RESEARCH BRIEF

Clinical and Molecular Characterization of Patients with Gross Hypotonia and Impaired Lower Motor Neuron Function

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Spinal muscular atrophy (SMA) represents the second most common fatal autosomal recessive disorder after cystic fibrosis. Due to the high carrier frequency, the burden of this genetic disorder is very heavy in developing countries like India. The aim was to study the clinical and molecular characteristics of patients suspected with SMA. It was a cross sectional study of 105 cases from January 2008 to August 2012. Patients' demographic and presenting features and PCR findings were noted. 65 (62%) cases had a confirmed diagnosis of SMA. Werdnig Hoffman disease (SMA type I) was the commonest variant seen in 34 (52.3%) children. Molecular analysis demonstrated deletion of both exon 7 and 8 of SMN1 gene in 83.1% of cases.

Key words: *Hypotonia, India, Spinal Muscular Atrophy, SMN1, SMN2.*

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Spinal muscular atrophy (SMA) is the most common genetic cause of infant mortality [1] with an incidence of 1 in 6,000-10,000 live births [2]; and a carrier frequency of 1:50 [3]. It results from homozygous deletions of exon 7 and 8 involving the *SMN1* gene located on chromosome 5q13 [4]. Homozygous *SMN2* detection, although found in 5-9% of normal control, may be associated with disease phenotype in selected cases [2,4,5]. Most cases of SMA have autosomal recessive inheritance; however, autosomal dominant and *X*-linked inheritance has also been documented [6].

SMA phenotype varies, depending on the age of onset and motor development milestones [7]. Since in India, SMA remains highly under-diagnosed, present study aimed to analyse the clinical characterization of patients with gross hypotonia and impaired lower motor neuron function and their further molecular confirmation by SMN gene study.

METHODS

This is a cross-sectional study carried out on patients referred between the period of January 2008 to August 2012 from Gujarat and its vicinity. The main clinical phenotype were marked hypotonia, diffuse proximal muscle weakness, tongue fasciculation along with absent or greatly decreased deep tendon reflexes and electromyographic (EMG) evidence of denervation on systemic examination. After approval from the ethical committee, 105 subjects were included in the study and their name, age, gender, other demographic findings, presenting signs and symptoms, family history were noted. Molecular analysis was carried out after taking informed written consent.

Gene analysis: Genomic DNA was isolated from blood samples using salting out method [8]. Deletion of exon 7 and 8 of *SMN1* and *SMN2* gene was carried out by polymerase chain reaction (PCR) amplification and restriction endonuclease digestion. PCR products of exon 7 were digested with Dra I, and exon 8 with Dde I and run on 2.5% agarose gel electrophoresis at 100v and observed under UV transilluminator.

RESULTS

Of 105 subjects presenting with suspicion of SMA, 65(62%) were consistent with the clinical diagnosis of SMA. Out of these, 34(52.3%) children were in the acute infantile group with onset within 6 months of age, 12 (18.5%) were in the range of 7-18 months falling in the category of chronic childhood, 18 (27.7%) subjects belonged to the chronic juvenile category while only 1 subject (1.5%) was more than 30 years of age at the time of presentation as shown in ***Table* I**. Males had a greater preponderance than females in our study in the ratio of 1.5:1. This skewed ratio in favour of males was most striking in SMA type I subjects.

Molecular study demonstrated that, 83.1% of cases showed deletion of both the exons 7 and 8 of *SMN1* gene while 6.2% and 4.6% cases showed deletion of only exon 7 and exon 8, respectively. Exon 7 and/or 8 deletion in *SMN2* gene was observed in 4.6% of cases. Deletion of both exons of *SMN1* and *SMN2* gene was detected in one case. Out of 12 cases with SMA type II, none showed deletion in exon 7 and/or 8 of the *SMN2* gene while the only case of adult onset SMA (type IV) had deletion in exon 7 of *SMN2* gene (***Fig.* 1**).

DISCUSSION

In our study majority of patients presented within the first 6 months and were of SMA type I (53.3%) which is similar to the observations in other studies [6.9]. Males had a greater preponderance which is in concordance with earlier study [6] while a female preponderance was reported in an Egyptian cohort [9].

Different genes and microsatellite markers have been identified in the 5q region that can be deleted in SMA patients. Homozygous deletions of the *SMN1* gene are detected in more than 90% of the SMA type I to III patients and only exceptionally in SMA type IV [10]. The number of *SMN2* copies correlates with the SMA subtype, age of onset, and length of survival [11]. It has been observed that 95% of SMA type I patients have only 1-2 copies of *SMN2*, whereas almost all patients belonging to type III had 3 or more copies and a less severe disease course [12]. The most common deletion found in our study was of *SMN1* gene with deletion of both exons 7 and 8 in majority of cases (83.1%). Similar observation was made in the cohort of Pakistani and Egyptian children [6,9]. Deletions of either exon 7 or exon 8 of *SMN1* gene and *SMN2* gene were observed only in around 5% of cases. Other studies have reported exon 7 deletion of *SMN1* gene in 18.2% of cases while none of the cases had deletion of only exon 8 [9] and homozygous deletion of *SMN2* gene in a childhood onset SMA [13]. A study in Korean population demonstrated an association between sporadic motor neuron disease and *SMN2* deletion in adults [14]. With increasing identification of the underlying genetic defects, clinical spectrum and presentation, the awareness of the disease has improved. However, because no cure is yet available, genetic counselling and prognostic considerations are of great importance. With the availability of genetic testing, it is now possible to diagnose these children early so that appropriate counselling can be offered to the family on the risk of future pregnancies. Early identification by genetic testing has also made early antenatal diagnosis possible. Thus spreading awareness about this lethal but preventive disease becomes imperative.

TABLE I SUMMARY OF CONFIRMED CASES OF SMA

Type of SMA	*Most common presentation*	*Number of cases*
Type I	Hypotonia and decreased limb movement	34 (22 males)
Type II	Delayed motor milestones and hypotonia	12 (7 males)
Type III	Proximal muscle weakness	18 (9 males)
Type IV	Weakness in lower limbs	1 male

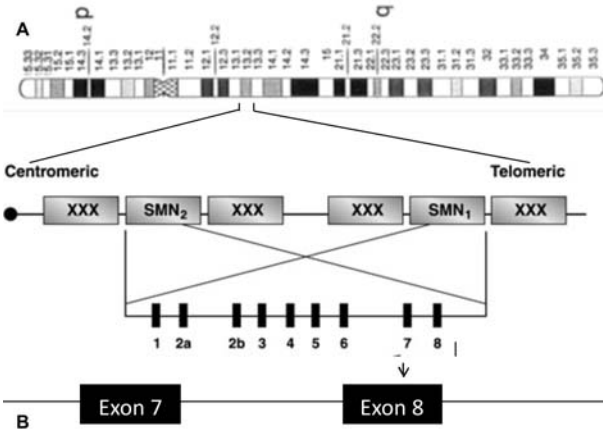

FIG. 1 *Schematic representation of SMA region on chromosome 5 and localization of SMN1 (telomeric copy) and SMN 2 (centromeric copy) on 5q13.*

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WHAT THIS STUDY ADDS

- Patients presenting with hypotonia and clinical features of lower motor neuron disease carry high level of clinical suspicion for SMA and need further confirmation.

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