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A Review on Spinal Muscular Atrophy: Its Cause, Diagnosis and Treatment

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Abstract:

The spinal muscular atrophy is considered as one of the familiar autosomal recessive disorder. Children suffering from spinal muscular atrophy suffer with diminished ability of normal growing processes like crawling, sitting, walking and controlling head movements. Even if SMA becomes severe, it may lead to damage of muscles helpful in breathing and swallowing which life is threatening. It was found that SMA occurs due to improper production of SMN (the survival motor neuron protein). The mutations on SMN1 and SMN2 gene are responsible for the improper production of SMN protein. The deletion of SMN1 gene is regarded as the primary level diagnostic test. Other conformation tests are electromyogram and Multiplex ligation-dependant probe amplification (MLPA) test. Treatment of SMA was done initially by using drugs like Quinozoline derivatives, aminoglycosides and neuroprotective drugs. However, these drugs increased the life span of SMA patients but could not offer relief from the disease. Hence, gene therapy came in to focus and found useful in the treatment of SMA. Currently used 3 forms of gene therapy are Nusinersen (Spinraza), Onaswmnogene abeparvovec – xioi (Zolgensma) and Risdiplam (Evrysdi). Stem cell therapy is used which involves the transplantation of stem cells.

Keywords: Evrysdi, Multiplex ligation-dependant probe amplication, Nusinersen, Risdiplam, Spinal muscular atrophy, Survival motor neuron protein.

1. Introduction:

Spinal muscular atrophy is the disorder that affects neurons and muscles which occurs due to alpha motor neurons degeneration of spinal cord that finally leads to muscle weakness and paralysis[1]. Children suffering from spinal muscular atrophy suffer with diminished ability of normal growing processes like crawling, sitting, walking and controlling head movements[2]. Even if SMA becomes severe, it may lead to damage of muscles helpful in breathing and swallowing[3].

1.1 Types of SMA:

1.1.1 SMA type 0: It is the rare and severe type of SMA which results in joint deformities and weak muscle tone of babies[4]. Due to weak muscle tone, the affected foetus cannot move freely in the womb itself[5]. The respiratory muscle tone is also very week in the affected infants[6]. These patients also seem to have congenital heart defects[7].

1.1.2 SMA type I: It is the most commonly occurring SMA and it is also called as Werdnig-Hoffmann disease. This type of SMA can be identified at birth time or first few months of their life. The affected children have swallowing problems which leads to feeding difficulties further leading to improper growth. The affected child also faces the problem in controlling their head movements and they cannot sit unassisted[8]. They also have bell shaped chest which does not allow their lungs to expand properly which leads to respiratory failure[9]. SMA type I is classified in to 3 groups based on the severity of clinical manifestations. They are i) severe weakness and head control is not possible, ii) weakness occurs after birth within 2 months and head control is not possible iii) severe weakness is seen but head control is possible[10].

1.1. **3 SMA type II:** It is also referred as Dubowitz disease. This disease develops in children at an age group of 6 to 12 months. At first, children affected with that disease can sit without assistance. At later stages in childhood, the patients require support to sit; these children cannot stand or walk without support. These children have curves in spinal cord which is termed as scoliosis, tremors and respiratory muscle weakness which is life-threatening[11]. On an average, the lifespan of individuals suffering with this condition is 20-30years.

1.1.4 SMA type III: It is also referred as Kugelberg-Welander disease[12]. In this condition, the patients can stand and walk without assistance, but during later stages of life, the patient needs wheel chair assistance[13]. These patients are having normal life expectancy as that of healthy individuals[14].

1.1.5 SMA type IV: it is a rare condition where the patient suffers from mild muscle weakness[15], tremors and mild respiratory problems[16]. These patients are having normal life expectancy as that of healthy individuals[17].

