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Ibrahim, S., Moatter, T., Saleem, A. F. (2012). Spinal muscular atrophy: clinical spectrum and genetic mutations in Pakistani children. *Neurology India*, 60(3), 294-298.

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Article in *Neurology India* · May 2012

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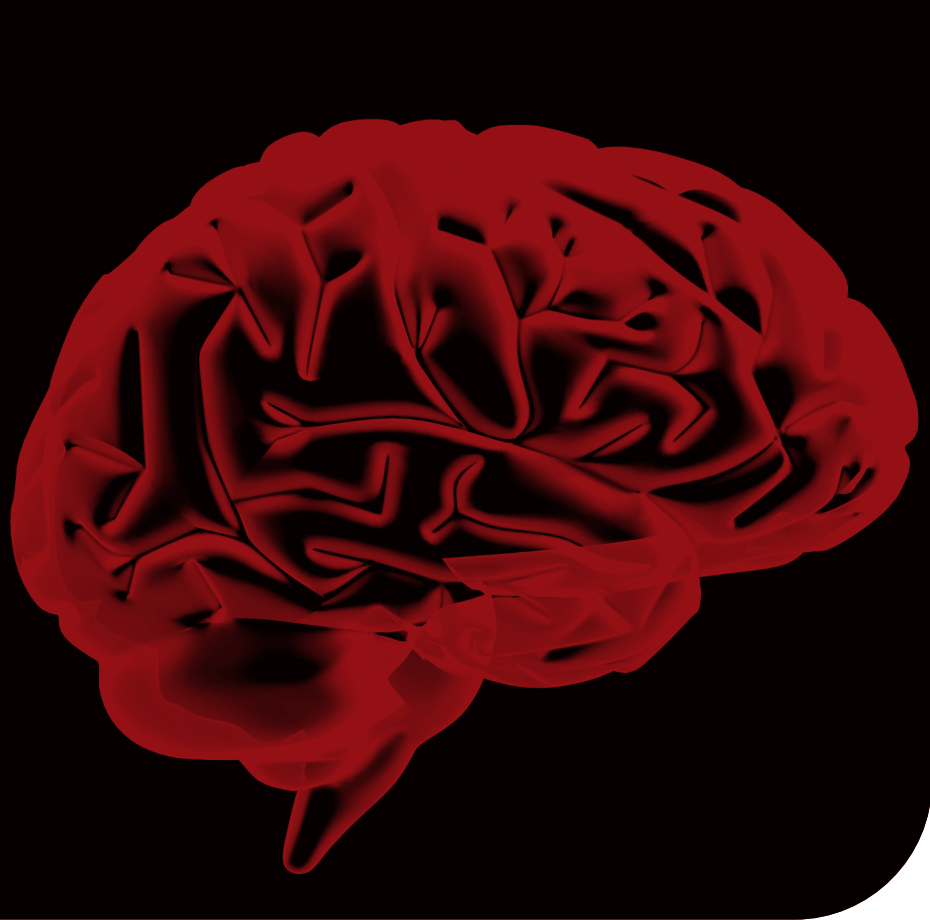


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ISSN 0028-3886

Impact Factor® for 2010:
0.834

May-June 2012 / Vol 60 / Issue 3



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Spinal muscular atrophy: Clinical spectrum and genetic mutations in Pakistani children

