The Effect of Nusinersen Therapy on Laboratory Parameters of Patients with Spinal Muscular Atrophy

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Abstract

Introduction We evaluated the effect of nusinersen on clinical and laboratory parameters and presented its safety and effect on laboratory parameters.

Methods Two groups were formed from among patients with spinal muscular atrophy (SMA) followed up between September 2017 and June 2021: group 1, SMA type 1; group 2, SMA type 2 and 3. The laboratory parameters were evaluated in groups 1 and 2 between doses. Motor scale tests were performed on patients before each dose of nusinersen.

Results Twenty seven patients (group 1; $n = 13$, group 2; $n = 14$) were included. The mean age ($\pm$standard deviation) at the onset of symptoms was 3 ± 1.21 (range, 1.5–6) months in group 1 and 12 ± 4.27 (range, 8–24) months in group 2. No significant laboratory treatment-related abnormalities and adverse effects were observed. The cerebrospinal fluid protein levels and the frequency of conventional LP were higher in group 1. Serum creatinine (Cr) levels were higher in group 1 before the first dose and higher in group 2 before the fifth dose ($p < 0.05$). With treatment, the Cr levels of group 1 decreased and group 2 remained constant or increased. We observed that the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders and Hammersmith Functional Motor Scale-Expand scores increased as our patients received treatment ($p < 0.05$).

Conclusion Our results support the safety and efficacy of nusinersen. However, changes in Cr levels according to the clinical type and treatment suggested that serum Cr could be a candidate marker for treatment follow-up.

Keywords
- nusinersen
- spinal muscular atrophy
- safety
- effectiveness
- creatinine

Introduction

Spinal muscular atrophy (SMA) is a genetic disease that occurs as a result of a mutation in the survival motor neuron (SMN) gene and causes clinical findings such as motor neuron degeneration, progressive muscle atrophy, and weakness by affecting the anterior horn of the spinal cord. Its incidence is reported as 1 in every 10,000 live births, and its prevalence as 1 in 100,000.[1] The pathogenesis of this disease, which is frequently an autosomal recessive disease, is explained by rapidly progressive apoptosis involving the spinal cord anterior horn cells and brain stem motor nuclei. With the mutation in the SMN1 gene, the expression of SMN protein, which protects organisms against apoptosis, decreases. In addition, SMN2, a paralogous gene, provides the production of 10% of the required SMN protein by alternative splicing by removing exon 7. Therefore, the SMN2 copies are directly related to the clinical condition of patients.[2]

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Nusinersen is an antisense oligonucleotide (ASO) that acts by increasing the production of the SMN protein by binding to mRNA on the SMN2 gene.[3,4] It is known that this drug, which is administered intrathecally with four loading doses in the first 2 months, followed by maintenance doses every 4 months, significantly contributes to the quality of life and motor functions of patients.[5] In addition to the efficacy of nusinersen, the reliability of the treatment is the determining factor for the success of the treatment. Elevated liver transaminase enzyme levels, renal failure, coagulation abnormalities, or thrombocytopenia are the adverse effects reported with the use of ASOs.[6–9] Studies examining the effect of nusinersen on the laboratory results of patients published in 2021 showed a positive safety profile of the treatment.[10,11] However, studies on this subject are limited to a small number of patients and have short follow-up periods. Nusinersen was approved in our country for use in patients with SMA type 1 in 2017, and patients with SMA type 2 and 3 in 2019. In this process, we observed positive effects of nusinersen on the clinical course in SMA, causing difficulties in phenotypic heterogeneity, prognosis, evaluation of disease activity, and monitoring of treatment response. We examined the laboratory findings and motor functions of our patients with SMA who received nusinersen in our study. Thus, by evaluating the effects of nusinersen on patients' motor functions and laboratory parameters, we aimed to evaluate its efficacy, safety, and whether there was a certain parameter that it affected.

Methods

Electronic records obtained from the data analysis unit and files of patients with SMA who were followed up in the Department of Pediatric Neurology of Dokuz Eylul University Faculty of Medicine between September 2017 and June 2021 were retrospectively analyzed. Age, sex, age of onset of clinical findings, age of genetic diagnosis, SMN2 copy number, type of SMA disease, nusinersen treatment status, age of onset of nusinersen treatment and the number of doses, and laboratory parameters obtained from patient files, and system data were recorded.

