European Review for Medical and Pharmacological Sciences 2023; 27: 7935-7945

Deletions of *SMNI* gene exon 7 and *NAIP* gene exon 5 in spinal muscular atrophy patients in selected population

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Abstract. – OBJECTIVE: Spinal muscular atrophy (SMA) is common among various populations because the genetic makeup is monogamous due to consanguineous marriages. Two genes, i.e., survival motor neuron *(SMN1)* and neuronal apoptosis inhibitory protein *(NAIP)* are mapped to the SMA vicinity of chromosome 5q13. The main objective of the study was to develop a solitary advanced genetic tool for the diagnosis of SMA by using SMN1 gene exon 7 and NAIP gene exon 5.

PATIENTS AND METHODS: This study involved SMA patients (n=84) belonging to different clinical features and socio-economic status. The identity of the intact *NAIP* gene is primarily based on the amplification of exon 5 only in those SMA patients that have a deletion of *SMN1* gene exon 7. Healthy controls (n=84) were also included in this study. The mutational analysis was observed through the Sanger sequencing method, where chromatograms were observed by using Chromas version 2.6.0.

RESULTS: This study showed a higher prevalence of SMA in females than in males. *NAIP* gene is considered a phenotype modifier as most SMA patients (94.90%) have *SMN1* exon 7 deletion along with a deletion in exon 5 of the *NAIP* gene. Single nucleotide conversion C-T in exon 7 of *SMN1* gene leads to its complete deletion. Mutated proteins encoded by *SMN1* and *NAIP* genes also result in degeneration and muscle weakness in SMA patients.

CONCLUSIONS: These SMA-associated gene deletions can be used as a molecular evaluation tool for pre- and postnatal diagnosis of SMA. This will be valuable when there is a need for precise and consistent results with a strong focus on quantification.

Key Words:
Spinal muscular atrophy, Autosomal, Neuronal apoptosis inhibitory protein, Survival motor neuron, Phenotypes.

Introduction

Spinal muscular atrophy (SMA) is a rare neurodegenerative disorder that causes generalized muscle weakness and motor neuron loss due to a disruption in the *SMN1* gene on 5q13[1,2]. The prevalence is 1-2 per 100,000 people, while the incidence is 1 in 10,000 people, according to the WHO[3]. Based on achieved neural function, SMA is classified into five phenotypes: type-0, type-1, type-2, type-3, and type-4[3,4]. SMA type 0 is a prenatal congenital condition characterized by severe weakness at birth, profound hypotonia, facial diplegia, areflexia, early respiratory failure, and joint contractures. SMA type 1 symptoms appear from birth to 6 months of age and include loss of head

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control, hypotonia (frog leg-like posture), weakness of intercostal muscles (breathing through the belly), tongue fasciculation (difficult swallowing), and congenital cardiac disorder (hypoplastic left heart syndrome). Patients with SMA type 2 can learn to sit but cannot walk. They have proximal weakness, hypotonia, areflexia, progressive scoliosis, joint contracture, ankylosis of the mandible, and hand tremor. SMA type 3 patients can normally walk, and most of them can live productive adult lives. It causes progressive proximal weakness, respiratory difficulties, and little to no scoliosis. SMA type 4 symptoms of the disorder's weakness appear before the age of 21. Adults often have the ability to walk without any respiratory or nutritional issues[5,6]. Molecular genetics has revealed[7] that the *SMN* gene, which occupies a 500-kilo-base region of chromosomes, is the primary cause of SMA. In the event of a disorder, the tel-SMN (*SMN1*) loses its function, whereas the *SMN* gene retains one copy of *SMN2*. The *SMN1* gene contains a total of 9 exons that aid in the progression of molecular machinery for the full-length production of *SMN* protein. A total of 95% of SMA patients are involved in *SMN1* gene homozygous deletion, and 90% of cases represented exon 7 deletion following a homozygous manner in tel-SMN (*SMN1*) because the most common mutation causing SMA is a homozygous deletion of the *SMN1* exon 7, which can be easily detected and used as a sensitive diagnostic test[7,8] as the severity of the disorder has been seen in skipping of whole exon 7. In spinal muscular atrophy (SMA), multiple *SMN1* and *SMN2* gene copies are common. Often, there are two *SMN1* and one *SMN2* copies. An *SMN1* DNA change (C-T) prompts similar changes in other *SMN2* copies, leading to shorter *SMN* proteins. Detecting exon 7 loss in 95% of cases lacking *SMN1* is the initial diagnostic step. Numerous DNA assays based on the c.840C>T difference between *SMN1* and *SMN2* allow for the detection of the absence of *SMN1* exon 7[9,10]. The *NAIP* gene spans 56 kb of chromosome 5's region as the genomic sequence with 6,100 bp transcript incorporates numerous discrete copies on chromosome 5 and 17 exons, and these copies are available in the form of reverted duplication, and this replicated area encircles a minimum of four genes as well as repetitive constituents which makes it vulnerable towards rearrangements and results in deletions as well as mutations. The sequence's repetitiveness and intricacy have also influenced the structuring of this controversial genomic area, but *NAIP* contains a complete gene copy, while other duplications with internal mutations and rearrangements can be identified in chromosome location[11]. Complete deletion of *NAIP* gene exon 5 in all types of cases of SMA can be used as a medical and genomic tool for diagnosing and verifying SMA, as it has a role in mediating neuronal survival in pathological conditions. Severe and critical types of SMA account for absolute deletion of *NAIP* exon 5 as these forms carrying homozygous deletions of *NAIP* exon 5 are more prevalent than moderate or chronic forms of SMA. *NAIP* deletions are recognized in 45.5% of type 1 SMA cases and 18.1% of types 2 and 3 SMA cases[12]. The main goal of the current study was to explore the mutation of *SMN1* gene exon 7 and *NAIP* exon 5, which are involved as a cause of SMA among Pakistani patients.

