Assessment and molecular biomarkers in myotonic dystrophy type 1

Doctoral (PhD) thesis

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1. Introduction - Myotonic dystrophy

1.1. Epidemiology, genetic background and classification

Myotonic dystrophy (DM) is one of the most common adult-onset autosomal dominantly inherited muscle diseases. The worldwide prevalence is 5/100000, with significant variation between geographical areas.

Two subtypes can be distinguished on the basis of the genetic mutation underlying the disease, and this is the most widely accepted form of disease classification. Type 1 (DM1) is caused by a CTG trinucleotide expansion in the DMPK (dystrophia myotonica protein kinase) gene, while type 2 (DM2) is caused by CCTG tetranucleotide repeats in the ZNF-9 (zinc finger protein-9) gene. Within type 1, four further subgroups can be distinguished according to the onset, severity and progression of the disease, i.e. congenital, childhood, adult type and asymptomatic myotonic dystrophy.

1.2. Pathomechanism

The underlying pathomechanism, in type 1 and type 2, is a loss of function involving several different mRNA molecules due to disruption of the alternative splicing mechanism. The abnormal mRNA molecule, transcribed from the DMPK and ZNF-9 genes, is trapped in the nucleus where various proteins (so-called RNA binding proteins such as the MBNL family of proteins), which play a key role in the maturation of RNA molecules, bind to it, forming intranuclear foci. As a result, the post-transcriptional maturation of many mRNA molecules in which intranuclear RNA-binding proteins would play an important role in the splicing process is disrupted. Among the impaired proteins are CLCN-1 (chloride ion channel), troponin proteins (TNNT1,2,3), insulin receptor (IR), and titin (structural and functional sarcomeric protein).

1.3. Clinical picture

In DM1, the clinical picture is dominated by a distal focus, slow but steady progression of muscular atrophy and paresis, and a usually severe form of grip myotonia and associated facies myopathica, which give the patient a characteristic appearance. The progression of the muscle involvement eventually extends to the bulbar muscles, causing dysphagia and speech disorders. The consequent malnutrition and caloric deprivation lead to worsening of symptoms, and the risk of aspiration further increases the risk of respiratory failure in advanced patients, due to the already existing respiratory muscle involvement.

Perhaps the most characteristic symptom of the condition is myotonia, which is a difficulty in relaxing the muscles, with the patient unable to release the object held in the hand. In its background, a CLCN-1 mRNA splicing disorder is suspected. It is considered to be the greatest impact on patient's quality of life.

Muscle symptoms are associated with other abnormalities due to the multisystemic involvement of the disease, which are commonly referred to as extramuscular manifestations.

These include the damage to the conduction system of the heart, which results in a high rate of supraventricular arrhythmias, including a not insignificant number of atrial fibrillation and atrial flutter. It is worth noting that, according to the reports of previous studies, despite the high prevalence of these arrhythmias in the patient population, the underlying myocardial damage (ischaemia, inflammation, etc.) is not apparent.

Carbohydrate metabolism disorders are also common, mainly in the form of impaired glucose tolerance (IGT) or type 2 diabetes mellitus. It is explained by a splicing disorder

involving the insulin receptor mRNA molecule. The HOMA-IR index (Homeostasis Model Assessment - Insulin Resistance) can be used to monitor carbohydrate metabolism and has been shown in several studies to be higher in the patient group compared to the healthy control group.

Cataracts are common in patients, often developing at a very young age (3-4 decades) and in more than one case requiring early bilateral surgery.

Fat metabolism is also significantly affected. This is primarily hypertriglyceridemia, and to a lesser extent LDL-hypercholesterolemia, and overall shows a picture of a lipid metabolism disorder of an atherogenic nature. The exact mechanism of its development is not known.

The disease is also characterized by a restrictive pulmonary dysfunction due to the involvement of the respiratory muscles, resulting in a progressive decrease in static respiratory parameters and sometimes requiring oxygen support and NIV (non invasive ventilation). Based on the results of available studies, timely NIV may help to slow the progression of disease in this area and reduce mortality.

The vast majority of patients also experience sleep disturbances, as it is generally in the cases of neuromuscular diseases. The most common sleep disorders in DM1 are sleep-dependent breathing disorder (SDB), hypersomnia and restless leg syndrome. There is also a significant prevalence of obstructive sleep apnoea syndrome (OSAS).

It is known, the central nervous system is not unaffected in myotonic dystrophy. Most studies report diffuse, non-specific white matter lesions, the clinical significance of which is still unknown, as is the exact mechanism of their development. A significant proportion of patients, particularly in the childhood and adolescent forms, present with cognitive deficits and, in severe cases, mental retardation. Neuropsychological tests show that in the majority of cases, the impairment affects several cognitive domains simultaneously, with a high degree of individuality in severity.

Although it is less talked about, we should also mention the dysfunction of the endocrine glands, which mainly affect the adrenal glands, testes and thyroid gland, but the dysfunction of the hypothalamic-pituitary-adrenal axis has also been demonstrated. Early frontal baldness and abdominal-type obesity are considered to be typical manifestations of hormonal disorders.

The incidence of certain malignancies was found to be higher in DM1. These include, firstly, carcinomatous lesions of the skin, followed by malignant processes of the lung. Several studies also mention malignancies of the thyroid, endometrium, colon and testis.

In DM2, as in type 1, extensive alternative splicing disorder underlies the multisystemic symptoms. The main difference is that atrophy and paresis are predominantly proximal, muscle symptoms are milder than in DM1, and myotonia is less frequent. Multisystemic involvement is similar to DM1, but again the abnormalities are generally less severe.

