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Research Article

CLINICAL AND CHROMOSOMAL INVESTIGATIONS IN INTELLECTUALLY DISABLED CHILDREN OF GUJARAT

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ABSTRACT

Background: The etiology of intellectual disability (ID) can be genetics or environmental including neurological disorders, gastrointestinal disorders and behavioral/psychiatric problems. Therefore, current study was aimed to diagnose possible health disparities by collective results of clinical manifestations, urinary biochemical and chromosomal analysis. **Materials and methods:** The clinical manifestations were studied for all 34 ID affected children from Gujarat. The results obtained from biochemical and chromosomal investigations were recorded and values were calculated and compared with all clinical data sets to point out significant findings. **Results:** Urine analysis revealed the presence of abnormal concentration of glucose, ketones, nitrites, bilirubin and urobilinogen in different groups of children with ID. The results from specific gravity test revealed hydration and dehydration status of individuals. Giemsa banding (G-banding) analysis confirmed the suspected Down syndrome (trisomy 21) in 5 children and no other chromosomal anomalies were detected from remaining samples. **Conclusion:** Microcephaly, macrocephaly, seizure, speech abnormality, short stature, oral cavity defect and facial dysmorphia were more frequently observed in Down syndrome (DS) children as compared to unclassified intellectually disabled (ID). Comparatively more number of DS children showed urinary glucose, ketone and nitrite excretion than ID. Bilirubin, blood and urobilinogen were more frequently excreted in ID children.

INTRODUCTION

Intellectual disability (ID) is one of the serious unsolved problem in medicine. It is characterized by impairment of skills (cognitive, language, motor, and social abilities) manifested during the developmental period (before 18 years age) and contribute to the overall level of intelligence (Schalock *et al*., 2010). On the basis of intelligence quotient (IQ) score, WHO classified ID into four subcategories, which includes mild (50-69), moderate (35-49), severe (20-34) and profound (<20). Intellectual disability is an etiologically heterogeneous group of disorders affectingaround 2-3 % of the general population (Koirala, Kumar, and Bhagat, 2012). Intellectual disability can be either, only consistent handicap (called non-syndromic intellectual disability) or maybe combined with other physical and/or behavioral abnormalities, called syndromic intellectual disability (Raymond, 2006).

The etiology of Intellectual disability can be genetic, environmental or both. The main etiological groups include chromosomal abnormalities, metabolic causes, single gene disorders, teratogens, mitochondrial disorders and multifactorial causes (Panchani, 2013). However, despite numerous diagnostic efforts, about 50% of cases of ID remains unexplained. Metabolic causes are responsible for around 3% of ID, which is a very low proportion when compared with other causes such as cytogenetic anomalies (12%) and other monogenic & known syndromic conditions (20%) (Winnepenninckx, Rooms, and Kooy, 2003). The possibility of an underlying metabolic disorder increases whenID is associated with other neurological signs, such as psychiatric disturbances, cerebellar dysfunction, and epilepsy (Sempere, Arias, Farré, & García-villoria, 2010).

The diagnosis of ID is often primarily performed based on clinical manifestations. According to the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-V), three criteria must be met for a diagnosis of intellectual disability: i) IQ below 70, ii) significant limitations in two or more areas of adaptive behavior (as measured by an adaptive behavior rating scale, i.e. communication, self-help skills, interpersonal skills, and more), and iii) evidence that the limitations became apparent before the age of 18.

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The recently developed genetic diagnosis also play a major role to point out genetic causes of intellectual disability. The most prevalent genetic conditions include Down syndrome, Klinefelter's syndrome, Fragile X syndrome (common among boys), Neurofibromatosis, congenital hypothyroidism, Williams's syndrome, Phenylketonuria (PKU), and Prader-Willi syndrome(Donald and Pratt, 2005). Other genetic conditions include Phelan- McDermidsyndrome (22q13del), Mowat-Wilson syndrome, genetic ciliopathy, and Siderius type X-linked intellectual disability (OMIM 300263) as caused by mutations in the PHF8gene (OMIM 300560). In the rarest of cases, abnormalities with the X or Y chromosome may also cause disability. The 48, XXXX and 49, XXXXX syndrome affect a small number of girls worldwide, while boys may be affected by 47, XYY; 49, XXXXY or 49, XYYYY(Salvador-Carulla *et al*., 2011).

Since, people with intellectual disability have higher rates of adverse health conditions such as epilepsy, neurological disorders, gastrointestinal disorders and behavioral/ psychiatric problems as compared to people without disabilities(Derakhshan and Khaniani, 2016), the present study was aimed to find out possible health disparities by collective results of clinical manifestations, biochemical analysis of urine and chromosomal analysis.