Table 1: Types of SMA occurring due to mutations of SMN gene

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

S.No	Type of SMA	Other name	Age of onset	Clinical manifestations	Life expectancy
1	SMA type 0	-	Before birth	Joint deformities Weak muscle tone, respiratory muscle weakness	Do not survive past infancy
2	SMA Type I	Werdning-Hoffmann disease	Zero to six months	swallowing problems, feeding difficulties, improper growth, bell shaped chest, profound hypotonia, symmetrical flaccid paralysis	Weak respiratory muscle tone leads to death of patient
3	SMA type II	Dubowitz	7-18 months	scoliosis, tremors, respiratory muscle weakness	20-30years
4	SMA type III	Kugelberg-Welander	Above 18 months	Unable to stand, walk during later stages of life	Normal life expectancy
5	SMA type IV	-	Early adulthood	mild muscle weakness, tremors, mild respiratory problems	Normal life expectancy

1.2Epidemiology:

The spinal muscular atrophy is considered as one of the familiar autosomal recessive disorder[18], which occurs in atleast one in 10,000live births[19].

1.3 Etiology:

The mutations that occur in SMN gene leads to disorder called spinal muscular atrophy. These SMN genes are usually located in nucleus and cytoplasm of motor neurons present mainly in the spinal cord[20]. SMN protein is present in association with cajal bodies and appears as a dot like structure in nucleus[21]. SMN gene is located on 5q13 chromosome[22]. Even though the complete role regarding survival motor neuron protein in the focalisation of spinal muscular atrophy is still unclearly, the patients suffering with SMA are found to contain lesser number of gems compared to healthy people. There are two types of SMN gene; SMN1 gene and SMN2 gene.

SMN1 gene: SMN1 gene mutations are the causative factors of almost all types of SMA. The SMN1 gene is completely functional and does not degrade rapidly. These genes provide necessary inputs for the production of SMN protein. Mostly SMN1 gene alone produces the functional SMN protein. Usually, two copies of SMN1 gene are present in every cell of human being[23].

SMN2 gene: The mutations that occur on the SMN2 gene leads to gradual development of disorder to its severe stage. The SMN2 gene is not completely functional and it degrades very rapidly. It shows the transition in the coding exon7 that results in alternative splicing and skipping of exon7 which makes the SMN2 gene unable to produce adequate amount of transcripts in full length. It results in the production of unstable protein. Decreased production of stable SMN protein causes the degeneration of motor neurons which leads to muscle weakness, limb paralysis, respiratory failure and eventually to death. As SMN1 gene, these genes also regulate the production of a protein called survival motor neuron (SMN) [24]. However, SMN2 gene produces only least number of functionally active survival motor neuron protein as only one type of SMN protein remains functional even though the SMN2 gene produces many types of SMN protein. Usually, one or two copies of SMN2 gene are present in every cell of human being but in some individuals, the number of copies of SMN2 gene varies up to eight[25].

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SMN protein: SMN protein is nothing but the complex of proteins which is also known as SMN complex. This complex is essential for the functioning of motor neurons which are helpful for the transmission of impulses from brain to skeletal muscles. Due to deficiency of SMN protein production, the motor neurons are unable to function properly which leads to improper functioning of skeletal muscles. Thus the non-functioning skeletal muscles undergoes atrophy gradually which results in spinal muscular atrophy. It is evident from several SMA cases that those people lack a piece of SMN1 gene which leads to deficiency in SMN protein. Hence, the death of motor neurons occurs in those patients which results in the improper transmission of impulses to skeletal muscles. Therefore, the muscles cannot contract properly and becomes weak and waste away leading to SMA[26].

It is identified that the SMN protein deficiency occurring due to mutations on SMN1 gene can replace the SMN protein produced by SMN2 gene; there we can understand that SMN2 gene accounts for severe features of SMA. It is identified that the mutations of SMN1 gene are responsible for the occurrence of SMA but SMN2 gene accounts for the severity of SMA. People suffering from Type 0 SMA contain one copy of SMN2 gene in each cell, people suffering from Type I SMA are known to contain one or two copies of SMN2 gene, people suffering from Type II SMA usually contain 3 copies and people suffering from Type IV SMA contain 4 or more copies.

SMN disruption has been done in models like Zebra fish, yeast and mouse to understand the pathogenesis of disease and to understand the pathways of SMN protein and also to offer platform where the genetic and drug screens can be done [27]. Anyways, SMA induced mice which die immediately after birth gives the analysis of preclinical testing of drug and pathogenesis mechanisms [28].

