of arsenic but the arsenic level of the parents was 224±98.26 i.e. normal level of arsenic, as shown in Table V.

Table IV. Level of Arsenic estimated in DS cases and their parents

No. of Families	Type	Level of Arsenic(µg/kg)[mean±SD]
84	Child	1328.17±1145.68
	Parents	759.45±472.88

Table V. Level of Arsenic estimated in healthy babies and their parents

No. of Families	Type	Level of Arsenic(µg/kg)[mean±SD]
12	Child	711.67±356.32
	Parents	224±98.26

SD: standard deviation.
n.b.: normal level of arsenic: 80-250 µg/Kg., toxic level of arsenic: >1000 µg/Kg., body burden level of arsenic: 250-1000 µg/Kg.

4. DISCUSSION

This study investigated the relationship between DS and level of arsenic and other risk factors including socio-economic status and maternal age of DS cases. To the authors' knowledge, this is the first study to find out the effectiveness of arsenic on DS cases in West Bengal, India.

As most of the families of Down syndrome cases as well as healthy babies in our study came from very poor socioeconomic condition, no definite association between low socioeconomic status and DS could be established. Our study differs from the opinion of Hunter et al. where it postulated that a maternal history of low socioeconomic condition, specifically low household income, is associated with having an infant with DS (Hunter et al., 2013).

Previous studies showed that increasing maternal age is a well known risk factor for having DS baby (Hunter et al., 2013). The present study corroborates with the previous findings as maximum numbers of Down syndrome cases were born to mothers above 30 years of age.

Previous studies reported that environmental factors (like socioeconomic status leading to poor nutrition, environmental pollutant) have their influence on only specific stages of meiosis (Torfs CP et al., 2003). Several reported environmental exposures, as said earlier, associated with chromosome 21 nondisjunction appear to differ between different stages of meiotic errors, suggesting that different aspects of the meiotic machinery may be more or less vulnerable under different conditions (Hollis ND et al., 2013). The accumulation of environmental pollutants i.e. arsenic over time may interact with the vulnerable pericentromeric recombination pattern to increase the occurrence of a nondisjunction event leading to increase in the birth of DS babies.

Environmental pollutants have some potential to cause Down syndrome through preconceptional mutagenic action (maternal or paternal) or postconceptional teratogenic action (maternal). It was reported in the village of Hungary in 1990s (Czeizel et al., 1993) to increase in teratogenic births, including that of DS. Water contamination with pesticide trichlorfon has been reported to cause an outbreak of DS birth incidence.

The present study aimed to measure the levels of arsenic in DS child and their parents, using atomic absorption spectrophotometry and to find out whether arsenic has any role in pathogenesis of Down syndrome. The existing study states that body burden level of arsenic was present in the parents of DS baby whereas all DS child had high concentration of arsenic in the toxic level. Concomitant exposure to arsenic may have severe ill effects on neurodevelopment of children.

It has been found that the parents of DS child have body burden level of arsenic whereas the parents of normal child have normal level (BDL) of arsenic. As arsenic can easily cross placenta during pregnancy, it can cause harmful effects to the fetus even at low exposure. In the present study high concentration of arsenic in the hair was detected in most of the DS cases, whereas most of the healthy babies have body burden level of arsenic. So from the above findings it can be inferred that arsenic may be a predisposing factor for development of DS. Although a more elaborate study with increased number of samples is needed for further confirmation.

5. CONCLUSION

The present study can conclude that high arsenic concentration in the parents may be a potential risk factor for development of DS in the offspring. In order to prevent DS child, one must reduce exposure to potential teratogens (especially arsenic) before pregnancy and prenatal service providers need to be aware of the uncertainties regarding environmental pollution when addressing parental concerns.

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