Patients who received nusinersen were divided into two groups consisting of patients with SMA type 1 (group 1) and SMA type 2 and 3 (group 2). Glucose, protein, potassium (K), chloride (Cl), and sodium (Na) levels, and leukocyte (white blood cell [WBC]) and erythrocyte (red blood cell [RBC]) counts in cerebrospinal fluid (CSF) samples; blood urea nitrogen (BUN), creatinine (Cr), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyltransferase (GGT) levels, hemoglobin (Hgb) levels, WBC, neutrophil and lymphocyte counts, mean platelet volume (MPV), platelets (PLT), prothrombin time (PT), international normalized ratio (INR), active partial thromboplastin time in serum samples; and protein and Cr levels in spot urine samples were examined before each nusinersen treatment in patients in these two groups. The interdose status of the above-determined laboratory parameters of the patients in groups 1 and 2 was compared with each other. In addition, to determine whether laboratory parameters were affected by nusinersen, glucose, protein, K, Cl, Na, BUN, Cr, Hgb, WBC, neutrophil, lymphocyte, MPV, PLT in serum, and protein and Cr levels in spot urine were compared between doses. Motor scale tests were performed on patients by a physical therapy and rehabilitation specialist before each dose of nusinersen. The Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP-INTEND) tests were performed for type 1 SMA and Hammersmith Functional Motor Scale-Expand (HFMS-E) for type 2 and 3 SMA. In addition, the effect of treatment onset time (age) on the motor scale test scores was investigated.

Based on the treatment national protocol used in our country's Ministry of Health, four loading doses on day 0 and the 14th, 28th, and 56th days in patients with SMA type 1, and day 0 and the 28th, 84th, and 273rd days in patients with SMA type 2 and 3 were administered. Following the four loading doses of nusinersen, maintenance doses were administered every 4 months. Intrathecal injection of 12-mg nusinersen was performed in each dose. Patients who might have problems during the intrathecal injection procedure due to the presence of scoliosis were treated by interventional radiology through fluoroscopic lumbar puncture (LP). CSF and serum samples were collected before each nusinersen injection and the relevant parameters were studied in the medical biochemistry and medical microbiology laboratories of Dokuz Eylul University Faculty of Medicine. Blood samples were collected 5 days$^{1-14}$ before each dose, and CSF samples were taken just before drug administration during LP for injection. The relevant predose laboratory parameter of all patients was enumerated with that dose (such as Cr level before the first dose; Cr1).

To determine changes in the laboratory values of patients with SMA under treatment, the mean level of change from baseline for each parameter was examined. To assess long-term changes, the most recently measured value available was compared with the corresponding baseline value in each patient and the value at each dose with each other. In addition, it was evaluated whether the parameter of each treatment dose deviated from the lower or upper limit of normal. The necessary permission for the research was obtained from the Ethics Committee of Dokuz Eylül University Faculty of Medicine (Date: January 18, 2021, Decision number: 2021/02-02).

Statistical Analysis

The data obtained in the study were entered into a database created in the SPSS 22.0 program, and statistical analyses were performed using the same program. Mean, standard deviation, median, minimum, and maximum values of continuous variables were calculated. The conformity of these variables to normal distribution was investigated. Considering the sample diameters, it was decided that normal distribution fitness conditions could not be met in all variables, so nonparametric methods were used. Comparisons of repeated measurements were made using the Friedman test and Wilcoxon test, and comparisons of independent subgroups were made using Kruskal–Wallis and Mann–Whitney

test methods. *p*-Values were calculated using Bonferroni correction, adjusted *p* (adj *p*). The relationship between independent variables was examined using the nonparametric correlation Shearman's rho method. In all statistical comparison tests, the margin of error for type 1 was determined as $\alpha = 0.05$ and was tested bidirectionally. If the *p*-value was less than 0.05, the difference between the groups was considered statistically significant.

Results

Patient and Treatment Characteristics

Twenty-seven patients with SMA followed in our clinic were included in the study. In total, 13 (48.1%) patients had SMA type 1 and 14 (51.8%) had SMA type 2 and 3. In total, 13 (48.1%) of the patients were female and 14 (51.9%) were male. The mean age (± standard deviation) at the onset of symptoms was 3 ± 1.21 (range, 1.5–6) months in group 1 and 12 ± 4.27 (range, 8–24) months in group 2. The mean age at the onset of treatment was found as 9.5 ± 34.55 (range, 4–133) months in group 1 and 72 ± 56.14 (range, 30–218) months ($p < 0.001$) in group 2. Of the patients receiving nusinersen treatment, 48.1% ($n = 13$) had SMA type 1, 44.4% ($n = 12$) had SMA type 2, and 7.4% ($n = 2$) had SMA type 3. A total of 164 (3–13 doses) intrathecal nusinersen injections were performed between September 2017 and June 2021. A total of three doses for two patients with SMA type 1 and a total of 23 doses for five patients with SMA type 2 were administered with fluoroscopy (►**Table 1**).

When the intrathecal treatment methods of the patients in groups 1 and 2 were compared, the rate of performing fluoroscopic procedures in the patients in group 2 was found to be significantly higher (►**Fig. 1**) ($p < 0.05$).

The patients were followed up for an average of 8 (range, 6–24) hours after treatment. The most common adverse event in the posttreatment follow-up was restlessness in group 1 ($n = 42$; 26%) and low back pain ($n = 50$; 58%) in group 2. The demographic characteristics of our patients are shown in ►**Table 1**, the treatment characteristics are given in ►**Table 1**, and the median values of CHOP-INTEND and HMSF-E scores for each dose are given in ►**Fig. 1**. It was observed that CHOP-INTEND and HMSF-E scores increased significantly as our patients received treatment ($p < 0.05$). A boxplot graph showing the distribution of the minimum and maximum quartiles of the median values of the CHOP-INTEND and HMSF-E scores at the relevant dose is presented in ►**Fig. 2**.