There were two main hypotheses that were in use to describe the SMA pathogenesis. They are

Hypothesis (a): It states that survival motor neuron proteins are responsible to synthesis of snRNPs which are small nuclear ribonucleoproteins. SMN protein is also responsible for mRNA splicing. Hence, reduction in the SMN protein leads to perturbation in snRNP assembly. This hypothesis has been evident in many experiments. SMNP is a member of high molecular weight complex containing another 8 proteins and it is also essential for the formation of smithclass core proteins[29]. These smithclass core proteins are extremely important for the formation of spliceosomes which in turn helps in the formation of pre-mRNA splicing. It is still confusing that why motor neurons are affected more even though the survival motor neuron protein is present at overall vegetative cells. Some studies provide an assumption that the survival motor neuron protein plays a unique responsibility in motor neuron functioning [30].

Hypothesis (b): It states that SMN protein has a specific motor neuron function in which the snRNPs assembly is not involved. It is reported by various evidences. Numerous studies suggested about the survival motor neuron protein is extremely important for the proper functioning of motor neurons as the SMN proteins promotes the axonal transport and also maintains neuromuscular junctions[31]. Hence the reduction of SMN protein may lead to improper axonal transport as well as improper interaction with skeletal muscles. Some studies suggested growth cones and ribonucleoprotein granules contain higher amounts of SMN protein[32]. Few authors suggested that the transportation of ribonucleoprotein complexes is also performed by SMN protein.

It has been observed that the SMA induced mouse has shown some changes in its morphology including proprioceptive reflexes and reduced functioning of synapses on motor neurons during the early stages of the disease. The above morphological changes start primarily in motor neurons that enter muscles of hind limb and axial muscles[33].

1.4 Other forms of SMA without the interference of SMN gene:

There are other forms of SMA which occur due to the mutations of other genes rather than SMN gene. They are listed in the following table along with their affected gene and clinical manifestations.

Table2: several forms of Spinal muscular atrophy differentiate with the survival motor neuron protein

S.No	Form of SMA	Gene	Age of Onset	Clinical manifestations
1	Scapuloperoneal SMA	AD 12q24.1-q24.31	15-30years	Gradual wasting of laryngeal and scapuloperoneal muscles occurs
2	Pontocerebellar SMA	X-linked Xp 11.3-q11.2 UBA1	At birth or first 3 months of life	Contractures occur from birth or at infant stage
3	Respiratory distress type I SMA	AR-IGHMBP2	6 weeks - 6 months	Feeding difficulties, respiratory weakness, repeated episodes of pneumonia
4	Congenital distal SMA	AD12q23-q24	3- 15 months	Contractures occur
5	Distal SMA-V/CMT2d	AD7p15GARS	At birth or by the age of 6 months	Weakness of hands mainly when exposed to cold conditions

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2. Diagnosis:

Clinical manifestations give high degree sensitivity for the diagnosis of SMA. Muscle weakness is seen in those children much in legs when compared to arms. Intensity of weakness depends on the age of onset of SMA. However the intellect and attentiveness is good. If the disease is severe, other clinical manifestations like weak cry, cough, feeding and swallowing difficulties, impaired head control etc. are also included. If the infant is suffering from SMA more severely, the symptoms like tongue fasciculation and atrophy are seen. Such infant depends much on diaphragm for breathing when compared to healthy infant. The primary test for the SMA suspected child is SMN1 gene deletion search[34] that is SMN1 7 exon absence[35] which conforms SMA. If the first level of diagnostic test shows negative, then further conformation can be done by performing the following tests. Blood test can be done to observe the missing or broken genes which can cause SMA.

Blood test can also be done to observe the level of creatine kinase in the blood (creatine kinase is the enzyme that leaks out of the weakening muscles; the level of this enzyme in the blood indicates the level of muscle damage).

To assess the respiratory problems, breathing observation, cough effectiveness evaluation, gas exchange monitoring should be done. Cough effectiveness tests can be done by measuring respiratory muscle function tests and by using suction pump and cough assisting devices. Nocturnal hypoxemia can be screened by using pulse oximetry for complete night along with recording on chart. Polysomnography for measuring carbon dioxide can be used to measure nocturnal oxygen levels.

Electromyogram can be done; for performing electromyogram, doctor puts minor patches on the skin of child and sends electrical impulses to children and checks whether the nerves are conducting impulses to the muscles or not.

Computerised tomography (CT) can be done which gives detailed image of body of child.

Magnetic resonance imaging (MRI) can be performed by sending the radio and magnetic waves to see the images of organs of child.