MATERIALS AND METHODS

Subjects and Clinical Data

Clinical and cytogenetic investigations were carried out in 34 intellectually disabled children from Surat and Anand district of Gujarat state in India. The clinical features identified by a pediatrician and occupational therapist. The diagnostic criteria followed from the Diagnostic and Statistical Manual of the American Psychiatric Association (DSM-V, 2015) and, the International Classification of Disease (Division of Mental Health and Prevention of Substance Abuse, World Health Organization, 1996) for intellectual disability. Anthropometric examination and birth history as well as family history including pedigree analysis were donebefore the sample collection process. According to the clinical symptoms, the children were grouped intoDown syndrome and intellectual disability.

The present study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki, 2001)for experiments on humans. The Ethical approval for this study was obtained from Human Research Ethics Committee of H. M. Patel Centre for Medical Care and Education, Karamsad, Gujarat, India. Additional informed consentswere obtained from all individual participants/their parents included in the present study.

Biochemical analysis

Biochemical investigation was performed for all 34 intellectually disabled children to observe inborn error of metabolism. The reagent strips for urinalysis (Multistix® 10 SG, Seimens) was used to test Bilirubin, Blood, Glucose, Ketones, pH, Leukocytes, Nitrites,Protein, Specific gravity and Urobilinogen in the urine of ID affected children. The standard procedure given in the product manual to perform urinalysis was followed for the analysis.

Chromosomal analysis

Peripheral Blood Lymphocytes of individuals were cultured by following standard procedures. The media was prepared using RPMI 1640, 12% of fetal bovine serum, 200 mM L-glutamine, 100mg/ml streptomycin and penicillin and Phytohemagglutinin-M. The cells were cultured for 72 hours in 5% of CO_2 at 37°C incubator until harvesting process. At 69 hours, the cells were treated by Colchicine reagent for 55 minutes, following exposure to hypotonic solution (0.075M KCL) for 20 minutes and then fixed with Carnoy fixative (methanol:aceticacid, 3:1), and achieved chromosomes in metaphase stage.

Table 1 Observed clinical features and anthropomatric measurements of children with ID

Clinical features	Male (n=24)	Female (n=10)	Down Syndrome+ID (n=5)	NS-ID (n=29)
Male	24	-	04	20
Female	-	10	01	09
Median age (y)	10	13	8	13
Average age (y) (SD)	8 (2.67)	8 (3.22)	7.6	11.6 (3.82)
Median Birth Weight (kg)	2.22	2.5	3.5	2.25
Average Birth Weight (kg) (SD)	2.4 (0.79)	2.5 (0.77)	3.21 (0.60)	2.5 (0.82)
Median Height (cm)	123.4	116.8	107	126
Average Height (cm) (SD)	124 (19.37)	112.5 (16.84)	106.8 (9.95)	127.4 (17.55)
Median Weight (kg)	27	29	19	25
Average Weight (kg) (SD)	24.8 (13.85)	23.3 (9.28)	19.4 (3.30)	27.5 (11.62)
Microcephaly	07	04	02	09
Macrocephaly	01	01	01	01
Seizure	07	02	03	06
Speech Abnormality	14	07	05	16
Autistic Disorder	05	03	01	07
Short Stature	09	06	04	11
Skeletal Abnormality	11	04	01	14
Oral Cavity Defects	09	03	03	09
Facial Dysmorphism	12	06	05	13
CNS Anomalies	09	01	01	09
Mild	12	03	02	13
Moderate	08	06	03	11
Severe	04	01	00	05
Under Weight	07	03	00	10
Over Weight	05	01	02	04

(y=Years; SD=standard deviation)

The slides were prepared and conventional G-banding technique performed to all the samples. Thirty metaphase spreads of each sample were analyzed at 400 band resolution and karyograms were prepared using Ikaros Karyotyping System.

RESULTS AND DISCUSSION

Clinicalfeatures and anthropometric examinations

In the present study, a total 34 children having intellectual disability were evaluated for clinical characteristics and measurements followed by urinalysis and chromosomal abnormalities to diagnose disease relation with abnormal clinical conditions. Previously there were several studies reported the significant results for urinalysis as well as cytogenetic and clinical examinations of intellectual disability (Baker *et al.*, 2002; Moeschler and Shevell, 2014; Romano, Citt, and Romano, 2010; Khimsuriya *et al.*, 2016). However, the present study combined clinical characteristics, urinalysis and chromosomal analysis together to rule out possible disease relevance in the studied population.

