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Analysis Of Deletion Mutation In SMN I Gene For Spinal Muscular Atrophy In A Tertiary Care Centre, Kolkata

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Abstract

The loss of lower motor neurons in the ventral horns of the spinal cord is the hallmark of spinal muscular atrophy (SMA), the second most prevalent autosomal recessive disorder in children. Some genes cause SMA. Among them, the mutation in the SMN1 gene is the most common, especially exon 7 and exon 8. SMN gene has at least one homologous but inverted copy in the chromosome. From newborns to adults, mutations in the SMN1 gene can impact people in a wide spectrum of severity. Types I, II, III, and IV are the four variations of SMA. The SMN2 gene can be found in each cell of certain individuals with SMA types II and III in three or more copies. The condition is less severe because of these numerous copies of the SMN2 gene. In our tertiary center, we have tried to find out the pattern of deletion and type in the patients. We investigated them depending on their clinical manifestation by PCR of exon 7 and exon 8 followed by RFLP with restriction enzymes DraI & DdeI respectively, for molecular diagnosis. Through molecular study, we have confirmed forty patients with deletions in the SMN1 gene. Among them twenty-five cases showed both deletions of exon 7 and exon 8, thirteen cases were detected with exon 7 deletion, and only two cases with deletion of exon 8.The patients with confirmed deletions were divided into three groups: 16 patients with type III, 19 patients with type II, and 5 patients with type I, based on the clinical symptoms and age of onset. It was commonly observed that the majority of the patient who showed both deletions had a severe phenotype, even if there was some overlap between the deletions detected and the type and severity of SMA.

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Keywords: Spinal Muscular Atrophy (SMA), DraI, DdeI, PCR

1.0 Introduction

SMA is a neuromuscular condition that runs in the family and is autosomal recessive. It is characterised by progressive muscle weakness and is one of the most common genetically inherited causes of child deaths. One in 10,000 live births[1] are estimated to be affected by SMA1, and one in fifty[2] people are carriers of the

disease.. Most occurrences of SMA are attributed to the spinal motor neuron gene (SMN gene), which has been found to reside on chromosome 5q11.2-q13.3. The 500 Kb inverted duplication contains this gene.The duplicated region consists of a minimum of four genes and repetitive elements. This makes it vulnerable to rearrangements and deletions[3]. There are at least two SMN genes, namely, telomeric and centromeric. Mutations in the telomeric copy or SMN 1 gene cause SMA in 90-95% of patients[3]. The SMN1 gene's exons 7 and 8 can be homozygously deleted to provide a molecular diagnosis of a patient[1].

SMA has been categorised into four primary groups based on the age at which symptoms initially manifested and the maximum level of motor function obtained[4]. About 60%[4] of SMA cases are SMA type I (infantile-onset SMA), and symptoms usually appear during the first six months of life[5]. Type II is approximately 30% of total SMA patients, exhibiting symptoms by 18 months of age. The symptoms appear after achieving the motor milestone of sitting[6]. Type III SMA is defined as when the typical symptoms start to develop after 18 months of age, and about 10% of cases fall under this category[7]. Type IV of SMS is the mildest kind, having signs that appear in the second or third decade of life [8].

An observational cross-sectional study was conducted at Kolkata's Bangur Institute of Neurosciences (BIN), a tertiary referral facility. From the regular outpatient department, we picked a few patients who appeared to have SMA and sent them to the BIN Neurogenetic clinic. This study comprised 40 cases with SMA with genetic proof. History and clinical examination were carried out and the findings were recorded. NCV, EMG, and MRI are carried out in all cases. Genetically proved SMA cases were included in the study.