Patients and Methods

The project titled "Deletions of *SMNI* gene exon 7 and *NAIP* gene exon 5 in spinal muscular atrophy patients in selected population" has been evaluated by the Department of Research Ethics and Biosafety Committee. The Committee could not find any Biosafety and Ethical concerns related to the proposed work and its execution at the Institute of Microbiology and Molecular Genetics, New Campus, University of the Punjab, Lahore, Pakistan. Verbal and written consent has been taken from the SMA patients before filling out the proforma under the supervision of the physician. Blood of the SMA patients (n=84) from different cities of Pakistan, especially from Punjab, was drawn through sterilized syringes and stored in a vacutainer. A total of 3 ml of whole blood was drawn from every patient and control. The blood stored in the refrigerator at 4°C was further used for DNA extraction and amplification. There are high chances of having pneumonia in SMA patients, and it is required to check for the presence of *Legionella pneumophila*. Therefore, the ELISA test was performed in patients with SMA (n=84). Virion/Serion ELISA Kit was used for this purpose (Catalog #: ESR106G), and the results revealed the presence of pneumonia in SMA patients.

Analysis of SMN1 Exon 7 Deletion

The DNA extraction of 84 patients and 84 healthy controls was done following the reported[5] methods. Polymerase chain reaction (PCR) was done by applying forward and reverse primers in order to amplify the coding sequence of the

SMN1 gene. *SMN1* exon 7 coding sequence was targeted for amplification of PCR using primers (CCAGATAATTCCCCCACCACC) and (TGTCAGGAAAAGATGCTGAGTGAT). The PCR program consisted of denaturation, annealing, and elongation at 95°C for 3 minutes, annealing at 57-60°C for 1 minute, elongation at 72°C for 1 minute, and elongation at 72°C for 3 minutes. 4°C was chosen as the holding temperature for PCR vials, with a 30-cycle program for exon 7 of the *SMN1* gene. After gradient PCR, the bands of exon 7 were seen at an annealing temperature was 57.0°C in controls (n=84). After viewing bands on a 1.5% agarose gel with a UV trans-illuminator, the PCR product was stored at 4°C. For this program, a total of 40 cycles were used. The PCR product was visible on a 1.5% agarose gel at 55°C using a trans-illuminator. The mutational analysis has been observed through the Sanger sequencing method to confirm the deletion in patients' samples and to compare it with samples of controls where chromatograms were observed using Chromas version 2.6.0 (South Brisbane QLD 4101, Australia).

Analysis of NAIP Exon 5 Deletion

The primers for amplification of exon 5 of the *NAIP* gene were developed using 436 bp of the *NAIP* gene region. *NAIP* exon 5 coding sequence was targeted for amplification of PCR using primers (CTCTCAGCCTGCTCTTCAGAT) and (AAAGCCTCTGACGAGAGGATC). The *NAIP* gene's exon 5 spans a 100-bp area. To formulate the primers, a DNA segment spanning 436-bp around exon 5 was selected, which included some side region from exon 5 in it to avoid any false positive results. To amplify the entire area, including exon 5, specific primers were created. The goal of developing these primers was to see if there was a difference in the size of the amplified product in controls and patients' samples, as the product size of patient samples was estimated to be 336 bp and 436 bp for controls to visualize the clear difference of 100 bp. The forward and reverse primers each have 21 nucleotide bases and are connected to the side region of the *NAIP* gene's exon 5.

PCR was done by applying forward and reverse primers in order to amplify the coding sequence of the *NAIP* gene. The program of PCR followed a melting temperature (Tm) of 58°C and was used with the following conditions: denaturation at 94°C for 60 seconds, annealing at 58°C for 60 seconds, and extension at 72°C for 90 seconds. 4°C was considered a holding temperature for PCR vials and a 30-cycle program for exon 5 of the *NAIP* gene. After viewing bands on a 1.5% agarose gel with a UV trans-illuminator, the PCR product was stored at 4°C.

Results

Gender distribution involved females and males, which showed more prevalence in females than males. There were 46 (54.76%) SMA female patients and 38 (45.23%) SMA male patients (Figure 1A), and the types of SMA in this study involved SMA type-1, SMA type-2 and SMA type-3 which included 30 (35.71%) type 1, 38 (45.23%) type 2 and 16 (19.04%) type 3 (Figure 1B).

The prevalence of cousin marriage and siblings involved in SMA disease were 34 (40.47%) and 18 (21.42%), respectively. The impact of SMA on patients results in the severity of the disorder, cardiac issues, respiratory problems, swallowing problems, abnormal chest shape, and jerky movements. These issues were further depicted in the SMA patients of the study where 86.01% severity of disorder was observed, 6.67% swallowing issue and jerky movements were observed, 62.01% respiratory issues, 40.50% abnormal chest and 7.14% cardiac defect were observed in SMA patients (Figure 2A). The socioeconomic distribution involved four classes of society to which SMA patients belong. The lower class to the average class of society involved the majority of the SMA patients (Figure 2B).

Patients further opted for treatment to cure the disorder, including medical drug treatment, conventional treatment, and surgical treatment. There were 8 (9.52%) SMA patients involved in getting conventional treatment, while drug and surgical treatment was chosen by 21.40% and 7.14% of SMA patients (Figure 3A). The psychological impact of SMA revealed that the prevalence of psychological stress was 88.10%, social rejection was 95.24%, and loneliness was 90.50% among patients of SMA (Figure 3B).