The evaluation of the anthropometric measurements as well as clinical features often found in intellectual disability among the study groups is summarized in Table 1.

The results revealed that there aremore number of male individuals (70%) as compared to female (30%) counterparts. We found variably distributed clinical features among studied population such as microcephaly (32%), macrocephaly (6%), seizures (26%), speech abnormality (62%), autistic behavior (23%), short stature (44%), skeletal abnormality (44%), mouth defects (35%), facial dysmorphism (53%) as well as mild (45%), moderate (41%), and severe (14%) intellectual disability.

The etiology of intellectual disability of studied group was identified followed by detailed clinical and physical examination. The median (3.5) and average birth weight (3.21±0.60) was higher in Down syndrome group as compared to unexplained intellectual disability. There was no significantdifference between male and female group for median and average birth weight. Microcephaly and macrocephaly was observed more frequent in DS and female as compared to ID and male in the current study. Seizures and mouth defects were observed more frequent in DS as compared to NS- ID. All the individuals with DS were observed with speech abnormality and facial dysmorphia while, only 55% and 45% of intellectually disabled observed with speech abnormality and facial dysmorphia respectively. Similarly, short stature was observed in 80% of DS and only 38% of NS-ID.

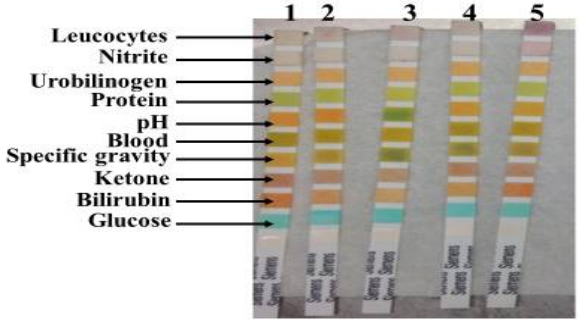

Figure 1 Representative reagent strips of urinalysis

In contradictory to that, skeletal abnormality and CNS anomalies were more frequent in ID than DS group.

Urinalysis

All the 34 patients were included and screened for urinalysis using commercially available reagent strips (Multistix® 10 SG, Seimens). The positive test for abnormal urinary components in patients were observed and recorded (Figure 1).

The presence of Glucose (n=4), Bilirubin (n=2), Ketones (n=6), Blood (n=3), Protein (n=0), Urobilinogen (n=1), Nitrites (6), Leukocytes (7) in the patients was presented in the form of bar diagram (Figure 2).

The results of urinalysis revealed that Glucose, ketone and nitrite excreted more frequently in Down syndrome than unexplained ID. Billirubin, blood and urobilinogen excreted less frequently in urine of DS individuals as compared to unexplained ID. Abnormal value of urine specific gravity (USG) was confirmed in 3 patients with relative hydration and in 5 patients with relative dehydration. The pH of all collected urine samples of patients were found in normal range (4.5 to 8) in our study population.

Comparatively more number of females showed urinary ketone, blood, urobilinogen and nitrite excretion than male in the present study. While, glucose and bilirubin was more frequent in urine of male children (Figure 2).

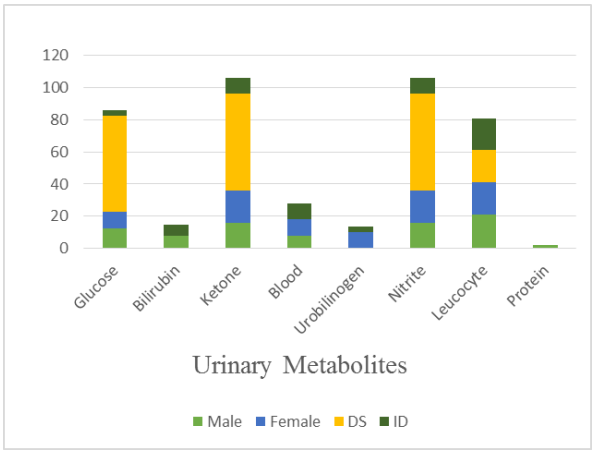

Figure 2 Summary of urinary metabolite excretion in different groups

Chromosomal abnormalities

Based on the clinical features, a total 5 cases (male-4; female-1) were suspected to have Down syndrome and 29 cases predicted unexplained for specific type of intellectual disability. Down syndrome (DS), due to trisomy 21, is the most common aneuploidy. G-banding analysis at 400 band resolution confirmed that all the five suspected individuals are of Down syndrome. Figure 3 showed representative male and female karyograms of the normal as well as trisomy 21 (DS) patients. No other chromosomal anomaly was observed in rest of the 29 samples and hence considered as idiopathic intellectual disability.

