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Arsenic May Play an Environmental Risk Factor to Give Birth a Down Syndrome Child

Podder Gargi*, Chakraborty Amit Kr. and De Madhusnata

Department of Genetics, Ramakrishna Mission Seva Pratishthan, Vivekananda Institute of Medical Sciences, 99 Sarat Bose Road, Kolkata-700026

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Address for Correspondence
Department of Genetics, Vivekananda Institute of Medical Sciences, Ramakrishna Mission Seva Pratisthan, 99, Sarat Bose Road, Calcutta - 700026, India
E-mail:
gargi.podder86@gmail.com

ABSTRACT

There are many different causes of mental retardation, both biological and environmental. Down syndrome occurs in one out of every 600-700 births worldwide. Arsenic is a naturally occurring metalloid that has been associated with increased incidence of different genetic anomalies in certain highly exposed populations. This present work aimed to study whether arsenic plays any role in the increased incidence of Down syndrome among residents of West Bengal, India, which is one of the worst arsenic-affected areas of the world. Our study group included thirty Down syndrome patients and their father and mother. We studied the arsenic concentration in the hair samples of our study group. We found in some area like Kolkata and Nadia higher arsenic concentration in the biological tissues of patients, which may correlate with their greater incidence of chromosomal aberrations. Thus, arsenic may act as a predisposing factor for giving birth of Down syndrome baby.

Keywords: Arsenic, Down syndrome, risk factor.

Objective:

1. To identify trisomic down syndromes and their families from our hospital.
2. To study the effect of environmental pollutions like arsenic in the etiology of the genetic disease especially down syndrome.

INTRODUCTION

Down Syndrome (DS), or Trisomy 21, is the most common serious autosomal chromosome aberration in which affected individuals survive beyond infancy. It is the most frequent form of mental retardation and is characterized by well-defined and distinctive phenotypic features and natural history. Down children have a widely recognized characteristic appearance. The head may be smaller than normal and

abnormally shaped. Prominent facial features include a 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. Retardation of normal growth and development is typical, and most affected children never reach the average adult height. The average mental age achieved is that of an 8 year old. The severity of the syndrome includes congenital cardiac malformation, immune system disorders, gastrointestinal malformation such as esophageal and duodenal atresia**,** and slow physical development[1].

Research on how many people with DS have certain conditions or diseases**, or** what interventions or medications work for people with DS would help to update medical guidelines such as Health Supervision for Children with DS. This in turn would improve how doctors, parents/caregivers, and in some instances self-advocates themselves handle the health care needs of people with Down syndrome.

Risk factors which could mediate the impact of socioeconomic status on the prevalence of congenital anomalies include nutritional factors, lifestyle, environmental and occupational exposures, access to and use of health services, parity and maternal age, and ethnic origin.

Educational levels in developing countries tend to be low, as measured by literacy rate (average: 71%; range: 57-87%, depending on the region; compared to 98% in developed countries) and the proportion of children reaching grade 5 of primary school (average: 75%; range 59-91%, depending on the region; compared to 99% in developed countries)[2]. All educational indices are significantly lower for women.

A karyotype test is a critical step in determining if someone has Down syndrome. People with DS have an extra number 21 chromosome. This extra number 21 chromosome can occur in three different ways - full trisomy 21, translocation Down syndrome and mosaic Down syndrome. Only a karyotype test can tell which form of Down syndrome your child has and what the chance is to have another child with DS.

Till now, 3000 inherited genetic diseases in humans have been reported. In addition to the clinical, biochemical and cytogenic detections that are available for some diseases, measurement of environmental toxicant like arsenic, lead on genetic disorder patient are necessary in the present day medical research.

Environmental pollution can in principle cause congenital anomalies through preconceptional mutagenic action (maternal or paternal) or postconceptional teratogenic action (maternal). Preconceptional mutagenic effects may include chromosomal anomalies and syndromes as a result of new mutations. Postconceptional action, the main focus of this paper, depends on the precise timing of exposure: in embryonic and fetal development, each normal development process occurs during a specific period of a few days or weeks, and it is during this 'sensitive period' that exposure to a teratogenic agent may lead to an anomaly. Thus, a particular chemical may cause a congenital anomaly after exposure in, say, the sixth week of development, but exposure during the previous or the succeeding week may have no effect, or an anatomically distinct effect. Where a child has more than one anomaly ('multiply malformed'), this may be because the exposure has covered a number of sensitive periods for different congenital anomalies, or because exposure at one developmental stage has a number of different effects on organogenesis.[3]

A study of all landfill sites in England, Scotland and Wales, investigated the risk of congenital malformations, and low and very low birth weight outcomes in

populations living within 2 km of a landfill site, open or closed. The study included over 9000 landfill sites. The study found that 80% of the population of Great Britain lived within 2 km of a landfill site. Statistically significant, but small (<10%) increases in risk were reported around all sites combined for all congenital anomalies, neural tube defects, hypospadias, abdominal wall defects, and low birth weight. Findings for sites that were licensed to take special (hazardous) waste were generally similar to non-special sites. In this study, only 20% of the country was available as reference population and the comparability of the 'landfill' and 'reference' areas therefore raises questions. Also, if risks were associated with a particular group of 'high-risk' landfill sites such a finding would be lost in the overall comparison of over 9000 sites in this study. Excess risks of some specific anomalies were found in the period before the opening of the landfill sites in the subgroup of sites that opened during the study period[4].