Biopsy of muscle tissue can be done where the doctor removes muscle cells by using needle and tested for abnormalities. Muscle biopsy is useful for diagnosing infants suffering from muscle weakness like congenital myasthenic syndromes, metabolic myopathies, congenital myotonic dystrophy etc as well as various other genetic syndromes like Prader-Willi syndrome, neonatal sepsis and acute hypoxic ischemic encephalopathy.

If the above tests report any motor neuron abnormality, further genetic tests for SMN mutations should be done. Real time PCR and Multiplex ligation-dependant probe amplication (MLPA) test gives quick and trustable results. Semiquantitative assays offer 98% sensitivity for the diagnosis of SMA.Sequence analysis is mandatory for the patient having single SMN1copy to identify the second causative mutation[36]. However, in about one third of patients having a only one copy of survival motor neuron protein i.e.SMN1, usually the second mutation will be absence at survival motor neuron protein coding area. For example, second mutation is not found in SMA type III which might be because of having intronic mutations. In some child, who born to consanguineous parents and has two SMN1 copies, SMN1 gene analysis of sequence has been suggested[37].

The sick individual cases having two SMN1 copies, SMN1 mutation related tests for diagnosis are not suggested. In their case, other motor neuron related disorders like SMARDI, distal SMA etc are examined[38].

If above tests give negative results for SMA, child must be considered for re-examination or other diagnostic tests should be done which help to diagnose other disorders.

Mostly, carrier testing is referred to parents of SMA children to help them to plan second child that is pre-implantation or prenatal diagnosis. In this case, during 11^{th} to 13^{th} week period of pregnancy, chorionic villi sampling can be done[39]. Screening of new born is done to the infants even before the appearance of symptoms. Screening of newborn can also be done to improve the quality of life of children having several genetic disorders[40]. As other genetic disorders, this SMA also did not have a proper treatment. Anyways, many drugs for the treatment of SMA are under clinical trials[41].

The following figure serves as a guide for the diagnosis of SMA

Figure 1

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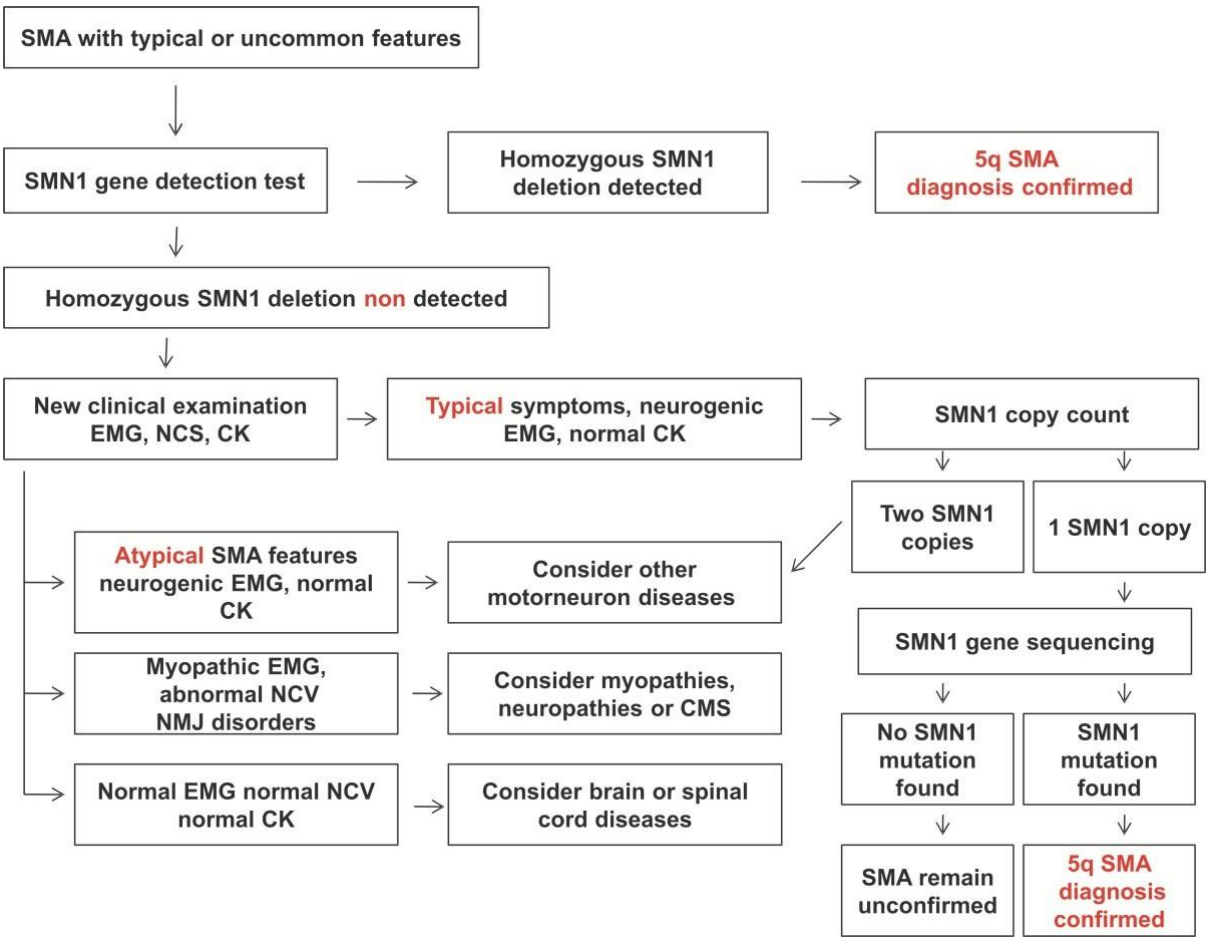