2.0 Methodology

Each patient had five millilitres of venous blood drawn, which was then stored at -20°C in ethylenediaminetetraacetic acid (EDTA).The phenol-chloroform method was used to extract the DNA from these blood samples, which was subsequently frozen at -20°C.Using the polymerase chain reaction (PCR), two exons—exons 7 and 8—were investigated.
Forward 5'- CTA TCA ACT TAA TTT CTG ATC A -3' and reverse 5'- CCT TCC TTC TTT TTG ATT TTG TTT -3 are the primers for Exon 7. A final reaction volume of 25 µL was used for the PCR amplification, which included approximately 100 ng of genomic DNA, 1× PCR buffer, 2 mM MgCl2, 0.2 mMdNTPs, 10 pmol forward primer, 10 pmol reverse primer, 1 unit of Taq polymerase, and distilled water to adjust the volume. The PCR technique calls for 35 cycles of amplification (94°C for 2 minutes, 55°C for 1 minute, and 72°C for 1 minute) after an initial denaturation step of 3 minutes at 94°C. For ten minutes, the last extension was done at 72°C.
The forward and reverse primers for exon 8 are, respectively, CTA CAA CAC CCT TCT CAC AG -3' and GTA ATA ACC AAA TGC AAT GTG AA -3'. The final reaction volume of the PCR amplification consisted of 100 ng of genomic DNA, 1 PCR buffer, 2 mM MgCl2, 0.2 mMdNTPs, 10 pmol forward primer, 10 pmol reverse primer, 1 unit of Taq polymerase, and 25 L of distilled water. A three-minute initial denaturation stage at 94°C is required for the PCR process. Following this, there are 35 cycles of amplification that last one minute each at 94°C, sixty°C, and 72°C. The last extension happened at 72 °C for ten minutes. Then the PCR products were placed for restriction digestion. Restriction enzyme Dra1 was used for SMA Exon 7 products and restriction enzyme Dde1 for SMA Exon 8 products. The total volume of each restriction digestion reaction was 30µL containing 10µL of PCR product with 17 µL nuclease-free water,2µl of 10X buffer, and 10 units of respective restriction enzyme. The reaction mixtures for restriction digestion were incubated for one night at 37°C. The resulting digested products were compared to a 100 bp DNA ladder using a gel documentation system (BIO-RAD) and electrophoresed on a 4% agarose gel under ultraviolet (UV) light.

3.0 Results

The extent of deletions i.e., SMN1 gene exons 7 and 8 in 40 patients were studied along with the clinical history and symptoms. Five types I SMA patients were diagnosed out of which 4(75%) showed deletion of both exon 7 and 8 from the SMN1 gene. While 14(73%) of SMA II patients had deletions of bothand 7(43.5%) SMA III (mild phenotype) showed deletion of both exons.
Only one SMA I patient was diagnosed to have exon 7 deletion who was male, with the age of onset at 6months with no family history. This patient had weakness in all four limbs and without trunk and wasting of proximal limb but not EDB. Fasciculation, DTR in lower limbs, scoliosis, twitching of muscles, pseudohypertrophy, tremor and perinatal asphyxia need for ventilator support were absent. DTR in one upper

limb was 1 with hypotonia of lower limbs only. He had repeated respiratory tract infections with neither chest deformity nor abnormality of higher mental function. Denervation patterns were noted in all limbs without a trunk in EMG with low CMAP in lower limbs only.There was occasional dysphagia. MRI brain showed no gross abnormality.
Four patients of SMA I were found to have a deletion of exons 7 and 8 both and their age range was 3 months to 3 years with an age of onset of 3 to 6 months. Though there was an overlap in deletions seen and the severity and type of SMA, on the whole, it was observed that the majority of patients showing more deletions resulted in a severe phenotype. Consanguinity was present in three of them and 1(25%) had a positive family history.
One patient had weakness in all four limbs and the other 3 (75%) had weakness in all limbs and trunk. Generalized limb wasting was noted in 3 (75%) and 1 had only proximal limb wasting.EDB wasting was noted in none of them. Fasciculation in all limbs and trunk and tongue was noted in 2 (50%) and 1(25%) patients respectively and absent in 1 patient. DTR in all limbs. Generalized hypotonia and scoliosis were present in 3 patients. One patient had hypotonia of the upper limb only. Repeated chest infection was present in all and chest deformity was found in all 3(25%) of them. They showed a denervation pattern of all limbs and trunks and all four had low CMAP amplitude in NCS. Twitching and tremor were not found in them and higher mental function abnormality was found in one patient. History, of perinatal asphyxia was found in 3 (75%) and less fetal movement was reported by the mother of 2 (50%) patients. There was no need for ventilator support in any patient. Only one patient showed abnormality in MRI Brain.

