



ORIGINAL ARTICLE

Patterns of inheritance and diagnostic features of spinal muscular atrophy, in areas with high rates of consanguineous marriages.

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Article Citation: Haider SMA, Bashir T, Qurat-ul-Ain, Ali W, Siddiq S, Malik SF. Patterns of inheritance and diagnostic features of spinal muscular atrophy, in areas with high rates of consanguineous marriages. Professional Med J 2022; 29(12):1857-1861.
<https://doi.org/10.29309/TPMJ/2022.29.12.7081>

ABSTRACT... Objective: To explore the diagnostic features and inheritance patterns of spinal muscular atrophy (SMA) in children at three tertiary care public hospitals. **Study Design:** Retrospective study. **Setting:** HITEC Institute of Medical Sciences Taxila Cantt. **Period:** January 2022 to March 2022. **Material & Methods:** A retrospective review of the medical records of the past ten years from January 2011 to December 2020 of three tertiary care public hospitals was conducted. Socio-demographic characteristics, consanguinity, and diagnostic features (including genetic investigation) were also considered. **Results:** A total of 70 children were diagnosed with SMA during the research study period and the most prevalent variation found among them was Werdnig Hoffmanver illness (SMA type I), affecting 40-57 percent of the children. The study found that 67 percent of the research group showed a high level of paternal consanguinity. The deletion of a gene, survival motor neuron (SMN1) was discovered in 21 (88%) genetically tested children out of 24, while 17 (71%) genetically studied patients were found to be positive for the deletion of specific apoptotic proteins called neuronal apoptosis inhibitory proteins (NAIP). **Conclusion:** In our study, SMA type 1 is the most prevalent. This piece of literature emphasizes the importance of antenatal detection and the need to increase awareness among high-risk societies with prevalent consanguineous marriages like Pakistan to lessen the disease load.

Key words: Consanguineous Marriages, Consanguinity, Neuronal Apoptosis Inhibitory Proteins, SMN 1, SMA.

INTRODUCTION

SMA is a neurodegenerative disease characterized by the progressive degeneration of alpha motor neurons, resulting in muscle atrophy, paralysis, and even death.¹ The pattern of inheritance is mostly recessive, however autosomal dominant and X-linked inheritance have been observed.² The reported incidence is approximately one in per 10,000 live births, with a carrier frequency of one in every fifty (50).³ Survival motor neuron (SMN1) gene, the found culprit has been localized to the region 5q11.2–13.3, and the SMN gene exists in two forms: SMN1 and SMN2.⁴ In SMA patients, the most prevalent mutation is an SMN1 gene (exon 7) homozygous deletion; nevertheless, other categories of deletion and point mutation has also been described in various compound heterozygous population.⁵ Because each SMA

patients show a deficiency in functional gene SMN1, for protein SMN production they must rely on their gene SMN2, which is non-functional and briskly destroyed, resulting in particular destruction of cranial and spinal motor neurons.⁶

Spinal muscular atrophy has utmost phenotypic irregularities and is medically categorized as follows:⁷ Type I SMA (extreme form, Werdnig–Hoffman) with onset before age of 6 months, and generally, patients may not sit but are not able to celebrate their second birthday; type II SMA (intermediate form) with onset between the age of 7–18 months, they might sit but can never stand, and usually die after the age of 2 years; type III SMA (mild form, Kugelberg–Welander)³ The other variety is type 0 or embryonic SMA (lethal form), which is distinguished through the decrease in

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Article received on: 16/04/2022
Accepted for publication: 07/09/2022

the fetal movements of during 30–36 weeks of gestation and very brief life span.⁸ This disease's clinical signs and symptoms include atrophy, decreased muscle tone, proximal symmetrical weakness, and diminished to non-existing deep tendon reflexes.⁴ Multiple approaches have been developed like genetic studies using polymerase chain reaction (PCR)-based DNA for finding out deletions in the responsible genes of this disorder which are SMN and Neuronal apoptosis inhibitory (NAIP) genes.^{2,9}

SMA is an autosomal recessive condition with increased risk in patients with a history of consanguineous marriage. Increased incidences of SMA have been reported in states like Egypt, Saudi Arabia, and Iran, all of which have high rates of consanguineous marriage.¹⁰⁻¹³ Pakistan is considered among the top-ranking countries with the highest recorded rates of consanguineous marriages in the world, having roughly sixty-one (61) percent of marriages being consanguineous.¹⁴ We anticipate a large burden of SMA in Pakistan because of the high proportion of consanguineous marriages. In this report, demographic parameters, clinical features, and genetic analysis of children among the Pakistani population with SMA are described.