We also evaluated the effect of the treatment onset time (age) on the increase in the motor scala scores of our patients. In group 1, a statistically significant negative correlation was observed between the age of treatment onset and the increase of CHOP-INTEND, after the first four loading doses. Similarly, we found a statistically significant negative correlation between the increase in HMSF-E at all nusinersen doses and the age at onset of treatment in group 2 (►**Table 2**). In conclusion, the earlier the treatment was initiated, the greater was the increase in HMSF-E and CHOP-Intend scores. The change in maximum points on motor scales between the prefirst and preseventh doses is given in ►**Fig. 3**.

The Comparison of Findings of Patients with Spinal Muscular Atrophy Receiving Nusinersen Treatment According to Clinic Type and Treatment Doses

Cerebrospinal Fluid Examination

It was observed that there was no significant difference between the glucose, Na, K, Cl, WBC, and RBC levels in the CSF of both groups in comparisons between doses. It was observed that only the CSF protein levels of group 1 were significantly higher than those of group 2 before the first three doses and that the elevation continued in the following doses but not at a significant level (►**Table 3**).

Table 1 Demographic and treatment characteristics of the patients

Patients		Group 1 (SMA type 1)	Group 2 (SMA type 2 and 3)	*p*-Value
Number of patients (*n*)		13	14	
Sex		7F/6M	6F/8M	
Age (months)		9.5 ± 34.55 (range, 6–133)	72 ± 56.14S (range, 30–218)	**$p < 0.05$**
Age of onset of symptoms (median) (months)		3 ± 1.21 (range, 1.5–6)	12 ± 4.27 (range, 8–24)	**$p < 0.05$**
Age of treatment onset (months)		5 ± 34.55 (range, 4–133)	72 ± 56.14 (range, 30–218)	**$p < 0.05$**
Total number of treatment doses		79 (range, 3–13)	85 (range, 4–8)	
Treatment duration		September 2017–June 2021	March 2019–May 2021	
Treatment type	Conventional (without fluoroscopy)	76 (96.2%)	62 (72.9%)	**$p < 0.05$**
	Fluoroscopy	3 ($n = 2$) 37.8%	23 ($n = 5$ SMA type 2) 27%	**$p < 0.05$**
Posttreatment adverse event		Restlessness ($n = 42$)	Back pain ($n = 50$)	
Death (*n*)		3	0	

Abbreviation: SMA, spinal muscular atrophy.
Note: The statistically significant values are in bold ($p < 0.05$)

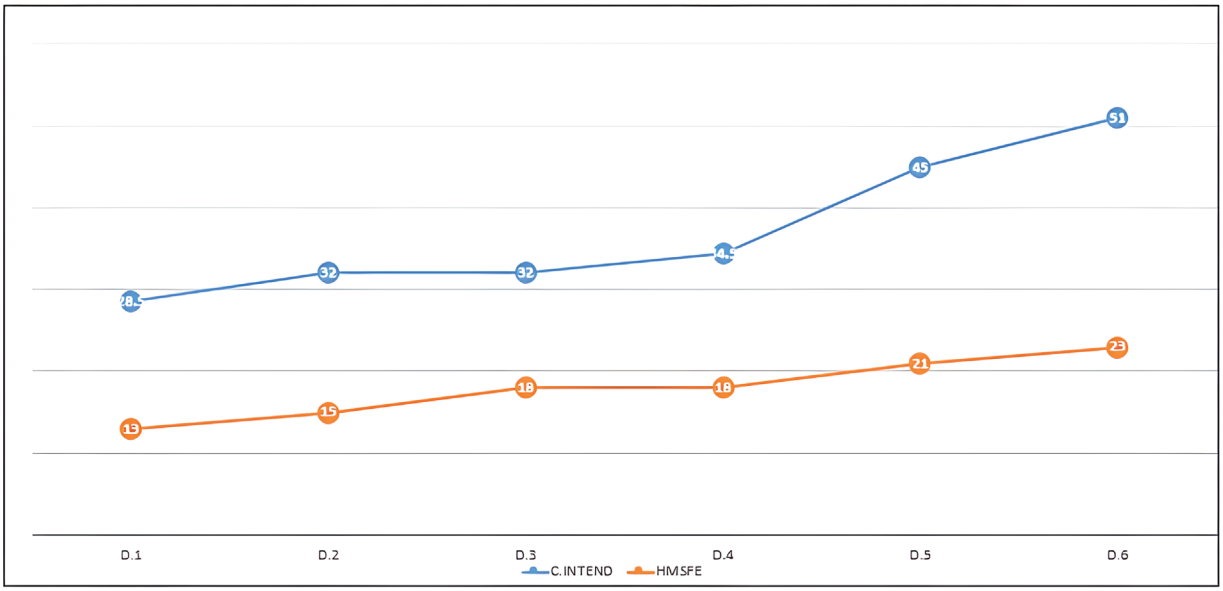

Fig. 1 Median values of CHOP-INTEND and HMSFE scores of the patients at relevant doses.