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Abstract

Background: In Pakistan the rate of consanguineous marriages is high, thus, the chance of incidence of autosomal recessive disorders is likely to be high. The aim of this study is to investigate the clinical characteristics and genetics of spinal muscular atrophy (SMA) in children who presented to Aga Khan University, Karachi. **Materials and Methods:** This study was a retrospective review of the medical charts of children (neonate: 15 years) with discharge diagnosis of SMA during last 10 years. Demographic features, consanguinity, and diagnostic analysis (including genetic analysis) were noted. **Results:** During the study period 67 children had a discharge diagnosis of SMA. Werdnig Hoffman disease (SMA type I) was the commonest variant seen in 37 (56%) children. Overall 68% were infants. High parental consanguinity was observed in 68% of the study cohort. The history of delayed development and undiagnosed early death was observed in the families of 19 children. Genetic testing was performed in 22 (33%) children. Survival motor neuron (SMN) 1 gene deletion was found in 19 (86%) of the 22 patients in whom the gene analysis was done and 13 (68%) were also positive for neuronal apoptosis inhibitory proteins (NAIP) deletion. **Conclusion:** SMA is not an uncommon neurodegenerative disorder in Pakistan and SMA type I was the most common type. SMN1 gene deletion was the most common genetic deletion found in this study. In addition, family history of developmental delay and frequent early deaths highlights the need for implementation of prenatal diagnosis for early detection, effective control, and management of this disorder in Pakistan.

Key words: Consanguinity, NAIP, SMN 1, spinal muscular atrophy

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Received : 03-04-2012


Review completed : 17-05-2012

Accepted : 10-06-2012

Introduction

Spinal muscular atrophy (SMA) is a neurodegenerative disease characterized by progressive degeneration

of alpha motor neurons, leading to muscle atrophy, paralysis, and even death.^[1] Mostly it has a recessive inheritance; however, autosomal dominant and X-linked inheritance has been documented.^[2] The reported incidence is about 1 in 10,000 live births with a carrier frequency of 1 in 50.^[3] The gene for SMA, survival motor neuron (SMN1) has been mapped on the 5q11.2–13.3 region and there are two copies of SMN gene: SMN1 and SMN2.^[4] Most common mutation found in SMA patients is homozygous deletion of SMN1 exon 7; however, many compound heterozygous subjects with different deletions and point mutation have been documented.^[5] All patients with SMA lack a functioning SMN1 gene

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and are thus dependent on their SMN2 gene for SMN protein, which is nonfunctional and rapidly degraded, leading to selective degeneration of spinal and cranial motor neurons.^[6]

Phenotypically SMA has extreme variability and clinically classified:^[7] SMA type I (severe variant, Werdnig-Hoffman) with age of onset before 6 months, may not sit, and usually die before their second birthday; SMA type II (intermediate variant) with age of onset between 7–18 months, may sit but never stand and death usually after 2 years of age; SMA type III (mild variant, Kugelberg-Welander) with age of onset ~18 months of age and death is in adult life; and SMA type IV (adult variants) presents in the second or third decade, individuals walk during the adulthood and death occurs in adult life.^[3] There other form is SMA type 0 or embryonic form (lethal variant), characterized by reduced fetal movements between 30–36 weeks of pregnancy with a very short life expectancy.^[8] The clinical characteristics of the disorder are: hypotonia, symmetrical proximal weakness, atrophy, and reduced to absent deep tendon reflexes.^[4] Genetic diagnosis by using polymerase chain reaction (PCR)-based DNA methods have been developed to detect deletions in both SMN and Neuronal apoptosis inhibitory (NAIP) gene.^[2,9] SMA is an autosomal recessive and consanguineous marriage increases the risk of the disease in the family. High prevalence of SMA has been reported in Iran, Egypt, and Saudi Arabia where consanguineous marriage rates are high.^[10-13] Pakistan has one of the highest reported consanguineous marriage rate in the world, approximately 61% marriages.^[14] With this high-rate consanguineous marriage, we expect a high burden of SMA in Pakistan. This report describes the demographic features, clinical characteristics, and genetic diagnosis of children with SMA in the Pakistani population.

Materials and Methods

Identification of study population

This was a retrospective chart review of children (neonate, 15 years) seen in the last 10 years (January 2001 to December 2010) and children with a diagnosis of SMA were evaluated. The sources of subjects included in this study were (i) inpatients discharge records with a diagnosis of SMA, ICD code (International Classification of Disease, 2008 (ICD9) 335.1 and 335.1 and (ii) data from the electrodiagnostic laboratory and genetic laboratory (SMA gene analysis). This study was approved by the Institutional ethical review committee; Aga Khan University, Karachi.