The following outlines the incidence and outcome for selected congenital anomalies in the Welsh population recorded by the Congenital Anomaly Register and Information Service (CARIS) (**Con Mals 1998-2003 Wales**).

See Table No. 1.

Assuming that the observed increased risk in proximity to landfill sites is due to environmental factors and using an EAF of 0.2 then the burden attributable to the environment would be a gross rate of 86 per 10,000 live and still births[5].

Arsenic has long been known to cause chromosomal damage, but most investigators have been unable to induce direct gene mutation. Arsenic exposure resultant frequency of chromosomal aberrations, has been explained by the concept that arsenic promotes genetic damage in large part by inhibiting DNA repair. The repair inhibition may be a basic mechanism for the comutagenicity and presumably the cocarcinogenicity of arsenic. Comparisons of chromosome aberration frequencies induced by trivalent and pentavalent arsenic have indicated that the trivalent forms are far more potent and genotoxic than the pentavalent forms[6].

MATERIALS AND METHODS

Identification of down patients and their families

The total number of cases of Karyotyping done in the cytogenetics unit of the Genetics Department of Ramakrishna Mission Seva Pratishthan is 167 from 1st March 2013 to 28th February 2014 (one year). Out of 167 cases, 30 cases were diagnosed as Down syndrome. The cases were referred from different Dist., Hospitals, Health Centers, Clinics of West Bengal and also Outdoor and Indoor Dept. of Ramakrishna Mission Seva Pratishthan.

Examinations of nose, eyes, mouth, tongue and palate were performed and recorded either as apparently normal or abnormal as observed from the clinical examination[7]. Abnormalities were recorded on specially prepared examination forms. Established clinically as an abnormally enlarged tongue this was protruding maximum time, when the ears were small, low set with an over folded helix, as a dysmorphic face, instead of two creases across their palms, people with Down syndrome frequently have a single crease and abnormal distance present between two eyes[7,8]. The examination also included, if the individuals have any clinical complication like congenital heart disease at present and jaundice at birth time.

Ethical Clearance

The studies involving human subjects were reviewed and approved by the ethical Committee of the Vivekananda Institute of

Medical Sciences. All individuals were included in this study only after the Informed Consent of their parents.

Cytogenetic analysis of the index patients

Blood cultures were made for chromosome preparations as per routine procedures[9,10]. 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 20% fetal bovine serum and PHA M (0.04ml/ml of culture media) and incubated at 37ºC for 72 hrs. At 70 h of culture colchicine was added. After 2 hrs of centrifugation at 1000rpm for 10 min, treated with pre-warmed KCl (0.075M) for 15 min. centrifuged at 1000rpm for 10 min and fixed in methanol: acetic acid (3:1). 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 chromosomal aberrations.

Measurement of arsenic level

Arsenic level was measured from biological sample like hair. Forty-four hair samples were collected from the nape of the head as near as possible to the scalp with a ceramic blade cutter (CL2, France) and stored in polyethylene bags. Hair samples were obtained from people who did not have colored or treated hair.

Biological samples (like hair) were collected from patients and their parents. Before estimation, the hair samples were digested with 5ml of concentrated nitric acid and 3ml of concentrated sulfuric acid. 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 (School of Environmental Sciences, Jadavpur University)[11,12].

Genetic Counseling

Genetic Counseling was done with the help of counselors and psychiatrist of Ramakrishna Mission Seva Pratishthan Hospital.

RESULTS

See Table no 2-6.

CONCLUSION

The DS baby in a family is economic and mental burden for the affected families. DS cases may have congenital heart defects, gastrointestinal atresias, Hirschsprung disease, leukemia, acquired hip dislocation, otitis media, eye disease including cataracts and severe refractive errors, hearing loss, obstructive sleep apnoea and thyroid disease etc. in future. So for the proper treatment of the above mentioned diseases early diagnosis is essential.

Environmental pollutant may have some role at the causation of the diseases. The aim of the study is to find out whether arsenic has any role the etiology of the disease. In this study it has been found that arsenic level with body burden of parents may give birth to DS child.

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Table 1.