In this study, it was observed that 24 (28.57%) out of 84 patients were positive for the presence of IgG antibody of *Legionella pneumophila* (Figure 4A). PCR products of *SMN1* gene exon 7 were shown on 1.5% agarose gel and clearly visible under trans-illuminator, which showed complete deletion of exon 7 (Figure 4B). This study showed complete deletion of *SMN1* gene exon 7 in SMA patients of Pakistan. The complete deletion of

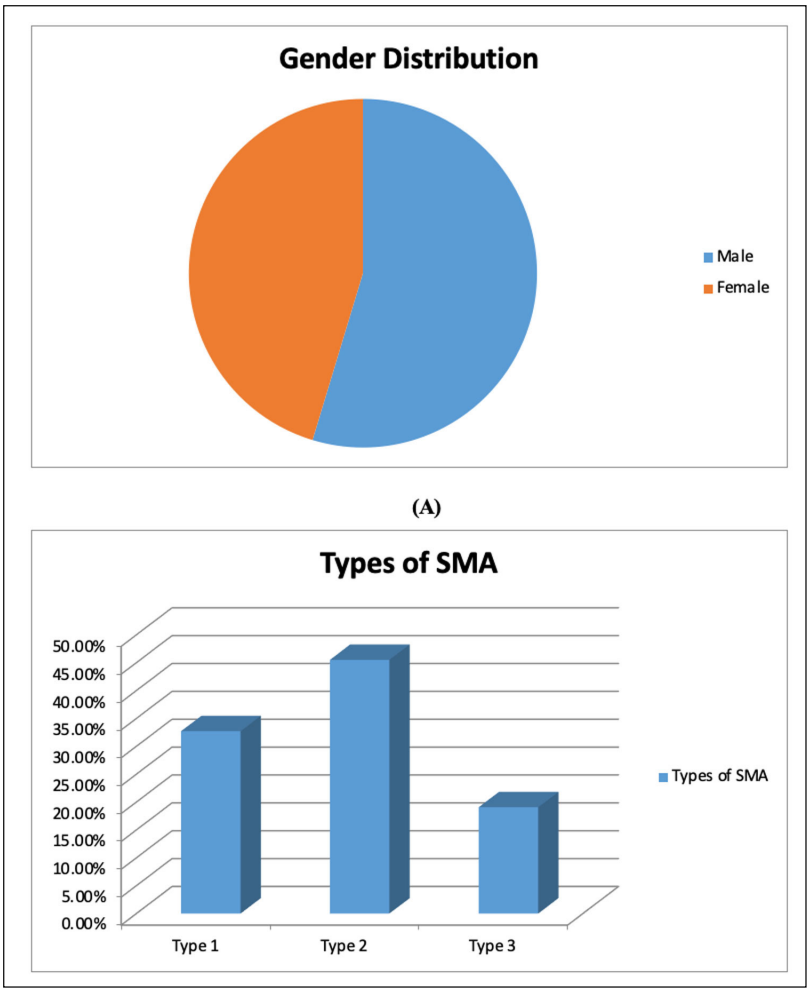

Figure 1. A, Gender distribution among SMA patients (n=84). **B**, Phenotypic prevalence of SMA patients (n=84).

exon 7 was concluded as a contributing factor of SMA in the Pakistani population. The exon 7 deletion contributed to the prevalence of SMA type-1, type-2, and type-3 in patients. All *NAIP*-deficient individuals lacked the *SMN* gene. In this present analysis, the overall frequency of *NAIP* exons 5 was 100%. Complete deletion of exon 5 was observed (Figure 5A). Mutation has been revealed by version 2.6.0 of the chromatogram in exon 5 of the *NAIP* gene where PCR product occupied 336 base pairs of length, showing the deletion of 100-bp of exon 5 length (Figure 6).

After Sanger sequencing of *NAIP* gene 5, the sequences were used to make a protein structure that shows abnormal protein structure. Using the Swiss Model tool, a mutated protein structure was made, which has differences compared to the normal structure of *NAIP* protein. The *NAIP* protein normally consists of Zinc Ion and Baculo-viral IAP repeats along with glycerol ligand. In the case of a mutated form of *NAIP*, these zinc ions and ligands are absent. The mutated structure is shown in Figure 7A, and a wild-type structure of *NAIP* protein with key features is added for comparison purposes in Figure 7B.

Discussion

SMA, an inherited autosomal recessive disorder which involves a genetic condition, has been prevailing in Pakistan[13]. The overall prevalence of the disorder is 1 in every 60,000 to 100,000 live births, while carrier frequency is much higher than the frequency of incidence, which is 1 in every 40 to 60 individuals. It has been seen[14,15] that the SMA is not gender specific. The only reason for SMA to be more likely in females is the deletion of the *NAIP* gene, which occupies 500 kb inverted repeats.