Case definition

Diagnosis of SMA was considered in any child with generalized hypotonia, diffuse symmetrical proximal

muscle weakness and atrophy, tremor of fingers and hands, tongue fasciculation along with absent or greatly decreased deep tendon reflexes^[4] and electromyographic (EMG) evidence of denervation, preganglionic anterior horn cells.^[3] Molecular genetic testing was aimed at detecting bi-allelic deletions or mutations in the survival motor neuron 1 (SMN1) gene on chromosome 5q13 (exon 7 and exon 8) and/or neuronal apoptosis inhibitory protein (NAIP) gene. Muscle biopsy was not done in any of the patients.

Gene analysis

Genomic deoxyribonucleic acid (DNA) was isolated from blood samples using column based DNA extraction kit obtained from Promega (USA). Deletion of exon 7 and 8 of SMN 1 gene was examined by polymerase chain reaction (PCR) amplification and restriction endonuclease digestion according to the method of Steege *et al.*^[15] PCR amplification was carried out for 35 cycles in PCR mixture containing 100 ng DNA, 75 ng of each primer, 200 μ M of dNTPs, 1.5 MgCl₂, 2.5U Taq polymerase in a final volume of 50 μ L. PCR products of exon 7 were digested with Dra I, and exon 8 with DdeI. The digestion pattern of the PCR product was evaluated according to the protocol described by Steege *et al.*^[15] The deletion of exon 5 of NAIP was identified by using the method described by Roy *et al.*^[16] Along with exon 5; exon 13 of NAIP was also amplified and used a control to monitor the performance of PCR assay.

Statistical analysis

The retrieved data were analyzed on SPSS version 19. Demographic features included age in days, weight, gender, presenting complaint at admission or health seeking, year of admission, consanguinity, perinatal depression, developmental delay or any other early death/s within the family were retrieved. Electromyography and nerve conduction study (EMG/NCV) and genetic analysis data were also retrieved and reported. SMN and NAIP gene mutations were reported among positive patients. Because of descriptive nature of the data only frequency and percentages were calculated.

Results

After chart review during the study period 67 children who fulfilled the inclusion criteria. Of the 67 patients 42 (63%) were infants and 7 were neonates. There was male predominance, 38 (57%) and male to female ratio was 1.2:1. Children were further divided according to the SMA types 1 to 3 [Table 1]. Developmental delay was the main presenting feature (45%) followed by, generalized weakness, hypotonia and respiratory difficulty. SMA type I accounted for 56% ($n=37$) of the cohort. Mean age at presentation in SMA-type 1 was 3.3 ± 1.7 months (median; 3 months). Overall

Table 1: Baseline features and genetic identification of children with spinal muscular atrophy

| Variables [^] | SMA type 1* (n = 37) | SMA type 2* (n = 15) | SMA type 3*(n = 15) |
|--------------------------------------|-------------------------|-------------------------|---------------------|
| Mean age of presentation (± SD) | 3.3 ± 1.7 mo | 15 ± 4 mo | 70 ± 2 mo |
| Male | 23 (62%) | 6 (40%) | 9 (60%) |
| Main presenting complaint | | | |
| Respiratory difficulty | 10 | 1 | -- |
| Developmental delay | 14 | 7 | 9 |
| Failure to thrive | -- | 1 | -- |
| Weakness / Hypotonia | 7 | 4 | 5 |
| Walking difficulty | -- | -- | 1 |
| Fever/ Lethargy/ Sepsis/ Poor intake | 6 | 1 | -- |
| Weight <5 th centile | 10 (33%) | 3 (40%) | 5 (36%) |
| Consanguinity in parents | 21 (66%) | 9 (82%) | 6 (60%) |
| Perinatal depression | 12 (35%) | 6 (54%) | 2 (15%) |
| Positive family history | | | |
| Diagnosed SMA/ | 12 (55%) | 4 (50%) | 3 (40%) |
| Developmental delay | 14 (47%) | 3 (27%) | 2 (18%) |
| Early deaths | | | |
| Genetic analysis of SMA patients | | | |
| Exon 7 and 8 deleted in SMN 1 gene | 2 | -- | 4 |
| Both NAIP and SMN 1 gene deleted | 10 | 3 | -- |
| Negative | 1 | 1 | 1 |

[^]Adapted from reference(6) *Because of missing values the data, the denominator was changed for each subcategory. Spinal muscular atrophy (SMA); survival motor neuron (SMN); neuronal apoptosis inhibitory (NAIP).