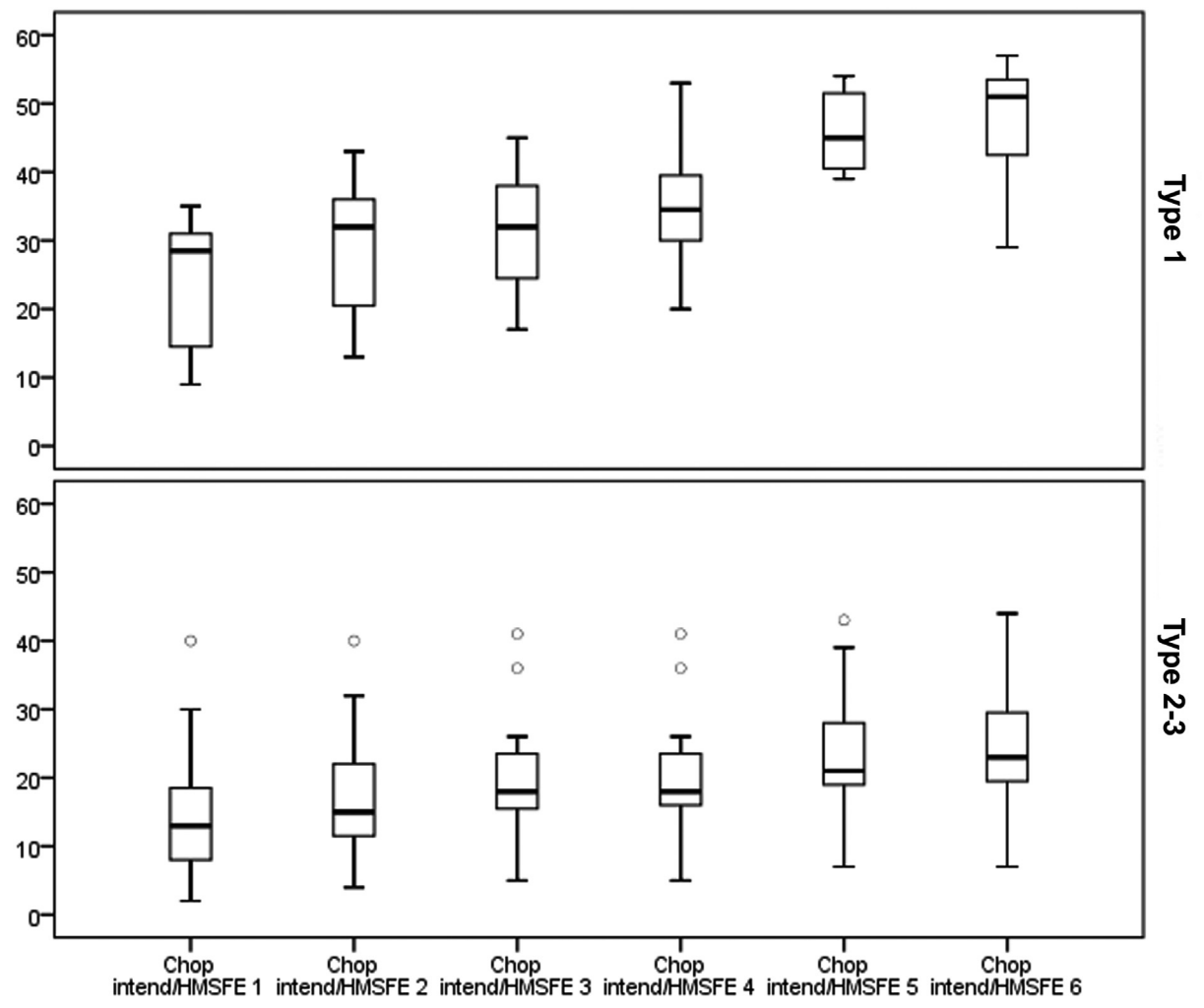

Fig. 2 Boxpilot chart giving the distribution of median values of CHOP-Intend and HMSFE scores of the patients at each dose.

Table 2 The effect of age at first application on increase of motor scale scores

Spearman's rho			CHOP-INTEND / HMSFE 2–1	CHOP-INTEND / HMSFE 3–1	CHOP-INTEND / HMSFE 4–1	CHOP-INTEND / HMSFE 5–1	CHOP-INTEND / HMSFE 6–1	CHOP-INTEND/ HMSFE 7–1
.	Group 1	Correlation coefficient[a]	0.152	0.003	0.001	−0.693	−0.663	−0.817
		Sig. (two tailed)[b]	0.620	0.993	0.996	0.018	0.073	0.025
		Patient number (*n*)	13	13	13	11	8	7
	Group 2	Correlation coefficient	−0.400	−0.656	−0.664	−0.740	−0.747	−0.739
		Sig. (two tailed)	0.156	0.011	0.010	0.006	0.008	0.009
		Patient number (*n*)	14	14	14	12	11	11

Abbreviations: CHOP-INTEND, the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders; HMSF-E, Hammersmith Functional Motor Scale-Expand.
[a]Correlation is significant at the 0.01 level (two tailed).
[b]Correlation is significant at the 0.05 level (two tailed).