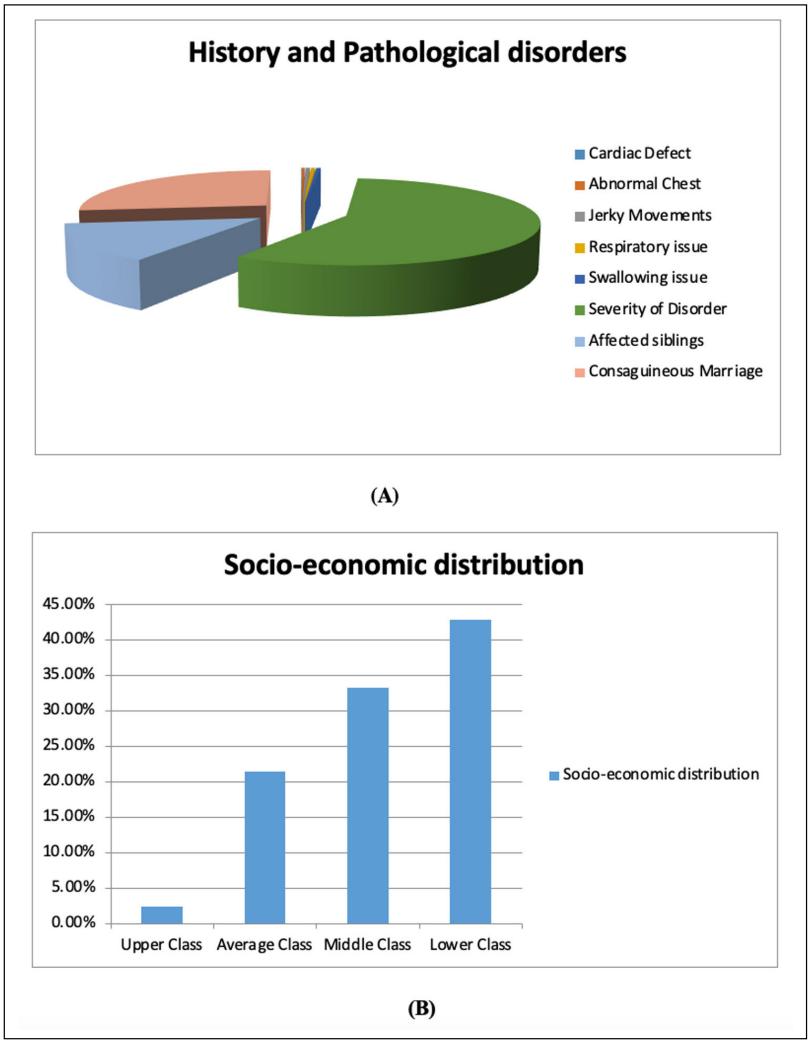

Figure 2. **A**, History and Pathological disorders among SMA patients (n=84). **B**, Socio-economic distribution among SMA patients (n=84).

This research included 84 patients diagnosed with spinal muscular atrophy (SMA), each representing a diverse range of clinical characteristics and socioeconomic backgrounds. The main focus was to identify the intact *NAIP* gene, primarily by amplifying exon 5 in SMA patients who had a deletion of exon 7 in the *SMN1* gene. As a comparison, the study also involved 84 healthy individuals as control subjects. The mutational analysis was conducted using the Sanger sequencing method, and the chromatograms were carefully examined using Chromas version 2.6.0.

This study showed a higher prevalence of SMA in females over males as female to male ratio is 23:19, which allows the *NAIP* gene to be deleted and also opened new ways towards the disorder versatility, which has a strong phenotypic impact on females. The disorder has been observed[16,17] in the UK-based Pakistani population, with the prime cause being consanguineous marriages. The *SMN1* gene exon 7 is highly important in order to understand the development of different phenotypic characters of SMA types. The deletion of *SMN1* gene exon 7 has been seen[18,19] in 96% of SMA type-1 patients, 94% in SMA type-2, and 82% in SMA type-3 patients. This study revealed the occurrence of three SMA types – type 1, type 2, and type 3 – in Pakistani patients, with frequencies of 35.71%, 45.23%, and 19.04%, respectively. However, SMA type 0 and type 4 were not detected, likely due to the uncommon nature of these disorders as subtypes (0, 4) which are indicated at the two extremes of clinical severity. Subtype 0 manifests with *in-utero* onset, restricted or absent movements, contractures, and infantile dependence on mechanical ventila-

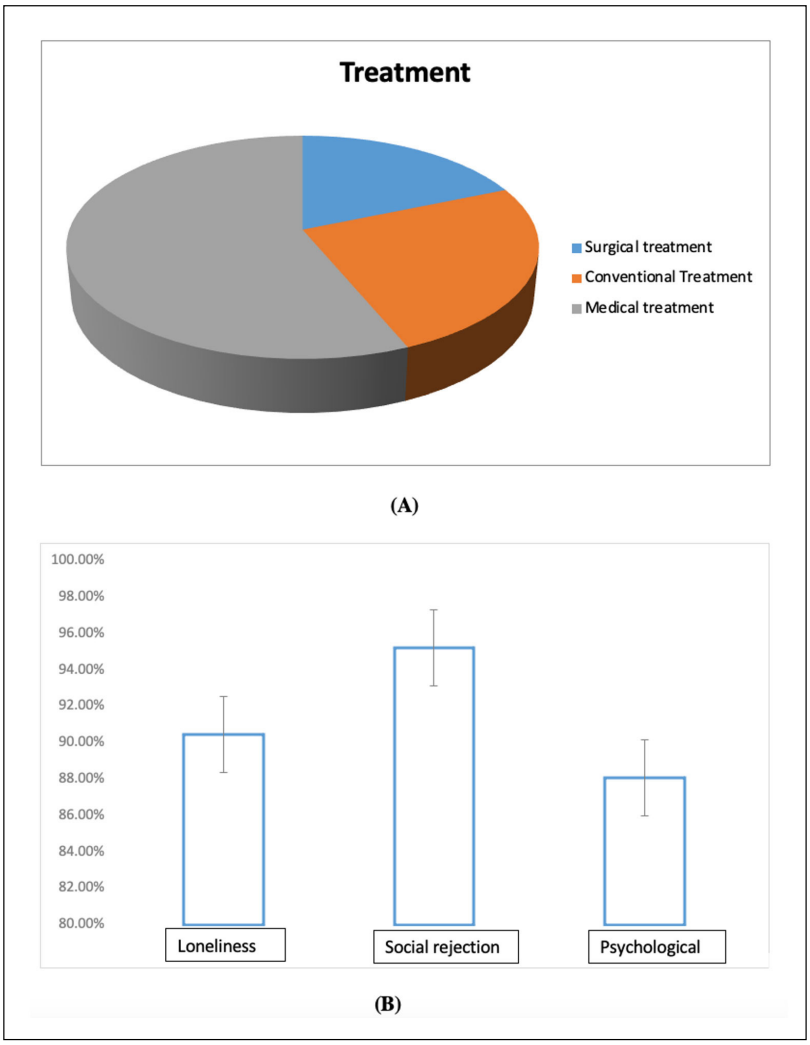

Figure 3. **A**, Treatment opted by SMA patients (n=84). **B**, Effects on mental health in SMA patients (n=84).

tion, while subtype 4 emerges in adulthood[20]. All *NAIP*-deficient individuals lacked the *SMN* gene. In this present analysis, the overall frequency of *NAIP* exons 5 was 100%. *NAIP* gene mutations were more familiar in patients of type-1 (87.1%) compared to type-2 (32.9%), consistent with prior studies[21] (types 2 and 3). The study revealed that cousin marriages and affected siblings contribute to a higher carrier frequency of the disease. In the case of SMA, mutation of *NAIP* exon 5 ends in the production of a mutant protein, lacking the Glycerol ligand and Zn^{2+} ion, which results in undesired inhibition with caspases, and ultimately leads to destabilizing the structural integrity and results in apoptotic process malfunction.