MATERIAL & METHODS

This was a retrospective analysis focusing on neonates up to fifteen years old adolescents with SMA over the last ten years (January 2011 to December 2020). Subjects for this study were obtained from inpatient medical records showing SMA as a diagnosis.

Case Definition

Any child with generalized hypotonia, diffuse symmetrical proximal muscle weakness and atrophy, tremor of fingers and hands, tongue fasciculation, absent or greatly reduced deep tendon reflexes⁴, and electromyographic (EMG) evidence of denervation, preganglionic anterior horn cells, was considered to have SMA.³ The main goal was to point out the bi-allelic deletions or mutations in the gene survival motor neuron 1 (SMN1) on chromosome 5q13 (exons 7 and 8), as well as in the gene neuronal apoptosis inhibitory

protein (NAIP) using molecular genetic studies. In any of the patients, the biopsy of muscles was not done.

By the use of the Promega column-based DNA extraction kit (USA), the genetic deoxyribonucleic acid (DNA) was recovered from samples of blood taken from patients. Exons 7 and 8 of the SMN 1 gene having deletions were investigated through the polymerase chain reaction (PCR) amplification and restriction endonuclease digestion, as described by Steege et al.¹⁵ The PCR product's pattern of digestion was examined using the methodology published by Steege et al.¹⁵ Using the method outlined by Roy et al., the deletion in gene NAIP exon 5 was discovered¹⁶ NAIP gene, exon 13 was amplified also, in addition to exon 5 and for monitoring the performance of PCR assay, a control was used.

Statistical Analysis

SPSS version 27 was used to analyze the collected data. Demographic information such as age (in days), gender, weight (in kgs), presenting complaints, admission year, consanguinity, prenatal depression, growth retardation, or any previous family history of premature deaths was obtained. Electromyography (EMG) and nerve conduction studies (NCV) data as well as genomic analyses were also obtained and described. Positive patients were found to have SMN and NAIP gene mutations. Because the data was descriptive, the calculation of only frequencies and percentages was done.

RESULTS

Based on chart review, 70 children met the inclusion criteria during the study period. 47 (67%) were infants out of the 70 patients, and neonates were only seven. The sample under observation showed a male majority of 40 (57%) and a 1.2:1 ratio of male to female. Children were then categorized under SMA types 1–3 (Table-I). The most common presenting feature was growth hindrance (45%), accompanied by widespread weakness, respiratory complications, and reduced tone. 56% accounted for SMA type I of the cohort (n=39). In SMA-type 1, the average age at presentation was 3.3+₋1.7 months (median;

3 months). In total, 21 (31%) of the children were seriously malnourished (weight for age; fifth percentile). There was a history of parental consanguinity in 51 (73%) of the offspring, and 35 (68%) of them gave a history of family marriages. In 19 (45%) out of the 42 children, decelerated developmental and/or spinal muscular atrophy was identified. 62 patients had undergone EMG/nerve conduction investigations, with all demonstrating positive sharp waves, fibrillation, and/or fasciculation, which agreed with the SMA diagnosis. Genetic investigations were performed on 25 children, out of which 22 got positive (86%) and three (13%) children had negative testing results surprisingly in all positive cases, the exons 7 and 8 of the SMN1 gene were found to be deleted. Exon 5 deletion of the NAIP gene was also discovered to be positive in 17(68%) of the 25 cases (Table-I). 25% of hospitalized children died. All of them died because of respiratory failure and were diagnosed with SMA type 1. The average death age was 3.9+ _1.2 months.