3. Management:

SMA involves various issues like respiratory problems, bone disorders, nutrition problems etc. Hence the role of primary care is important in coordinating the care and follow-up[42]. Follow-up involves expert management who is able to plan variety of interventions including orthopaedic, pulmonary and gastroenterology care. Respiratory failure is the main reason of morbidity and mortality of Spinal muscular atrophy patients mainly type I and type II SMA[43] patients. Patients of SMA suffer from chest infections very often, followed by nocturnal hypoventilation and hypercarbia[44]. Nocturnal hypoventilation can be treated by using non-invasive ventilation (NIV). NIV can replace the process of tracheostomy in children. Additionally, nutrition supplements can be given orally and medical therapy can be given or gastrostomy can be done to treat gastroesophageal reflux disease. Patients of SMA also suffer with the spinal deformity and formation of contractures, their daily way of living strongly gets effected with this. Therapy and surgeries depends on their family wishes. The doctor should evaluate the musculoskeletal functional deficits. Doctor should advise walking to the patient whenever possible by using assistive devices. Hip subluxation can be done if necessary; but it was identified that recurrence is observed in many cases. Scoliosis surgery can be done to the patients who survive more than 2 years of age but should be carefully done by observing pulmonary functioning. Spinal orthoses can be given for supporting posture and it cannot control the progression of curve. Bracing can be done to correct the spinal curve[45]. If bracing does not work properly, Vertical Expandable Posthetic Titanium Rib (VEPTR) or growing rods can applied to avoid arch growth in children[46].

3.1. Pharmacological therapy: Several drug trials have been done to treat SMA mainly by using neuroprotective drugs such as riluzole to prevent motor neuron damage, creatine is also used in order to improve the metabolism and the drug albuterol has been used to repair the SMN2 gene expression[47]. Many efforts were done to target SMN gene expression, pre-mRNA splicing and to enhance activity of SMN2 promoter.
Quinozoline derivatives were developed as SMN promoter-activating compounds. These compounds improved SMN in-vitro as well as in mouse model SMNΔ7.

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

Administration about SAHA enhanced lifespan of SMA induced mouse. Histone deacetylase (HDAC) inhibitors, shows positive results to the both SMA mouse models and patients. Sodium butyrate, Tricchostatin A and valproic acid have shown positive results in murine SMA models, but these drugs have not shown efficiency in clinical trials. HDAC inhibitor compounds like LBH589 has improved SMN levels in patients suffering from SMA.

3.2. Gene therapy:

Along with the drug therapy, gene therapy is found useful in the treatment of SMA. Gene therapy is done by using the viral vectors to replace SMN gene. In an experimental study, AAV8-hSMN self-complementary was intrathecally injected into the central nervous system of spinal muscular atrophy containing mouse. which shows that increased life span up to 50days than the untreated ones. Another of vectors which can be used are Adeno-associated virus (AAV) vectors. These vectors are useful to deliver Adeno-associated virus in to CNS by intrathecal route.