The symptoms of SMA are overlapped in SMA type-1, type-2, and type-3, which include respiratory problems, swallowing issues, abnormal chest shape, jerky movement, and cardiac defects[22,23]. Families of patients experience significant challenges due to their child's condition, with increased demands on time and added financial burden. This issue was commonly observed across the families in our study[24]. The patients of SMA often lose their opportunities in terms of their careers, and the hectic and painful schedule of medication and doctor appointments have a direct impact on their mental health[23,24]. Psychological stress has been observed[19] in SMA patients in the Pakistani population. Psychological distress, social isolation, lack of societal support, and rejection are prevalent in patients, with an occurrence of 88.10%, 97.24%, 90.50%, and 97.62%, respectively, contributing to complications in treating the disorder. In this study, *Leigonellia pneumophilia* was found to be an important factor

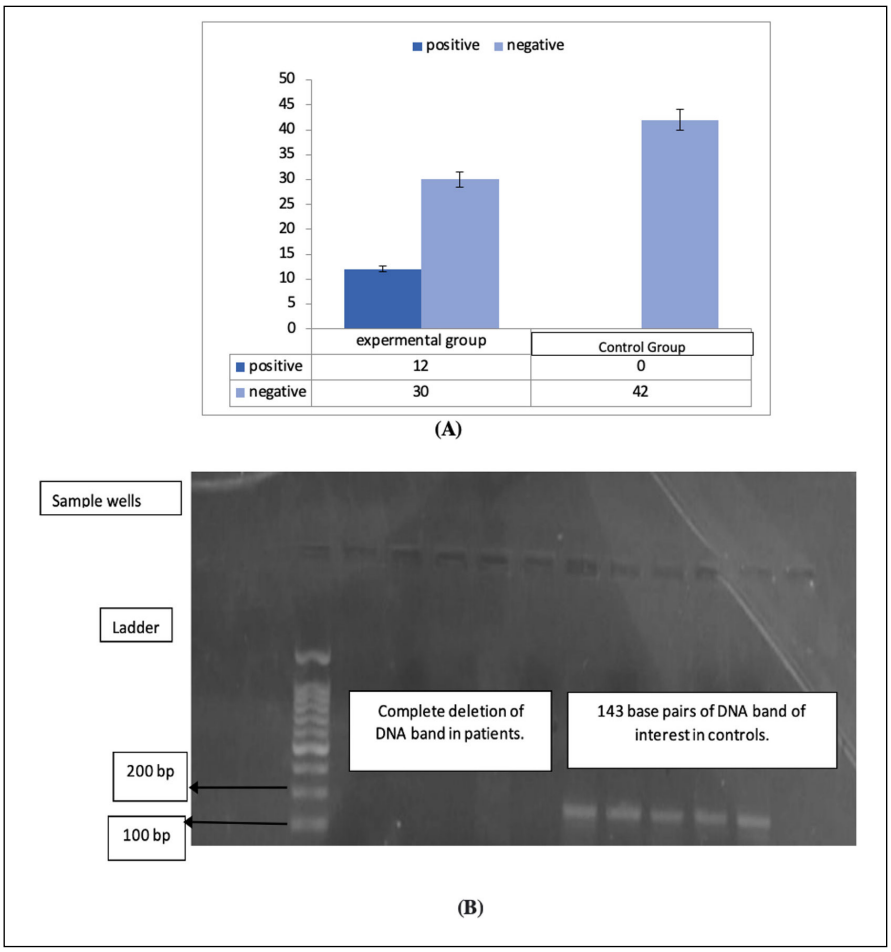

Figure 4. **A**, IgG antibody of *Legionella pneumophila* ELISA results' comparison among patients (n=84) and control group (n=84). **B**, Amplified DNA bands (143 bp) of *SMNI* gene exon 7 in controls and completely deleted in patients (n=84).

in causing pneumonia in SMA patients, another alarming bacterium in SMA patients.

This study found a total absence of *NAIP* gene's exon 5 in Pakistani SMA patients. Because exon 5 is the major coding region of the *NAIP* gene, a mutant form of *NAIP* protein is produced when the mutated *NAIP* gene is translated into *NAIP* protein which lacks glycerol ligand. The unstable protein interferes with the normal activity of the *NAIP* gene, which in turn interferes with the functioning of neighboring genes, as evidenced by the severity of the condition. Analyzing *NAIP* at the protein level in SMA tissues would help elucidate its role in SMA pathogenesis and, potentially, contribute to the development of conventional and genetic therapeutics for these severe diseases as it affects the functioning of nearby genes. Furthermore, the discovery of genes with homology to the *NAIP* locus as well as proteins that interact with *NAIP*, could aid in the continued understanding of apoptotic pathways in mammalian cells. The treatment for SMA is time taking and financially burdens the family, conventional or surgical. The therapies include hydrotherapy, which burdens financially and results in very little improvement[25]. In developing nations, medical drug therapy is frequently withheld due to the profound motor neuron enhancements caused by the medication. Commonly, drugs such as creatine, gabapentin, and hydroxyurea are utilized to alleviate movement-related discomfort[26]. The distribution of patients receiving medical drug treatment, conventional treatment, and surgical treatment was 21.42%, 9.52%, and 7.14%, respectively. The socioeconomic status of the majority of SMA patients in Pakistan is classified as lower class to average class, which clearly depicts the choice of treatment patients have opted for.