Table 3 Statistical evaluation of the laboratory parameters of group 1 and 2

	Dosage 1			Dosage 2			Dosage 3			Dosage 4			Dosage 5			Dosage 6		
	Mean ± SD Gr1	Mean ± SD Gr2	*p*-Value	Mean ± SD Gr1	Mean ± SD Gr2	*p*-Value	Mean ± SD Gr1	Mean ± SD Gr2	*p*-Value	Mean ± SD Gr1	Mean ± SD Gr2	*p*-Value	Mean ± SD Gr1	Mean ± SD Gr2	*p*-Value	Mean ± SD Gr1	Mean ± SD Gr2	*p*-Value
Protein (CSF) mg/dL	29.05 ± 12.44 (22–51)	21.80 ± 8.08 (15.90–46.70)	0.004	33.50 ± 46.62 (19–142)	22.40 ± 8.21 (15.70–46.10)	0.013	42.85 ± 57.43 (19–170)	22.10 ± 11.00 (14.20–48.30)	0.006	33.40 18.03 (10.00–57.00)	20.00 55.70 (11.70–198.90)	0.284	32.75 ± 14.68 (17.90–54.00)	23.80 ± 22.55 (16.10- 87.00)	0.248	25.45 ± 7.97 (14.90- 36.50)	23.00 ± 6.97 (14.50- 38.70)	0.964
Hgb (serum) g/dL	11,70 ± 1.3 (9.9–13.1)	12.3 ± 0.89 (11.3–14.00)	0.049	11.6 ± 1.29 (10.3–13.80)	0.89 ± (11.1–14.00)	0.044	11.80 ± 1.23 (9.70–13.20)	12.20 ± 1.09 (10.70–14.20)	0.410	11.6 ± 5 (0.99–10.80)	12.60 ± 1 (11.00–14.00)	0.226	11.4 ± 0.56 (11.10- 12.70)	13.00 ± 0.91 (11.30–14.60)	0.008	11.40 ± 1.31 (9.10–12.90)	13.30 ± 0.94 (12.00–15.10)	0.011
Cr (serum) mg/dL	1.25 ± 0.035 (0.080–0.170)	0.170 ± 0.067 (0.080–0.3)	0.011	0.115 ± 0.04 (0.060–0.17)	0.130 ± 0.048 (0.080–0.240)	0.125	0.105 ± SD (0.032–0.080)	0.130 ± 0.034 (0.080–0.190)	0.084	0.11 ± 0.021 (0.100–0.160)	0.120 ± 0.053 (0.070–0.250)	0.322	0.100 ± 0.018 (0.080–0.130)	0.130 ± 0.045 (0.070–0.230)	0.034	0.110 ± SD (0.041–0.060)	0.120 ± 0.051 (0.051–0.070)	0.820
BUN (serum) mg/dL	8.45 ± 4.66 (6.70–19.00)	11.10 ± 3.11 (8.00–17.30)	0.004	7.80 ± 4.45 (5.80–16.00)	11.20 ± 4.05 (7.60–20.60)	0.006	8.65 ± 1.84 (6.20–11.40)	8.90 ± 2.69 (6.00–14.00)	0.006	9.95 ± 4.32 (8.00–19.40)	10.20 ± 4.09 (6.20–18.00)	0.150	12.00 ± 4.31 (6.40–19.20)	10.90 ± 2.79 (4.90–13.50)	0.220	12.05 ± 4.01 (6.70–18.00)	10.20 ± 2.69 (7.30–15.70)	0.342
WBC (serum) $10^3/\mu L$	9.10 ± 1.57 (7.90–11.90)	8.40 ± 2.26 (6.70–14.30)	0.438	10.85 ± 1.92 (8.40–14.00)	7.60 ± 1.69 (5.00–10.50)	0.009	9.50 ± 1.86 (7.60–12.10)	6.90 ± 1.53 (4.50–10.60)	0.006	9.50 ± 1.86 (7.60–12.10)	6.90 ± 1.53 (4.50–10.60)	0.015	11.95 ± 2.22 (10.70–16.80)	7.50 ± 1.39 (5.40–9.30)	0.003	13.70 ± 4.58 (10.80–22.60)	7.50 ± 8.68 (6.20–36.30)	0.013
Lymphocyte (serum) $10^3/\mu L$	6.30 ± 1.27 (4.60–8.20)	3.70 ± 0.95 (1.00–4.40)	0.002	6.90 ± 1.02 (5.20–8.10)	3.20 ± 0.68 (2.20–4.20)	0.002	6.55 ± 1.91 (3.90–9.00)	3.30 ± 0.81 (1.20–4.20)	0.027	6.80 ± 1.48 (5.60–9.30)	3.50 ± 0.90 (2.50–5.20)	0.057	6.45 ± 1.32 (5.80–9.20)	3.50 ± 0.68 (2.50–4.90)	<0.001	5.90 ± 1.69 (4.10–8.30)	3.60 ± 0.57 (2.70–4.70)	0.001

Abbreviations: BUN, blood urea nitrogen; Cr, creatinine; CSF, cerebrospinal fluid; Gr, group; HGB, hemoglobin; LENF, lymphocyte; NEU, neutrophil; SD, standard deviation; WBC, leukocyte.