18 (31%) children were severely malnourished (weight for age; <5th percentile). The history of parental consanguinity was present in 53 (73%) children and them 36 (68%) had consanguineous marriages [Figure 1]. Perinatal depression was positive among 20 (34%) on the 58 children where such history was documented; while family history of developmental delay and/or spinal muscular atrophy was found in 19 (45%) of 42 children where such history was documented. The history of early deaths in the family was present in 19 (37%) of the 52 children with the data available. EMG/nerve conduction studies were performed in 62 patients, showing positive sharp waves, fibrillation, and/or fasciculation in all, and were consistent with the diagnosis of SMA.

Gene analysis was done in 22 children; among them 19 were positive (86%), genetic testing results were negative in 3 (13%) children. All positive patients showed a deletion of exon 7 and 8 in SMN1 gene. Exon 5 deletion of NAIP gene was also found positive in 13 (68%) of the 19 patients [Table 1]. Out of 20 hospitalized patients 5 (25%) children died. All died because of respiratory failure and were diagnosed as SMA type 1. The mean age at death was 3.9±1.2 months.

Discussion

Werdnig-Hoffman disease (SMA-1) is the most severe clinical type of SMA and prenatal onset of SMA has also been defined.^[10,17] It is associated with high morbidity and mortality. In our study majority of patients (61%) were infants at the time of presentation similar to the observations in other studies.^[10,17,18] Males had a slightly higher preponderance than females in our study and this also has been reported in the literature.^[10,17] Consanguinity was very high in our study. The reported consanguinity in the earlier studies ranged between 38.9% and 49%.^[10,17,19] High consanguinity would reflect the autosomal recessive inheritance of this disease. Table 2 compares the consanguinity rates among SMA children from different countries. Early or previous deaths in family and history of developmental delay may be the additional features indicating a genetic background of this disease in our study cohort.

More than 98% of patients with spinal muscular atrophy have a homozygous disruption of SMN1 by deletion, rearrangement, or mutation.^[3] The clinical severity is influenced by the number of copies of SMN2 gene. 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.^[20] Because of this, the children belonging to these groups have less severe form of disease.^[21] The most common deletion found in our cohort was of SMN1. Exon 7 and 8 in SMN1 gene was deleted in majority of positive children. Similar was the observation in the Egyptian cohort, in this study the frequency of homozygous deletion of exon 7 and 8 were present in 54.5–80% of patients irrespective of their clinical severity.^[22] In our cohort NAIP was the most common deletion (68%) among children tested for genetic testing; 10 (77%) of them were SMA type 1. The frequency of NAIP deletion was also found to be more frequent in SMA type I (80%) as compared to type II (22%) and type III (50%) in the Egyptian cohort.^[22] However, the incidence was much higher (100%) in the studies from Kuwait and Saudi Arabia.^[10,13] Interestingly all the three children with SMA II in our study had gene deletion for both NAIP and SMN1 gene. The reported frequency of NAIP deletion is relatively lower for both SMA II and III.^[17,23] Very few children with SMA II and SMA III had genetic testing and therefore we are unable to draw any significant conclusion from these results. Larger samples are needed to further explain the genetic basis of SMA type II and III patients in our population. Genetic testing was negative in three children. The 5q-SMA genetic testing is 95% sensitive and 100% specific.^[23] Fiver percent (1/20) of affected individuals with 5q-SMA have rare point mutation that are not detected by the SMN genetic testing. Most of these children have one SMN1 gene with SMN1 exon 7