Among the patients, the most common observation was the deletion of exon 7 in the *SMNI* gene, where single nucleotide conversion C-T in exon 7 leads to complete deletion of exon[27]. The

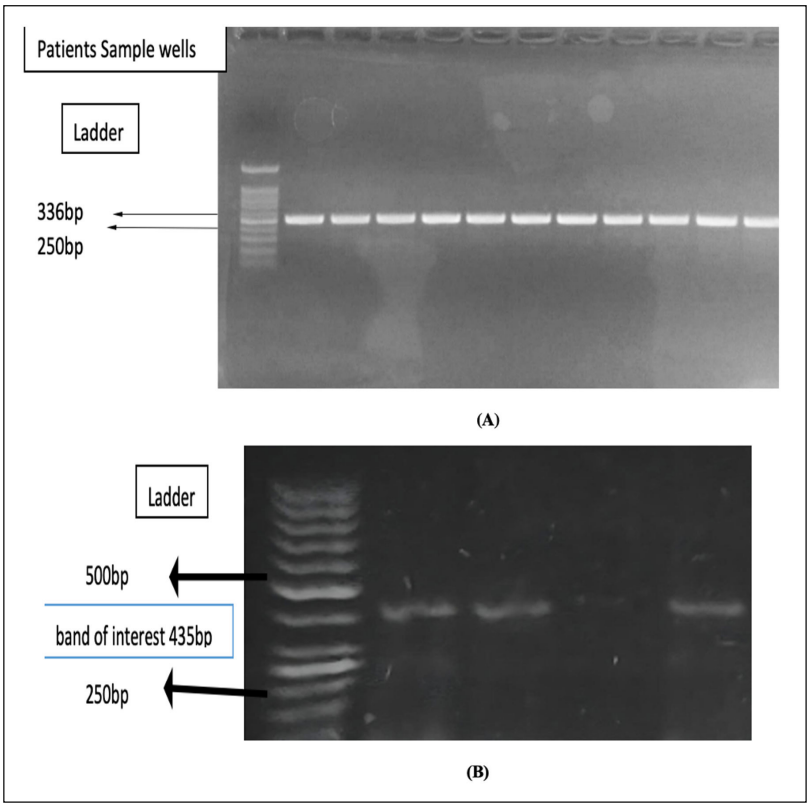

Figure 5. **A**, Patients sample DNA amplified bands of *NAIP* exon 5. **B**, Controls' sample DNA amplified bands of *NAIP* exon 5.

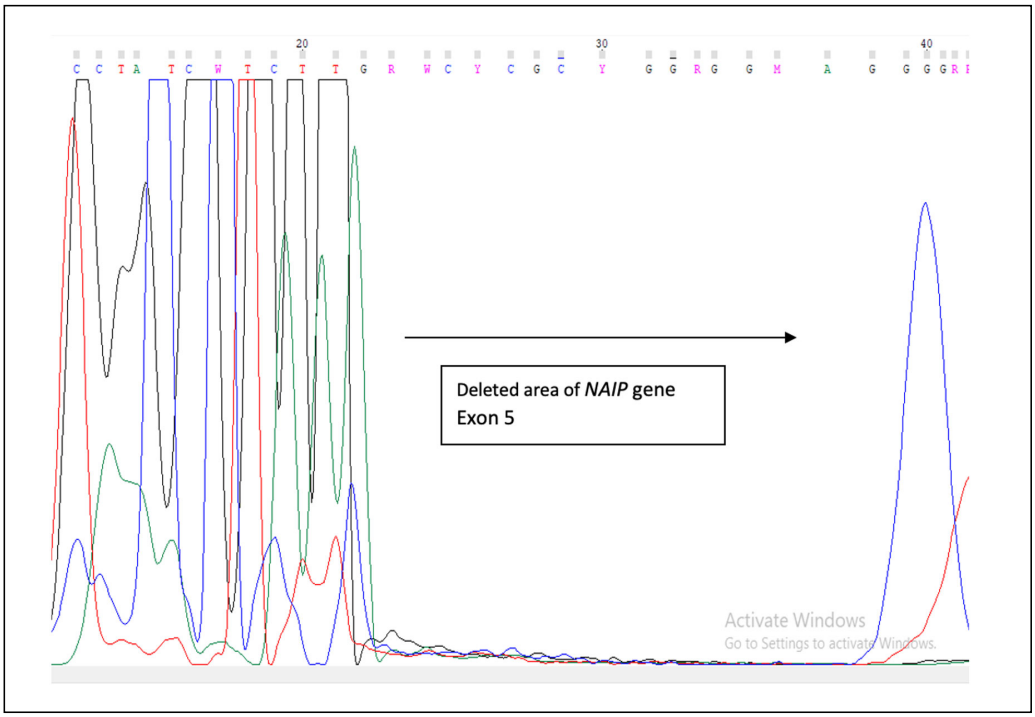

Figure 6. Chromatogram of *NAIP* exon 5 in SMA cases.