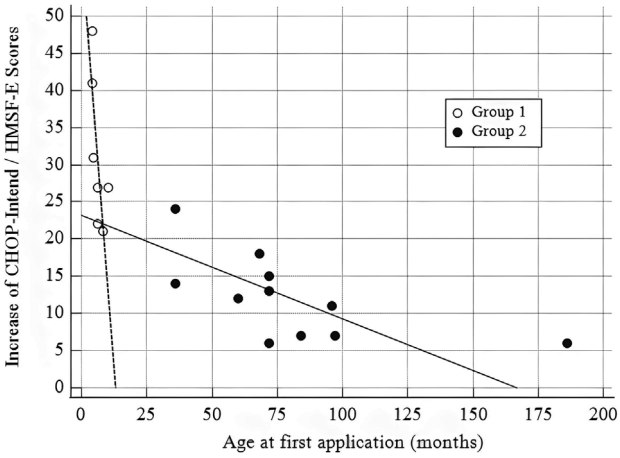

Fig. 3 Change in maximum points on motor scales (between pre-1st and pre-7th doses). Lines represent regression lines and suggest that early initiation of treatment corresponds to a better outcome (statistically significant both of two groups).

Serum Examination

There was no statistically significant difference between the doses in terms of the AST, ALT, GGT, lymphocyte, neutrophil, PLT, PT, MPV, PTT, and INR values of groups 1 and 2. Significant differences were found between doses in terms of blood Cr, BUN, HGB, WBC, and lymphocyte values. These differences are detailed in ►**Table 3**. Cr1 was higher in group 1 and Cr5 was higher in group 2 ($p < 0.05$) (►**Table 3**). BUN was higher in group 2 in the first four doses and in group 1 in the fifth and sixth doses (BUN1-2-3; $p < 0.05$) (BUN4; $p > 0.05$) (BUN5-6; $p < 0.05$) (►**Table 3**). Serum WBC counts of group 1 before the second, third, fourth, fifth, and sixth doses and lymphocyte counts of group 1 before the first, second, third, fifth, and sixth doses were higher than those of group 2 ($p < 0.05$) (►**Table 3**). MPV6 was higher in group 1 ($p > 0.05$) (►**Table 3**).

Urine Examination

There was no statistically significant difference in terms of spot urine protein and Cr levels.

Comparison of Laboratory Parameters of Patients Between Doses of Nusinersen Treatment

Cerebrospinal Fluid Parameters

There was no significant change in terms of the levels of glucose, protein, K, Cl, and Na in the cerebrospinal fluid between doses.

Serum Parameters

When the BUN, Cr, Hgb, WBC, neutrophil, lymphocyte, MPV, and PLT values of both groups were compared between doses, there were significant differences between MPV and Hgb values in group 2. When the MPV values of both groups were examined, it was observed that there was a slight increase in MPV values after the first dose of treatment, a decrease in the continued doses, and then an increase again toward the sixth dose. In the first group, MPV2 was significantly higher than MPV5 (adj $p < 0.05$). In group 2, MPV5 was significantly higher than MPV3 and MPV4 (adj $p < 0.05$); MPV6 was significantly higher than MPV4 (adj $p < 0.05$). In addition, NEU6 was found to be significantly higher in group 1 than in the other five doses (adj $p < 0.05$). When the Hgb levels of group 2 were evaluated between doses, it was seen that Hgb6 was significantly higher than Hgb1-2-3 (adj $p < 0.05$).

Urine Examination

There was no significant difference between doses in terms of Cr, protein, and protein/Cr levels in spot urine.

Discussion

Due to concerns about the safety of nusinersen, a new and promising treatment for SMA, its effects on laboratory parameters, and its adverse effects are topics of interest to patients and physicians. As nusinersen applications continue, new results have started to be reported about this drug. For the follow-up of patients, it is important to determine whether there is a specific parameter that nusinersen affects. Defects in liver function tests (transaminases) and coagulation parameters, renal failure, and thrombocytopenia are reported adverse effects of ASOs.[7,12–14] In our study, these adverse effects related to ASOs were not observed.