| Features | Type 1 N=39 | Type 2 n=16 | Type 3 n=15 |
|--|----------------|----------------|----------------|
| Mean Age + - s.d | 3.2+-1.8mo | 16+- 5mo | 71+- 3mo |
| Male | 24 | 7 | 10 |
| Difficulty in breathing | 11 | 2 | - |
| Growth deceleration | 16 | 9 | 9 |
| Early deaths | - | 3 | - |
| Decrease muscle tone | 8 | 5 | 6 |
| Bodyweight <5 th centile | 11 | 4 | 5 |
| Consanguin- eous parents | 23 | 10 | 6 |
| Diagnosis of SMA | 14 | 5 | 4 |
| Deletion of Exon 7 & 8 in SMN 1 | 4 | - | 5 |
| Deletion of both NAIP & SMN 1 gene | 12 | 4 | - |

Table-I. Features of children with spinal muscular atrophy

DISCUSSION

Werdnig–Hoffman disease (SMA-1) is the most extreme clinical form of SMA, and prenatal SMA onset has also been identified.^{10,17} It has a significant morbidity and fatality rate. In our analysis, at the time of presentation of patients, the majority (61%) were children, which is consistent with previous findings.^{10,17,18} In our study, males outnumbered females by a tiny margin, following the reported literature.^{10,17} In our study, consanguinity was extremely common. Preceding research shows the reported consanguinity ranged between 38.9 and 49 percent.^{10,17,19} The disease's autosomal recessive inheritance would be reflected in the high consanguinity. Family history with preliminary or foregoing fatalities and delay in development might be an additional marker in our study group indicating a hereditary basis for this condition. Homozygous SMN1 gene disruption because of mutation, deletion or rearrangement was present in more than 98 percent of patients with SMA³ while the clinical severity is highly under influence of the number of copies of the SMN2 gene. Data shows that about 95 percent of type I SMA individuals possess just SMN2 gene one-two copy, but practically all type III SMA patients possess three or more copies.²⁰ As a result, the children in these groups had a milder form of sickness.²¹

In our group, the most prevalent loss was SMN1. In most positive children, exons 7 and 8 of the SMN1 gene were deleted. Similarly, to the Egyptian cohort, in this investigation, the frequency of exons 7 and 8 homozygous deletions was found in 54.5–80 percent of individuals, regardless of clinical severity.²² NAIP was considered the most prevalent deletion (68%) among children screened for genomic studies in our sample; 10 (77%) of which had type 1 SMA. In the Egyptian cohort, the NAIP deletion frequency was likewise shown to be higher in type I SMA (80%) compared to type II SMA (22%), and type III SMA (50%).²² However, the incidence was substantially greater (100%) in the statistical analysis from middle eastern countries (Saudia Arabia and Kuwait)^{10,13} Surprisingly, in our investigation, all subjects with SMA II showed gene deletions for both the NAIP and SMN1 genes. NAIP deletion is found to occur

at a lesser frequency in SMA type II and type III.^{17,23} As a handful of children with SMA disorder type II and type III got tested genetically, we are unable to deduce any meaningful conclusions from these findings, so large sample sizes are required for a better understanding of the genetic underpinnings of SMA type II and III patients among our population. Three of the children tested negative for genetics. The genetic testing for 5q-SMA is 95% sensitive and 100% specific.²³ Five percent (1/20) of people with 5q-SMA have an uncommon point mutation that is not found by SMN genetic testing. Most of these youngsters have one SMN1 gene with a missing SMN1 exon 7 and a second SMN1 gene with an uncommon point mutation.²³ There is currently no cure for SMA; one viable intervention could be to educate the people to prevent consanguinity by arranging awareness campaigns on governmental levels. This will eventually cause the communication to be interrupted by lowering the carrier state. With the availability of genomic investigations, it has become possible to detect these children promptly to provide proper counseling to the affected families with the future risk of pregnancies, and these new genetic approaches have also paved the way for prenatal diagnosis.

SMA genetic analysis in Pakistan began in 2005 at Aga Khan University in Karachi; thus, statistics on genetic testing for patients were only available succeeding this time. Some families rejected these genetic studies for a variety of reasons. With these caveats, this is the second Pakistani study presenting clinical scope and genetic analysis of SMA patients.

CONCLUSION

In our study, SMA type 1 is the most prevalent. This piece of literature emphasizes the importance of antenatal detection and the need to increase awareness among high-risk societies with prevalent consanguineous marriages like Pakistan to lessen the disease load.

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AUTHORSHIP AND CONTRIBUTION DECLARATION

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| 2 | Tehniat Bashir | Manuscript writing. |  |
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