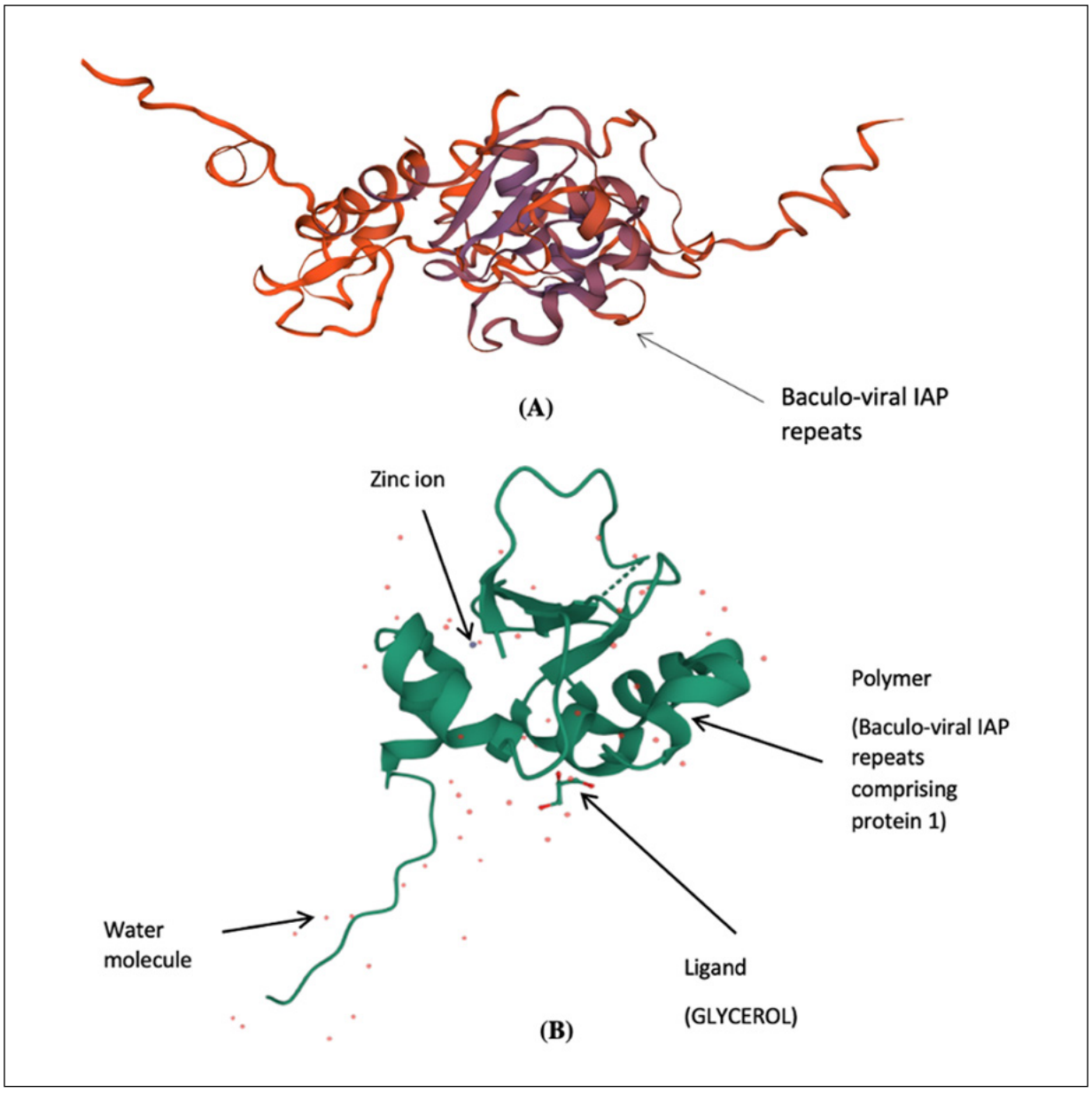

Figure 7. **A**, Mutated *NAIP* protein structure. **B**, Wild-type structure of *NAIP* protein.

prevalence of homozygous deletions in patients throughout the *NAIP* gene variates between SMA types 1, 2 and 3 (45% *vs.* 18%), which creates the hypothesis that the SMA acuteness and severity depend on the mutations and deletion of the coding parts of the *NAIP* and analyzing *SMN* and *NAIP* genes individually is challenging due to minimal distinctions between normal and mutated genes. There are multiple shortened and internally deleted variants that exist in a variety of copies. The amplification of exons 5, the primary coding exon, which is thought to be deleted in the distinct variants, is used to determine the identity of the intact *NAIP* gene[28]. The exon 5 of the *NAIP* gene is completely absent in patients with spinal muscular atrophy in Pakistan, according to this study.

Conclusions

Analyzing *NAIP* at the protein level in SMA tissues would help elucidate its role in SMA pathogenesis and potentially contribute to the development of conventional and genetic thera-

peutics for these severe diseases as it affects the functioning of nearby genes. Furthermore, the discovery of genes with homology to the *NAIP* locus as well as proteins that interact with *NAIP*, could aid in the continued understanding of apoptotic pathways in mammalian cells. There was a robust link between the telomere region of *NAIP* deprivation and the severity and acuteness of SMA, according to our findings. These findings show that mutations in the *NAIP* gene cause or contribute to the development of SMA phenotype by preventing a normally occurring suppression of motor neuron death. This study discovered that exon 7 of the *SMN1* gene is completely absent in Pakistani SMA patients, whereas exon 5 of the *NAIP* gene was also absent in such SMA patients.

Availability of Data and Materials
All the data generated in this research study has been included in the manuscript.

Authors' Contributions
Conceptualization: N. Hussain, and Bashir.; methodology, Iqbal.; software, T. Hussain.; validation, Alkhateeb.; formal analysis, A Shaer investigation, Al-Abbas resources, Alhomrani; data curation, S Alamri, and Aziz writing–original draft preparation, Alhomrani and S Alamri.; writing-review and editing, Shakoori and Labban; visualization, T. Hussain; supervision, Aziz.; project administration, Bashir; funding acquisition, Alkhateeb.

Ethics Approval
The Ethical approval for this study was approved by Departmental Research Ethics and Biosafety committee Institute of Microbiology and Molecular Genetics University of the Punjab dated 17th March 2022, no. D/356/mmg.

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Acknowledgements
Princess Nourah bint Abdulrahman University researchers Supporting Project number (PNURSP2023R294), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

Funding
The authors report specific funding in relation to this research from University of the Punjab. Princess Nourah bint Abdulrahman University researchers Supporting Project number (PNURSP2023R294), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

Conflict of Interest
The authors have declared no conflict of interest.

Informed Consent
Verbal and written consent has been taken from the SMA patients before filling out the proforma under the supervision of the physician.