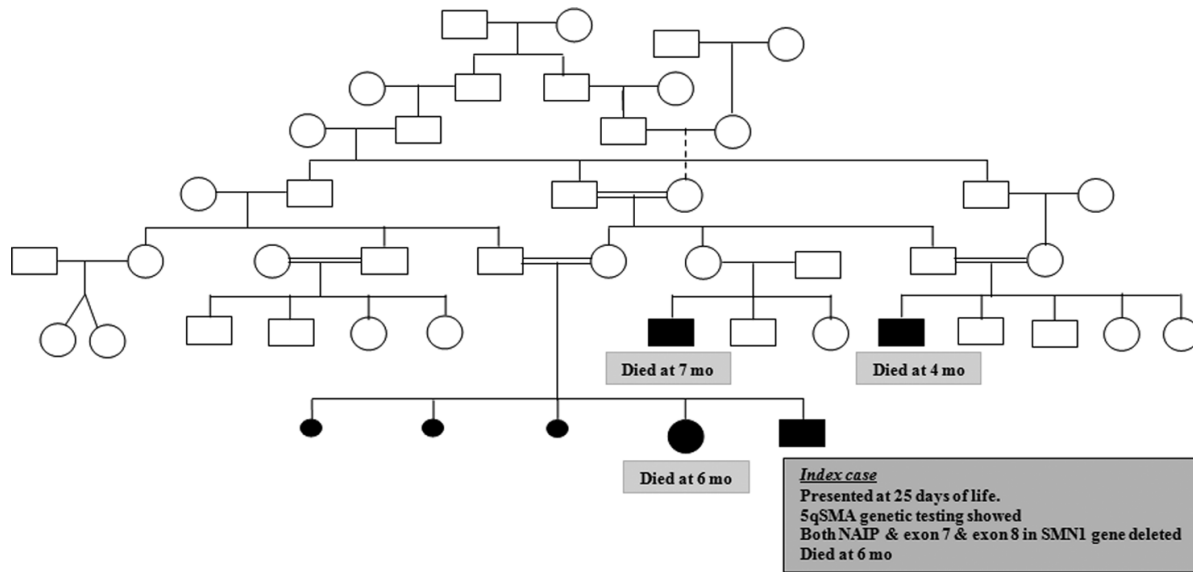


Figure 1: Family tree of a patient with SMA I

Table 2: Comparison of reported proportion of parental consanguinity in children with SMA

| Author name and study year | Reported proportion of consanguinity | Country |
|---------------------------------------|--------------------------------------|--------------|
| Shawky (2011) ^[10] | 46% | Egypt |
| Koul (2007) ^[17] | 49% | Oman |
| Salahshourifar (2007) ^[12] | 65% | Iran |
| Al Rajeh (1998) ^[13] | 81% | Saudi Arabia |
| Ibrahim et al. (our study) | 68% | Pakistan |

missing and a second SMN1 gene with a rare point mutation.^[23]

Currently there is no available cure for SMA; one possible intervention could be to reduce the consanguinity by educating the population. This will eventually interrupt the transmission by reducing carrier state. With the availability of genetic testing it is now possible to diagnose these children early so that appropriate counseling can be given to the family on the risk of future pregnancies. Early identification by genetic testing has also opened the way for early antenatal diagnosis.

Limitations

Genetic testing for SMA in our university was started from 2005; hence, data on genetic testing were only present for patients after this period. Some families denied genetic testing due to various reasons. With these limitations this is the first study from Pakistan that reports clinical spectrum and genetic testing of SMA patients.

Conclusion

SMA-1 is the commonest SMA type in our study population. Deletion of exon 7 and exon 8 in SMN1 gene is the commonest deletion in our SMA study cohort; however, an exon 5 deletion in NAIP gene is also very high. This study highlights the need for implementation of prenatal diagnosis in high risk consanguineous populations like Pakistan to reduce the burden of the disease.

Acknowledgments

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How to cite this article: Ibrahim S, Moatter T, Saleem AF. Spinal muscular atrophy: Clinical spectrum and genetic mutations in Pakistani children. *Neurol India* 2012;60:294-8.

Source of Support: Nil, **Conflict of Interest:** None declared.

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