When we compared the findings of our patients with SMA who received treatment, the CSF protein levels and the frequency of conventional LP were higher in group 1. Studies reported that total protein levels in CSF tended to increase with age, were highest in the neonatal period in childhood, and then decreased with age.[15–17] In a comprehensive study conducted to determine the variability of CSF total protein levels in childhood according to age, it was stated that the total protein level, which was highly variable between 0 and 6 months, was at its lowest values between 2 and 6 years of age and gradually increased from 6 years to 18 years of age. The authors stated that factors such as blood–brain barrier permeability, myelination, CSF flow rate, and the clearance rate of proteins in CSF might affect this result.[17] Wurster et al found no relationship between age (between 11 and 60 years) and CSF protein levels in their study in which they examined the CSF findings of patients with SMA receiving nusinersen treatment, and they added that they found no parameters affecting CSF protein levels.[18] By contrast, Müschen et al reported that intrathecal (such as conventional, fluoroscopy, or CT-guided) nusinersen in patients with SMA was effective on CSF protein levels.[19] This study was conducted on adult patients with SMA types 2 to 4, and higher CSF protein levels were found to be associated with SMA type 3, male sex, conventional LP, and SMN2 copy numbers $\geq$3. They attributed this result to the older age of the patients with type 3 SMA, with SMN2 copy numbers $\geq$3, and in whom conventional LP was performed. They also added that the CSF protein levels of most of their patients were within the reference range specified in the literature according to age (upper limit 500 mg/L for patients aged 18 to 30 years and 600 mg/L for patients aged $\geq$30 years).

However, it has been reported that CSF total protein levels in childhood are highly variable under 2 years of age,

especially 0 to 6 months, and gradually increase thereafter. Although the patients in the group with high CSF protein levels in our cohort were diagnosed as having infantile SMA type 1 and most were aged under 2 years (median 9.5 months), which might have caused high CSF protein levels in this group, we thought that the main reason was due to conventional LP, as Müschen et al stated that because conventional LP is open to trauma, it is often performed without anesthesia in wards and it may cause increased CSF protein levels. This result suggests that the LP method has a direct effect on CSF protein levels. Therefore, fluoroscopy should be preferred in patients to prevent traumatic LP if necessary. In addition, Müschen et al stated that there was a significant increase in CSF protein levels during regular intrathecally administered nusinersen, and this could be due to nusinersen or repeated LP procedures, and that intrathecal treatment could increase CSF protein levels.[19] However, in our patients, there was no significant change in CSF protein levels between doses. Our result suggested that nusinersen or repeated LP applications had no direct effect on CSF protein levels.

Abnormal liver function tests are a known adverse event with the use of ASOs.[9,10] However, regarding nusinersen, it was shown in the NURTURE study that transaminase levels were stable in presymptomatic children.[20] In the ENDEAR and CHERISH studies on the safety of nusinersen, there were deviations from normal in the levels of liver transaminases, but it was not found to be statistically significant when compared with control groups.[21,22] In addition, no significant deterioration was observed in liver function tests in pediatric and adult patient studies published in 2021.[10,11] In our study, the liver enzyme levels of our patients were within the normal range. This result suggested that nusinersen, an ASO, was safe in terms of hepatotoxicity, unlike other ASOs.

When we examined the renal function tests of our patients, there was no significant change in the serum Cr levels of patients in both groups receiving nusinersen treatment between doses. When the Cr levels of the two groups receiving treatment were compared with each other, it was seen that Cr levels were higher in group 1 before the first dose and group 2 before the fifth dose. Cr levels decreased in our patients with SMA type 1 who received treatment and it tended to remain constant or increase in patients with SMA type 2 to 3 in the follow-up. It is known that serum Cr levels are associated with skeletal muscle mass, SMA disease type, SMN2 copy number, motor function, and the severity of denervation.[23] Alves et al emphasized that the natural course of serum Cr levels tended to decrease in patients with SMA and that Cr levels were lower as the severity of SMA disease increased and the SMN2 copy number decreased in patients with SMA who did not receive nusinersen.[24] In addition, in a very recent study, the effect of nusinersen on serum Cr levels in patients with SMA type 3 aged over 18 years was examined, and it was reported that there was an increase in serum Cr levels with treatment.[25] Our result was similar to this study for patients in group 2, but the same result was not obtained in patients with SMA type 1. Decreased serum Cr levels in patients with SMA type 1 treated with nusinersen and increased serum Cr levels in patients with SMA type 2 and 3 treated with nusinersen were observed in our cohort.

We explain this positive effect of nusinersen on the Cr levels of our patients with SMA type 2 and 3 through the direct effect of nusinersen on maximum motor capacities. In patients with SMA type 1, the effect of nusinersen on Cr levels may not have been reflected in the laboratory due to the faster course of muscle breakdown. We thought that there was a decrease in Cr levels in this patient group due to the decrease in muscle mass secondary to rapid destruction. Given that the loss of muscle mass is less in SMA types 2 and 3, nusinersen may increase it by affecting Cr levels.

More comprehensive pediatric studies are needed, especially in patients with SMA type 1, regarding the relationship between nusinersen and Cr levels. In addition, it was observed that other renal function tests remained in the normal range throughout the treatment period except for one patient with transient proteinuria. In the literature, no significant difference was found between the patients treated with nusinersen and the control group in terms of the rate of proteinuria.[21,26] In another study, transient proteinuria was reported as the most common laboratory abnormality.[11] Our results were supportive of the renal safety of nusinersen in this respect.