References

1) Baccon J, Pellizzoni L, Rappsilber J, Mann M, Dreyfuss G. Identification and characterization of Gemin7, a novel component of the survival of motor neuron complex. J Biol Chem 2002; 277: 31957-31962.
2) Butchbach ME. Copy number variations in the survival motor neuron genes: implications for spinal muscular atrophy and other neurodegenerative diseases. Front Mol Biosci 2016; 3: 7.
3) Charroux B, Pellizzoni L, Perkinson RA, Shevchenko A, Mann M, Dreyfuss G. Gemin3: A novel DEAD box protein that interacts with SMN, the spinal muscular atrophy gene product, and is a component of gems. J Cell Biol 1999; 147: 1181-1194.
4) Charroux B, Pellizzoni L, Perkinson RA, Yong J, Shevchenko A, Mann M, Dreyfuss G. Gemin4: a novel component of the SMN complex that is found in both gems and nucleoli. J Cell Biol 2000; 148: 1177-1186.
5) Corry PC. Consanguinity and prevalence patterns of inherited disease in the UK Pakistani community. Human heredity 2014; 77: 207-216.
6) Cremers CH, Fischer MJ, Kruitwagen-van Reenen ET, Wadman RI, Vervoordeldonk JJ, Verhoef M, Visser-Meily JM, van der Pol WL, Schröder CD. Participation and mental well-being of mothers of home-living patients with spinal muscular atrophy. Neuromuscul. Disord 2019; 29: 321-329.
7) D'Amico A, Mercuri E, Tiziano FD, Bertini E. Spinal muscular atrophy. Orphanet J Rare Dis 2011; 6: 71.
8) Evans GA, Drennan JC, Russman BS. Functional classification and orthopaedic management of spinal muscular atrophy. J Bone Jt Surg 1981; 63: 516-522.
9) Gubitz AK, Feng W, Dreyfuss G. The SMN complex. Exp Cell Res 2004; 296: 51-56.
10) Gubitz AK, Mourelatos Z, Abel L, Rappsilber J, Mann M, Dreyfuss G. Gemin5, a novel WD re-

peat protein component of the SMN complex that binds Sm proteins. JBC 2002; 277: 5631-5636.
11) Kesari A, Misra UK, Kalita J, Mishra VN, Pradhan S, Patil SJ, Phadke SR, Mittal B. Study of survival of motor neuron (SMN) and neuronal apoptosis inhibitory protein (NAIP) gene deletions in SMA patients. J Neurol 2005; 252: 667-671.
12) Shin S, Park SS, Hwang YS, Lee KW, Chung SG, Lee YJ, Park MH. Deletion of SMN and NAIP genes in Korean patients with spinal muscular atrophy. JKMS 2000; 15: 93-98.
13) Hahnen E, Forkert R, Marke C, Rudnik-Schöneborn S, Schönling J, Zerres K, Creavin T, Wirth B. Molecular analysis of candidate genes on chromosome 5q13 in autosomal recessive spinal muscular atrophy: evidence of homozygous deletions of the SMN gene in unaffected individuals. Hum Mol Genet 1995; 4: 1927-1933.
14) Ibrahim SH, Moatter T, Saleem AF. Spinal muscular atrophy: Clinical spectrum and genetic mutations in Pakistani children. Neurology India 2012; 60: 294.
15) Jesmi AA, Jouybari L, Sanagoo A. The lived experiences of patients with spinal muscular atrophy: A phenomenological study. JNMS 2019; 6: 57.
16) Kocova H, Dvorackova O, Vondracek P, Haberlova J. Health-related quality of life in children and adolescents with spinal muscular atrophy in the Czech Republic. Pediatr Neurol 2014; 50: 591-594.
17) Kolb SJ, Battle DJ, Dreyfuss G. Molecular functions of the SMN complex. J Child Neurol 2007; 22: 990-994.
18) Liu Q, Fischer U, Wang F, Dreyfuss G. The spinal muscular atrophy disease gene product, SMN, and its associated protein SIP1 are in a complex with spliceosomal snRNP proteins. Cell 1997; 90: 1013-1021.
19) Lorson CL, Hahnen E, Androphy EJ, Wirth B. A single nucleotide in the SMN gene regulates splicing and is responsible for spinal muscular atrophy. PNAS USA 1999; 96: 6307-6311.
20) Finkel R, Bertini E, Muntoni F, Mercuri E, ENMC SMA Workshop Study Group. 209th ENMC international workshop: outcome measures and clinical trial readiness in spinal muscular atrophy 7–9 November 2014, Heemskerk, The Netherlands. Neuromuscular Disorders 2015; 25: 593-602.
21) Markowitz JA, Singh P, Darras BT. Spinal muscular atrophy: a clinical and research update. Pediatr Neurol 2012; 46: 1-2.
22) Paushkin S, Gubitz AK, Massenet S, Dreyfuss G. The SMN complex, an assemblyosome of ribonucleoproteins. Curr Opin Cell Biol 2002; 14: 305-312.
23) Pellizzoni L, Baccon J, Rappsilber J, Mann M, Dreyfuss G. Purification of native survival of motor neurons complexes and identification of Gemin6 as a novel component. JBC 2002; 277: 7540-7545.
24) Rudnik-Schöneborn S, Röhrig D, Morgan G, Wirth B, Zerres K. Autosomal recessive proximal spinal muscular atrophy in 101 sibs out of 48 families: clinical picture, influence of gender, and genetic implications. Am J Med Genet 1994; 51: 70-76.
25) Stark C, Duran I, Cirak S, Hamacher S, Hoyer-Kuhn HK, Semler O, Schoenau E. Vibration-assisted home training program for children with spinal muscular atrophy. CNO 2018; 5: 2329048X18780477.
26) Vaidla E, Talvik I, Kulla A, Sibul H, Maasalu K, Metsvaht T, Piirsoo A, Talvik T. Neonatal spinal muscular atrophy type 1 with bone fractures and heart defect. J Child Neurol 2007; 22: 67-70.
27) Verhaart IE, Robertson A, Wilson IJ, Aartsma-Rus A, Cameron S, Jones CC, Cook SF, Lochmüller H. Prevalence, incidence, and carrier frequency of 5q–linked spinal muscular atrophy–a literature review. Orphanet J Rare Dis 2017; 12: 1-5.
28) Samilchuk E, D'Souza B, Bastaki L, al-Awadi S. Deletion analysis of the SMN and NAIP genes in Kuwaiti patients with spinal muscular atrophy. Hum Genet 1996; 98: 524-527.