Currently, the following therapies are in use for the treatment of SMA as a part of gene therapy.

a. Nusinersen (Spinraza): This drug is useful in the medication of SMA in both paediatrics and adults. This drug changes the SMN2 gene expression and improves the SMN protein synthesis. This drug is administered to the patients of SMA into the space present around their spinal cord. The therapy needs to start by four loading doses, the first 3 loading doses should be administered for every 14 days and the last loading dose will be given after 30 days following third loading dose after the completion of loading doses, maintenance doses should be given for every 4 months. Before the administration, the drug need to be warmed at room temperature. It has been identified that this drug slows down the disease in approximately 40% of people. Being a very costly drug, therapy of SMA with Nusinersen needs high financial assistance. The major limitations of this drug usage are high cost, limited clinical data and doubts about the duration of treatment. The interesting point is this drug has not shown any interaction with cytochrome p450 enzymesindicating very less drug interactions. The recommended dose of Nusinersen for the treatment of SMA is 12mg. As we have studied that this drug is administered intrathecally; intraventricular administration can be used as an alternative. This drug cannot cross the blood brain barrier at therapeutic dose, hence if the drug is to be act systemically, the doctors need to inject 100 fold higher dose than normal therapeutic dose. The adverse effects involved in the Nusinersen usage are respiratory problems, constipation, pneumonia etc. However, the above adverse events are more common symptoms of SMA.

b. Onaswmnogene abeparvovec –xioi (Zolgensma): This drug is useful for the treatment of SMA in children less than 2 years of age. It is a process of replacement of SMN1 gene. The medical team will insert a catheter directly to vein in the hand of children for treatment and sends a copy of SMN gene in to motor neuron cells through the tube. This therapy is to be done only one time and it helps the children to overcome the effects of SMA like impaired head control and impaired posture. But the usage of this drug is associated with the development of severe liver disorder in SMA patients.

c. Risdiplam (Evrysdi): this drug is to be taken by the SMA affected child once a day orally after having a meal. Drug dosage will be determined based on the weight of the patient. This therapy helps to enhance the yields of SMN protein and increases the ability of protein to reach the nerve cells by blocking the SMN2 genes from disrupting the protein production. It was evident that 41% of the patients shown improved muscle function after 12 months treatment with this drug.

3.3 Stem cell therapy:

More than the pharmacological and gene therapy, Stem cell therapy offers promising role in the cure of Spinal muscular atrophy and attracted much attention. This therapy involves the Stem cell transplant. Neural primary stem cells which are originated by spinal cord were used in certain studies, which shows development of SMA phenotype in mice, supposing the limited translational applications.

Besides Pharmacological therapy, Gene therapy and Stem cell therapy medical team may suggest other ways to manage various symptoms of SMA. They are

- Breathing difficulty: Breathing difficulty [44]is the main problem associated with SMA especially type 1 and 2Breathing. Hence, the medical team suggests a special mask or mouthpiece. The child can use a machine that helps them to take breath.
- Swallowing and nutrition difficulties. The children affected with SMA faces the swallowing [3]difficulties as the muscles in their mouth and throat becomes weak. As a result, the child faces malnutrition. In that situation, the medical team generally suggests a feeding tube.
- Movement difficulty: The child suffering from SMA suffers alot in doing their daily activities because of the severe weakness of the muscles in their body. In that situation, the medical team suggests leg braces, a walker or an electric wheelchair[2].

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

- Spinal cord issues: When SMA starts in childhood, children can generally get a curve in their spinal cord. In that condition, medical team may suggest a back brace for the child to wear while their spine is still growing and after their complete growth, the can opt for surgery[11].

4. Conclusion:

Besides the effectiveness of drugs, there are other important factors like the drug availability and cost effectiveness to be considered. These drugs are beyond availability and are not affordable to lower middle class and middle class people. Hence, the Government should consider and make the drugs affordable to poor people also. We agree that this disease is rare but every life is expensive.

A team involving family members of SMA affected children, doctor, therapists can help the child to overcome the problems associated with SMA. The child may need lifelong treatment from pulmonologists, neurologists, orthopaedists, gastroenterologists, nutritionists and physical therapists.

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