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RESEARCH NOTE

Hypotonic infant with Pallister–Killian syndrome diagnosed by cytogenetic microarray, without pigmentary skin changes and malformations

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Abstract. Pallister–Killian syndrome (PKS) is a rare genetic developmental disorder characterized, by intellectual disability, seizures, streaks of hypo- or hyperpigmentation and characteristic dysmorphic features. PKS is characterized by the presence of cytogenetic abnormality in form of a supernumerary isochromosome 12p, in a tissue limited mosaicism. The isochromosome 12p is usually not detected in karyotype done from peripheral blood. Presence of patchy pigmentary skin lesions suggest the possibility of mosaicism and karyotype from skin is done which clinches the diagnosis. We describe an infant with severe hypotonia in whom trisomy 12p was detected by chromosomal microarray performed on peripheral blood. The karyotype from blood was normal and combining this information with three copies of 12p in microarray suggests the possibility of tetrasomy12p in mosaic form. The infant did not have any skin patchy pigmentary changes and malformations and hence, the diagnosis of PKS was not clinically suspected. Cytogenetic microarray is the first test for evaluation of cases with developmental delay and intellectual disability, PKS diagnosis may come as a surprise in unsuspected cases without characteristic skin pigmentary abnormality and malformations.

Keywords. Pallister–Killian syndrome; hypotonia; mosacism; Tetrasomy-12p; cytogenetic microarray.

Introduction

Pallister–Killian syndrome (PKS) is a rare sporadic disorder caused by mosaicism in tetrasomy of isochromosome of chromosome 12p (Pallister *et al*. 1977; Teschler-Nicola and Killian 1981). PKS is characterized by coarse facies with a prominent forehead, sparse frontotemporal hair, hypotonia, seizures, intellectual disability and anomalies like diaphragmatic hernia, cleft palate and polydactyly ('Pallister Killian Syndrome,' n.d.). Streaky hypopigmentation or hyperpigmentation is a clue to the mosaicism of chromosomal abnormality.

Detection of isochromosome 12p [i(12p)] is often complicated by the absence or low frequency of this supernumerary chromosome in phytohemaglutinin (PHA) stimulated peripheral blood lymphocytes. Diagnosis frequently requires skin biopsy followed by cytogenetic analysis of fibroblast cells by conventional karyotype, fluorescence *in situ* hybridization (FISH) or array comparative genomic hybridization (aCGH).

Case report

We report a case of a four months old male child, second in birth order, born of nonconsanguineous marriage, brought with complaints of feeding difficulties and not having neck control. He was delivered at term by caesarean section (indication being previous caesarean section in mother) with birth weight of 2.5 kg (Z score = −1.9). There was history of perinatal asphyxia and admission to intensive care unit and was managed by noninvasive ventilation. He had severe hypotonia, poor suck with episodes of cyanotic spells, so was initiated on nasogastric tube feeds, further evaluation showed laryngomalacia. At two months he had seizures and was initiated on sodium valproate and biotin. Family history was not contributory. On examination length was 52 cm which was less than third centile (Z score = −0.8), weight 3.4 kg corresponding to tenth centile and a normal head circumference of 37.5 cm (Z score = −5.2). He had subtle dysmorphism including prominent forehead, and tented

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upper lip. Scalp hair were sparse. There were no hypo or hyperpigmented skin lesions (figure 1). He had normal muscle bulk with profound hypotonia. There were no spontaneous movements of limbs. Cry was weak. Respiratory movements were weak with respiration being predominantly diaphragmatic. He had brief apneic spells with peripheral cyanosis. Liver and spleen were not enlarged. External genitalia were normal male type. Rest of the systemic examination was unremarkable. Radiograph of the chest and skeletal survey were normal.

CT scan head showed hypodensity in posterior periventricular white matter bilaterally; with normal age related myelination. Total creatine kinase was 67 units/litre. Karyotype was 46, XY with no abnormal cell line out of 20 metaphases counted. Skeletal survey did not show punctate ossification of epiphyses or any other abnormality. Multiplex ligation probe amplification (MPLA) for common microdeletion revealed no deletion/duplication in the probe set used for common microdeletion syndromes (which includes Prader Willi syndrome region). MLPA for spinal muscular atrophy showed no deletion / duplication in exon 7 and exon 8 of *SMN1* gene. A normal acylcarnitine and amino acid profile was reported on tandem mass spectrometry. Gas chromatography – tandem mass spectrometry analysis of urine for organic acids was normal. A normal very long chain fatty acids (VLCFA) ruled out peroxisomal disorders. No pathogenic variant could be identified on clinical exome sequencing. Chromosomal micro array (CMA) was done as the later part of evaluation using Affymetrix CytoScan 750 K microarray (Affymetrix, Santa Clara, CA, USA), it showed 37.7 Mb duplication on 12p.33q11 (arr[hg19] 12p13.33q11(173,786-37,874,855) x3) (figure 2). By the time the report was available the child was not alive. Karyotype from skin fibroblasts and FISH with probes for chromosome 12 could not be done as the child died at seven months of age.