Of the 19 SMA II patients, 5 (27%) had a loss of exon 7 alone; the remaining patients had deletions of both exons 7 and 8. Patients who had both exon 7 and exon 8 deleted ranged in age from 1.5 to 11 years, with 4 (28.6%) females and 10 (71.4%) males. Their age of onset was 6 months to 18 months and 12 of them had no family history. Weakness of lower limbs, all four limbs, and limbs with trunks was found in 1(7.1%), 5(14.3%), and 8(57.1%) respectively. Distal, generalized, and proximal wasting were noted in 1(7.1%), 9(64.3%), and 4(28.6%) patients respectively. EDB wasting was present in 11(78.6%) of them. Fasciculation in the tongue, limbs, and tongue and all limbs only was present in 4(29%), 6(42%), and 4(29%) patients respectively. DTR in lower limbs is 0 in most of them{0-10(71.4%)}, {1-4(29%}. Upper limb DTR was 0 in most of them{0 in 9(65%)},1 in 5(35%)}. Generalized hypotonia was present in {upper limb only-1(7.1%), lower limbs only-5(32.9%), generalized-8(60%)}. Scoliosis {Present-8(57%), absent-6(43%)}, repeated chest infection {Present-3(21.4%) absent-11(78.6%)}. Denervation pattern in EMG was found involving the trunk and all limbs{Lower limbs with trunk-1(8%), all limbs with trunk-10(71%), Upper limbs and lower limbs-3(21%)}. Low CMAP was found in all limbs in most patients {Upper limbs only-2(14.3%), all limbs-12(85.7%)}. None had higher mental function abnormality. The decreased fetal movement was reported in 3(21.4%) and the absence of simultaneous dysphagia and dysarthria in {Dysphagia-3(21.4%), Both-1(7.1%), Absent-10(71.4%) patients. None required any ventilator support and the MRI brain was normal in all.

Exon 7 deletions were seen in five (27%) of the 19 SMA II patients, whose ages varied from 3 to 18. Three (60%) were males and consanguinity was absent in 4(80%) patients. One patient had a positive family history. Three of the total(60%) had weakness of both limbs and trunk and the rest had weakness of all four limbs without significant truncal weakness. Limb wasting was generalized in 3 (60%)of them, the rest had only proximal limb wasting.EDB wasting, fasciculation in all limbs only, and fasciculation in all limbs along with the tongue were found in 3(60%) and 2(40%) patients respectively. DTR was 0 in 3 patients (60%) in both upper and lower limbs. Hypotonia was present in all{Upper limbs only-1(20%), Lower limbs only-2(40%), Generalized-2(40%)}. Scoliosis, repeated chest infection, and chest deformity were absent in 4(80%), 4(80%), and 3(60%) patients respectively. Denervation pattern in EMG in all limbs and trunk were found in 3(60%) and others have both upper and lower limbs whereas low CMAP in nerve conduction studies were found in all limbs in 4(80%) patients and 1(20%) patient in lower limbs only. Tremors and higher mental function abnormality were found in 2(40%) and 1(20%) patients respectively. No abnormality was found in the MRI of the brain in any of them.

Out of the total 16 SMA III patients, 7 (43.5%)had a deletion of exon 7 only and their age ranges from (5-35) years. 6 of them were males and consanguinity was present in 2(28.5%) of them. Their age of onset varied from (2.5-12) years. Weakness in all four limbs was present in 1(14.4%) and only in the upper limbs in 5(71.4%). EDB wasting and generalized limb wasting were found in 4(57.1%) and 2(28.6%) patients respectively whereas proximal wasting was found in 3 (42.8%) patients. The rest of the patients had weakness in the lower limbs(42.8%).EDB wasting was absent in all except 1(14.4%) patient. Fasciculation was present

in most of them. Upper limbs only-3(42.5%), Lower limb only-2(42.5%), Absent-1(15%)} along with weak DTR in both upper and lower limbs. Generalized hypotonia (28.6%) and scoliosis (28.6%)were present in 2 patients. Repeated respiratory tract infections were present in 1 patient(14.3%) but chest deformity was absent in all. 3 patients (42.9%) showed a denervation pattern in all limbs and trunks and 3 patients (42.9%) showed denervation of lower limbs and upper limbs only. Low CMAP were found in upper limbs and lower limbs in all 7 patients and tremor were present in 4(57.1%) of them. Higher mental function was normal in all except 1(14.3%) patient.