Among the hematologic parameters, the most intriguing parameter regarding nusinersen is PLT levels. Thrombocytopenia is one of the main reported adverse events of ASOs, which reduces LP safety because it increases the risk of hemorrhagic complications.[6–9,27] Accordingly, thrombocytopenia that may develop due to nusinersen is also important because it will put other doses at risk. In the ENDEAR and CHERISH studies on PLT counts under nusinersen treatment, no patient had persistent thrombocytopenia or bleeding and median PLT counts remained stable during treatment.[21,22] In the NURTURE study on nusinersen treatment in presymptomatic patients, it was shown that PLT counts remained stable.[20] Goedeker et al reported that no patients had a thrombocyte count of $<$100/nL in their study published in 2021.[11] In another study, mild and transient thrombocytopenia was reported in a single child treated with nusinersen.[28] These results showed that nusinersen did not cause persistent thrombocytopenia or thrombocytopenia that required transfusion. Our results were similar to these studies because we did not see thrombocytopenia in any patients.

When the blood Hgb levels of both groups receiving treatment were examined, the Hgb levels in the first three doses of group 1 were significantly lower than those of group 2. There was no difference in Hgb levels in the other three doses (fourth, fifth, and sixth) between groups 1 and 2. This situation might be iatrogenic, especially in patients with SMA type 1, due to frequent blood collection due to the shorter intervals between the first doses. In addition, when the Hgb levels of group 2 were compared between doses, the Hgb values of the first three doses were significantly lower than those with the sixth dose. This result suggests that Hgb levels increase as the doses increase.

These results might also be because our patients with type 1 SMA were in the period of infant physiologic anemia. However, as the drug doses of patients with type 2 and 3 SMA are increased, the increase in Hgb values supports the possibility of iatrogenicity. With these results, reducing the frequency of blood collection will reduce the psychosocial and medication burden on patients and their families and secondary treatment and examination costs. In addition, anemia may be associated with SMA disease and nusinersen. In an animal study, Szunyogova et al showed that the SMN protein was required for normal erythropoiesis.[29] It is known that SMN protein levels increase with treatment in patients with SMA.[3,4] We speculate that this result in Hgb levels in our study is due to the effect of SMN protein on erythropoiesis.

In other hemogram findings of our patients, serum WBC and lymphocyte counts of group 1 were higher than in group 2. We attributed this result to the fact that the patients in group 1 were younger than in group 2 because it is known that the normal values of WBC and lymphocyte counts in childhood vary with age and tend to decrease with increased age.[30]

Coagulopathy, another reported adverse event of ASOs, was not observed in our study. In the NURTURE study and recently published studies, it was reported that nusinersen caused no significant abnormalities in coagulation parameters.[10,11,20] Our findings showed that nusinersen had no negative effect on coagulation parameters.

Our results showed that the CHOP-INTEND and HMSF-E scores of patients with SMA improved during follow-up with nusinersen treatment. In studies conducted to determine the natural history of CHOP-INTEND and HMSF-E scores in patients with SMA who did not receive treatment, a decrease in scores was observed in the follow-up,[31,32] which suggests that nusinersen significantly contributes to the quality of life of patients with its effect on motor functions. In addition, when we evaluated the effect of the age of treatment onset on the increase in motor scores of our patients. We found that the earlier the treatment was started, the more positive were the motor scale results. We observed that this effect appeared especially after the first four loading doses in patients with SMA type 1. Similar results have been obtained in recent studies.[33] This result once again demonstrated the importance of early diagnosis and treatment of SMA patients. However, another important point is that these patients will reach their maximum CHOP-INTEND and HMSF-E scores as they receive treatment. We think that these scoring methods will not objectively reflect the clinical conditions of patients after a certain upper level. Therefore, it is necessary to develop new markers for the typing, prognosis, and treatment response of patients with SMA who have significant changes in their clinical presentation with new treatments.

Conclusion

We detected no persistent or significant laboratory abnormalities related to treatment in our unit, where we have been administering nusinersen for nearly 4 years. The laboratory abnormalities of our patients who received treatment were mostly mild and caused no significant change in our treatment plan. Our findings suggest that nusinersen, unlike other ASOs, does not affect the liver, renal function tests, and coagulation parameters, suggesting that it is safe. We think that the need for frequent laboratory monitoring in patients receiving nusinersen should be reevaluated considering the development of anemia.

It was observed that there was a significant increase in CHOP-INTEND and HMSF-E scores as the patients received treatment. This situation supports the positive effect of nusinersen on motor milestones, and we think that these scores will be insufficient for follow-up in patients who will reach maximum scores with further doses.

Limitations

The most important limitation of our study is the sample size. Therefore, studies with larger samples are needed to better evaluate the efficacy and safety of nusinersen.

Conflict of Interest
None declared.

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