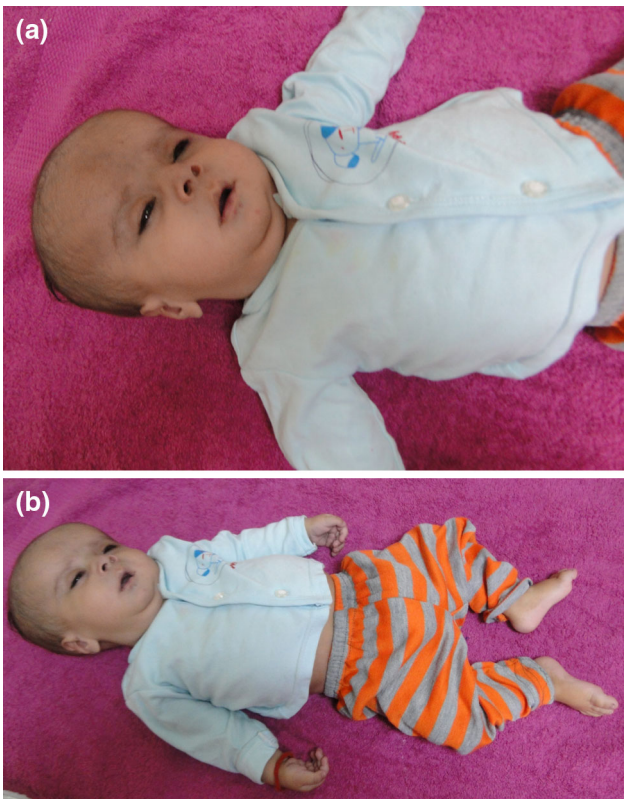

Figure 1. Photograph of the baby showing (a) hypotonic posture and (b) subtle dysmorphism.

Materials and methods

Informed consents were obtained from the parents of the patient for the molecular genetic analysis of the patients. Blood samples from both affected and unaffected individuals of the family were collected in ethylenediaminetetraacetic acid (EDTA) containing vacutainer sets. Genomic DNA from the blood samples was extracted using QIAamp DNA Blood extraction kit.

Discussion

The normal karyotype from blood and trisomy of 12p on cytogenetic microarray suggest the possibility of tetrasomy of 12p in mosaic form. This could not be confirmed by FISH or skin fibroblast culture as the child died before the microarray report became available. As the child did not have skin pigmentary abnormalities PKS or chromosomal mosacism was not suspected on clinical grounds. The predominant presenting feature of the child was profound hypotonia since birth needing respiratory assistance during neonatal period and tube feeding. The intercostal muscles were very weak and the respiration was predominantly diaphragmatic. He did not have any major malformation or polydactyly. The subtle facial dysmorphism with predominant hypotonia suggested the possibilities peroxisomal and other metabolic disorders, spinomuscular atrophy and congenital myopathies. Hypotonia due to Prader Willi syndrome was less likely as external genitalia were normal. These disorders were ruled out by appropriate investigations. Traditional karyotype and MLPA for common microdeletion syndromes were normal. Carnitine, amino acid profiles were normal. Organic acidemias and peroxisomal disorders were ruled out by normal urine GCMS and VLCFA respectively.

Cytogenetic microarray from blood sample was ordered after metabolic and neuromuscular disorders were ruled out. Inference that the child had PKS is based on the combination of karyotype and microarray report. It is supported by presence of subtle facial dysmorphism and hypotonia consistent with the diagnosis of PKS. Theisen *et al*. (2009) have reported diagnosis of PKS based on array comparative genomic hybridization (aCGH) in nine cases. Isochromosome 12p on cultured peripheral lymphocytes and FISH on uncultured lymphocytes could be demonstrated only in one

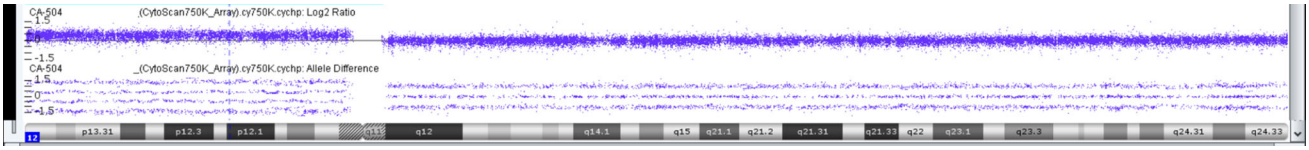

Figure 2. Snap shot of microarray showing Log 2 ratio of 1.36 of 12p and normal 1 for 12q.

case. Conlin *et al*. (2012) did aCGH on blood samples of 15 previously confirmed patients of PKS and showed that aCGH was able to detect tetrasomy 12p in 46% of peripheral blood cells. The study also showed that with advancing age frequency of the i(12p) decreases in blood, but not in fibroblasts. Lee *et al*. (2017) also reported two cases of PKS detected by aCGH. In one case aCGH detected 2 to 3 copies of 12p and in another case there were three copies of 12p. Obviously this will depend on the level of mosaicism. Blyth *et al*. (2015) studied 22 cases of PKS and suggested that aCGH only suggested the diagnosis in 15.8% but buccal FISH could have made the diagnosis in 75.0%. According to Conlin *et al*. (2012) mosaicism up to 5% was detectable. This is very important as now a days aCGH has become the first tier of diagnostic test and especially in absence of skin lesions, karyotype from skin fibroblasts will not be ordered. Skin pigmentary lesions were present in 66.7% of cases in the series of British patients (Blyth *et al*. 2015). The same study evaluated 22 patients and published literature in details and reported that hypotonia is present in 76.3% to 100% of cases. Malformations are common features; diaphragmatic hernia and cardiac malformations being reported in 34.8% and 8.7% respectively. Characteristic facial dysmorphism in the form of coarse facial features and temporal balding, short nose, micrognathia, low set ears usually become apparent with age (Blyth *et al*. 2015). Absence of major or minor malformations in this child and presence of severe hypotonia, lead to the differential diagnosis of non-chromosomal disorders. This case highlights the challenges of diagnosis of patients with developmental delay, especially during neonatal period and infancy. Presence of such severe degree of hypotonia which is a rare feature of PKS and absence of malformations and characteristic skin lesions caused delay in reaching aetiological diagnosis.

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