Congenital Malformation	Cases (n)	Gross rate(all cases)/10,000 Live&stillbirths
All cases	8146	429
Musculoskeletal	1429	75
Abdominal Wall Defects	192	10
Gastrochisis	94	5
Exomphalos	81	4
Cleft Lip and Cleft Palate	133	7
Cleft Lip	66	3
Cleft Palate	187	10
Upper Limb reduction defects	147	8
Lower Limb reduction defects	76	4
Anencephalus	143	8
Spina bifida	155	8
Encephalocele	39	2
Complex Cyanotic Disease (CHD)	134	7
Transpositional great vessels	79	4
Fallots	55	3
Ventricualseptal defects (VSD)	945	50
Atrioventicualseptal defects (AVSD)	113	6
Hypospadias	356	19
Chromosomal	908	48
trisomy 21 - Down syndrome	**377**	**20**
trisomy 18 - Edwards syndrome	89	5
trisomy 13 - Patau syndrome	53	3
triploidy / polyploidy	44	2
Turner's syndrome	83	4
Klinefelter's syndrome	19	1
other anomalies sex chromosomes	36	2
deletions	57	3
other chromosome anomalies	157	8

Table 2. Socio-Economic Status of the study group

Types	Total no. of individuals	<Rs 5000 p.m		Rs 5000- 10000 p.m		Rs 10000- 15000 p.m		>Rs 15000 p.m		BPL	
		No	% present	No	% present	No	% present	No	% present	No	% present
Patient	30	14	46.66	12	40	2	6.66	2	6.66	0	0

Inference: In case of Down syndrome patient's family, in our study group maximum were come from very low economic condition.

Table 3. Education of mother of the studied cases

Types	Total no. of individuals	Illiterate		Elementary education		Primary education		High School		College	
		No	% present	No	% present	No	% present	No	% present	No	% present
Patient	30	3	10	1	3.33	13	43.33	10	33.33	3	10

Inference: In case of Down syndrome patient's mother, maximum had elementary and primary education.

Table 4. Age of mothers and fathers and distribution of mothers having birth wastage before the birth of the Down syndrome child

Age group (years)	No. of mother	% present	Birth wastage					Any medicine taken During pregnancy	No. of father	% present
			Abortion			Still births	Total			
			Spontaneous		Induced (one)					
			one	two						
15-20	1	3.33	1	0	0	0	1	0	0	0
21-25	11	36.66	2	0	1	0	3	1	0	0
26-30	8	26.66	2	0	2	0	4	0	6	20
31-35	5	16.66	2	1	0	1	4	0	9	30
36-40	5	16.66	3	0	1	0	4	0	9	30
>40	0	0	0	0	0	0	0	0	6	20
Total	30	100	10	1	4	1	16	1	30	100

Inference: Maximum number of Down syndrome cases was born to mother of age group 21-25 years and father of age group 36-40 years. Previous spontaneous abortion is a risk factor for giving birth to Down syndrome baby.

Table 5. Chromosomal analyses of the Down syndrome cases
1000 blast cells were analyzed for each individual to study the aberration of chromosomes

Age group		< 12 months	1 yr- 4 yr 11 months	5 yr- 9yr 11 months	10 yr- 14 yr 11 months	15 yr- 19yr 11 months	>20 years	Total
Trisomy 21(Pure)	Male	9	3	1	1	0	0	30
	Female	9	2	4	1	0	0	

Inference: Cytogenetic testing revealed that all the Down syndrome patients had pure trisomy 21. No mosaicism or translocation had been seen among 15 DS child. Maximum patients were within 12 months age group.

Table 6. Down syndrome cases and their families from different areas and their Arsenic estimation

	Different District of West Bengal									
	Kolkata	Howrah	24Pargana (S)	24Pargana (N)	Burdwan	West Midnapur	Nadia	East Midnapur	Malda	Murshidabad
No. of patients	6	4	5	3	1	1	2	5	2	1
Arsenic (µg/kg) In Down syndrome patient	1548.535	520.222	572.48	481.86	-	-	1040.29	500.28	303.03	128.66
Arsenic (µg/kg) In Mothers	339.48	188.39	524.29	240.09	-	-	640.42	239.05	244.5	3351.5
Arsenic (µg/kg) In Fathers	347.28	296.28	338.17	326.32	-	-	652.9	575.09	199.71	268.63

Note: Arsenic estimation of 2 cases was not yet completed (patients from Burdwan and West Midnapur)

N.B- Normal level of Arsenic is 80-250 µg/Kg.
Toxic level of Arsenic is above 1000 µg/Kg.
Body burden level of Arsenic is 250-1000 µg/Kg.

Inference: From the above table it has been found that body burden level of arsenic was present in parents of DS child. All Down children had high arsenic content may be due to high arsenic level of their parents and this arsenic passes from parents to children. Maximum level of arsenic was found in the DS patient residing at Nadia and Kolkata and both areas are worst hit area by arsenic.