Polipalli and colleagues(2016) studied chromosomal abnormalities in 859 intellectually disabled patients in North Indian population and found 35% with Down syndrome, 6% with Turner syndrome, 0.5% with sex development disorders (45,X0/46,XX; 45,X0/46,XY) and 1.5% miscellaneous

abnormalities. However, present study observed only 14.7% with DS and remained were karyotypically normal.

A meta-analysis showed the sex ratio between male and female cases in Down syndrome reported male predominance(Kovaleva, 2002). The present study also revealed similar results.

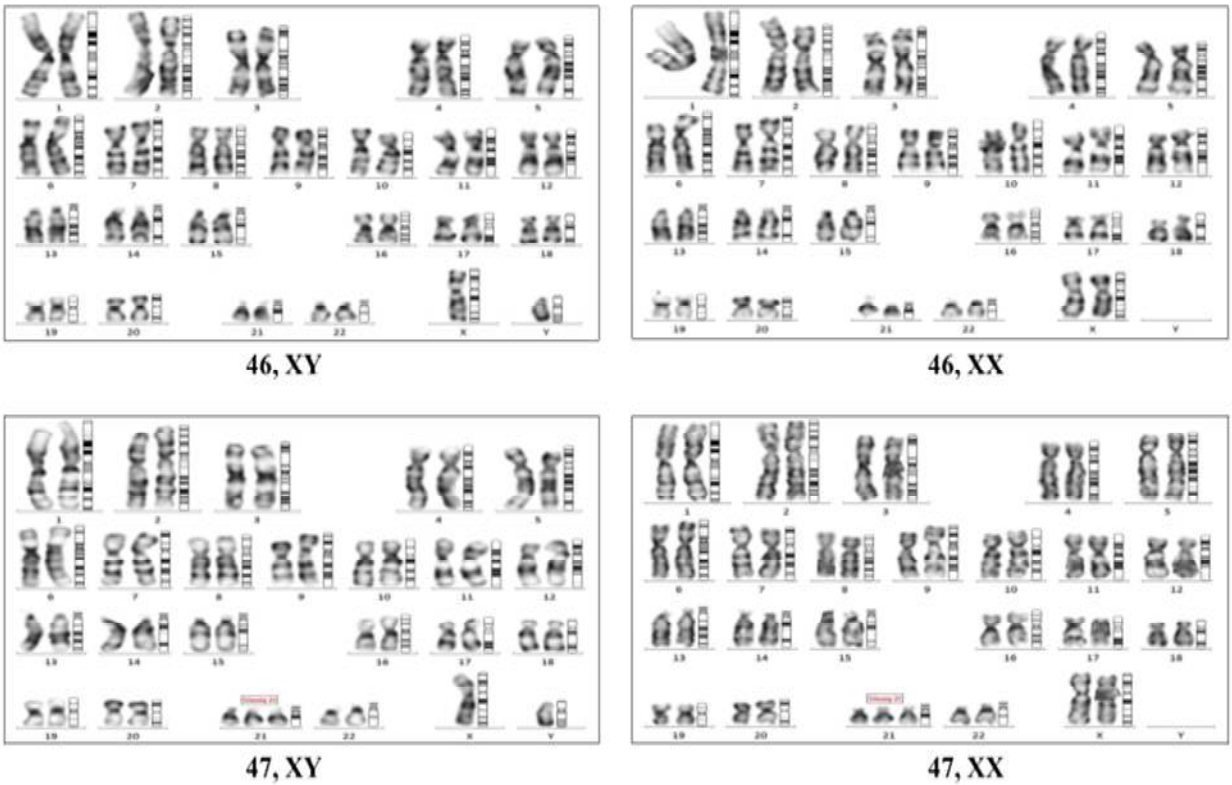

Figure 3 Karyograms of the normal (top) and trisomy 21 (bottom) children.

CONCLUSION

Microcephaly, macrocephaly, seizures, speech abnormality, short stature, oral cavity defect and facial dysmorphia were more frequently observed in Down syndrome (DS) children as compared to unclassified intellectually disabled children (ID). On the other side, skeletal abnormality and CNS abnormality were more frequently observed in ID children as compared to DS. Comparatively more number of DS children showed urinary glucose, ketone and nitrite excretion than ID. Bilirubin, blood and urobilinogen were more frequently excreted in other ID affected children.

Since, there is in a high proportion of trisomy 21 in studied population alarming us to screen the ID affected children for chromosomal analysis, especially for Down syndrome.

Conflict of interest: Authors declares no conflict of interest.

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