Ages ranging from 19 to 26 years old, two SMA III patients had exon 8 deleted, without any consanguinity and age of onset (12-13) years. There was no family history of a similar type of illness. One had weakness of all four limbs and 1 had weakness of lower limbs only although both had proximal limb wasting and EDB wasting. Fasciculation was present in the lower limb only in 1 patient and another one showed no fasciculation.DTR was 0 in the lower limb in one patient and 1 in another. For upper limbs, one had 0 DTR in the upper limb and 2 in another. Scoliosis was present in one of them but none had chest deformity/repeated respiratory tract infection. Denervation pattern was found in the lower limb only in one patient(50%) and lower limb with trunk in other patients (50%) whereas low CMAP in nerve conduction studies was found in the lower limbs of 1 patient and all limbs in another patient.
Seven (43.5%)of the 16 SMA III patients aged 3.5 to 28 years with age of onset 2 to 10 years had a deletion of both exon 7 and 8. Weakness of limbs and trunk were found in different combinations {Lower limbs only-1(14.4%), All four limbs-3(42.8%), limbs with trunks-3(42.8%)} along with limb wasting{Proximal-5(71.4%), Generalized-2(28.6%)} and EDB wasting {Present-2(28.5%), absent-5(71.5%)}. Fasciculation was present in all limbs in 1 patient(15%), lower limbs only in 3(42.5%), and upper limbs only in 1(42.5%) patient. DTR was 1 or less in most patients. Hypotonia in lower limbs in 3(42.8%) and generalized in 3(28.6%) patients. Scoliosis was absent in 6(85.7%) and neither repeated chest infection nor chest deformity was present in any one of them. Denervation pattern in EMG in lower limbs only in 2 patients(28.6%) and in all limbs and trunk in 5(71.4%) although low CMAP was found in all limbs in all 7 patients.Tremor and twitching were present in 6 patients(85.7%) and higher mental function abnormality was absent in all except 1(14.3%). MRI brain showed nonspecific signal changes in one patient.

4.0 Discussion

Our study found that deletion of both exon 7 and exon 8 in each type of SMA was associated with a more severe phenotype in almost all clinical parameters. Although there is a case report of SMA with the deletion of exon 8 only with milder clinical features, whether it is comparable to the deletion of exon 7 only is not known definitively. According to a research by Dastur et al[9], 27% of type I SMA patients and 46% of type II SMA patients had exon 7 and 8 deletions without NAIP losses. Along with 27% of SMA type II patients, two patients with type III SMA also had a single loss of SMN exon 7. Liang Y U et al[10] discovered that 32 SMA I patients and 76% (13/17) of SMA II patients had homozygous deletions for exons 7 and 8, and that all 13 SMA III patients and 24% (4/17) of SMA II patients had single deletions of SMN1 exon 7. In 2012, Ibrahim et al[11] reported that exons 7 and 8 both are deleted in 2 of the 37 SMA type1 patients and 4 of the 15 SMA type 3 patients. 35 individuals with type I SMA, 18 with type II SMA, 6 with type III SMA, and I with type IV SMA were included in another study conducted by Mrad et al[12]. Similarly, homozygous deletions of SMN1 exon 7 or exon 8, or both, were found in 43/57 families (75%) with the distribution of type I 11/14, type II 7/10, type III 24/31, and type IV 1/2. This information was gathered from a study conducted by Sifi Y et al[13]. Out of the 43 families that carried deletions, 36 of them carried deletions affecting both exons 7 and 8, four carried deletions affecting just exon 7, and three patients showed homozygous deletions limited to SMN1 exon 8. In our study, we have selected 40 patients with genetically proven SMA. We have tried to correlate their clinical parameters with the deletion pattern of exons 7 and 8 of the SMN1 gene. Our research revealed that a more severe phenotype in nearly all clinical parameters was linked to the loss of both exons 7 and 8 in each type of SMA. Although there is a case report of SMA with the deletion of exon 8 only with milder clinical features, whether it is comparable to the deletion of exon 7 only is not known definitively. Further study in the future will reveal more information about SMA which may be helpful in its therapy. Even though there was some overlap between the deletions found and the kind and severity of SMA, it was generally observed that most patients who had both deletions had a severe phenotype.

The limitation of our study is due to infrastructural constraints SMN 2 copy numbers could not be detected.

5.0 Conclusion

There was some overlap between the types and severity of SMA and the deletions observed, even though it was frequently found that most patients who displayed both losses had a severe phenotype.

6.0 Acknowledgements

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7.0 Conflicts of interest

No conflicts of interest exist.

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