1.4. Histopathology

Although a characteristic histological picture of DM1 would in many cases be sufficient for diagnosis, the role of muscle biopsy in the diagnosis of DM has nowadays diminished compared to the period before genetic tests were widely available. Histological findings are characterized by a high variability in muscle fiber diameter, ranging from a few micrometers to 100 micrometers. The leading variation is the muscle fiber atrophy with an accumulation of fat and connective tissue. DM1 is dominated by type I muscle fiber atrophy, DM2 by type II muscle fiber atrophy. Another characteristic feature is the high number of centralized nuclei (up to 50% of the nuclei, compared to less than 3% in healthy muscle). The histological picture is varied by the presence of regenerating fibers with basophilic staining, split fibers and the coexistence of fibrosis and fat deposition. The histopathological appearance of DM1 and DM2 is quite similar, the main differences being the predominance of vascularity of fibers I or II, the presence

or absence of angular type fibers in DM2. The histological picture, like the clinical picture, is characterized by a rather slow progression.

1.5. Diagnostics and patient care issues

Genetic testing is the basis for diagnosis. A fundamental problem in patient care today is the lack of a common, universally accepted standardized testing protocol for assessing and following patients. The importance of this is underlined by the multisystemic involvement described in previous chapters, which requires the early detection and treatment of a number of otherwise well-treatable co-morbidities in order to preserve patient's quality of life in an optimal way. There are currently no registered OTC therapies available, but a number of new drugs (mostly gene therapies) are in development with promising results. The expected expansion of the range of therapeutic options requires the identification of biomarkers that can be used to objectively measure disease severity and follow the effectiveness of therapy.

1.6. The present and future of therapy

In the development of therapeutic approaches for the treatment of disease, drug targets are the key steps of the pathomechanism, so we can basically talk about the following groups of therapeutic efforts under development: (1) mitigation of repeat instability; (2) promotion of degradation of mRNA molecules with abnormal structure; (3) suppression of aggregation of splicing regulator proteins; (4) correction of abnormal splicing mechanism; (5) modulation of factors responsible for alternative splicing mechanisms.

One of the main lines of drug development is ASO (antisense oligonucleotide) molecules, which are short, synthetic nucleic acids with modified structures. Their therapeutic potential has been raised in a wide range of diseases, including neurodegenerative diseases, malignancies and myotonic dystrophy. Another possible therapeutic direction seems to be the modulation of proteins key to alternative splicing mechanisms, in particular MBNL1, based on the fact that in myotonic dystrophy, sequestration of MBNL1 is known to play a fundamental role in the development of multiple mis-splicing events, but in a mouse model, overexpression of MBNL1 did not improve the pathological abnormalities of the muscle.

2. Assessment of muscle involvement in DM1 patients treated at the Department of Neurology of the University of Pécs

2.1. Aims

The aim of our studies was to assess the musculoskeletal involvement and motor performance of patients with myotonic dystrophy type 1, who are treated at the Department of Neurology of the University of Pécs.

2.2. Patients and methods

Our study included 31 patients. All patients were genetically tested for dystrophic myotonic type 1. The ratio of males to females in the study was 11:20, with a mean age of 43.2±14.1 years. The mean duration of disease was 15.8±12.2 years.

The inclusion criterion was a positive genetic test for the disease. Exclusion criteria were severe mobility impairment interfering with standard test performance, severe cardiopulmonary disease reducing exercise capacity, pregnancy, residual symptoms from other neurological disease that might affect the results of the tests. Participation in the study was voluntary. The control group, matched by sex and age, was selected on a voluntary basis from the staff of the Department of Neurology, who had no neurological symptom, no history of neurological disease, no other disease affecting the musculoskeletal system or reducing their exercise capacity.

The MRC (medical research council) scale, which is also used in standard neurological examination, was used, scoring the muscle strength between 0-5, where 5 indicates physiological muscle strength retained even against resistance, and 0 indicates complete absence of muscle contraction. After the traditional MRC scale was used, the results were converted to a 10-point scale to facilitate visualization of subtle differences, and this modified scale was used in the statistical analysis. For accurate measurement of the upper limb's distal muscle strength, a dynamometric measuring device was used and the grip strength was expressed in kg. The test was performed on both hands, using 3-3 measurements per hand, the results of which were averaged for each patient.

The 6-minute walk test (6MWT: 6 minutes walk test) was used to test the exercise capacity. Before the test was performed, the vital parameters (blood pressure, pulse, oxygen saturation) of the patients and an ECG were recorded at rest. To measure the distance, a 25 m long walking surface marked with cones at the ends and with a flat surface was used, and each walked 25 m section was recorded. Time was measured using a stropper watch. At the end of this time, or if the patient was unable to continue the test, the patient was stopped and the distance in meters between the last buoy crossed and the patient's finishing position was measured. Finally, the measured distances were added together to give the distance walked overall in meters in 6 minutes. At the end of the test and 30 minutes afterwards, the patient's vital signs were recorded again.

Fine motor performance was measured with the standard 9-hole peg test (NHPT), which requires the patient to insert 9 sticks into 9 holes on a specially designed surface, one by one, using only one hand. Then, after inserting all 9 sticks into the holes, the patient must remove the sticks from the holes one by one, again using only one hand. The time taken to complete the test was measured using a stopwatch (from the time you start inserting the sticks into the holes until the last stick is removed). The test was performed by patients with both hands.

To test for myotonia, we used relaxation tests accepted in the international literature: opening and repeatedly clenching the fist ten times, extending and retracting the tongue ten times, and closing and opening the eyes ten times. Time was also measured using a stopwatch during the tests. Test subjects were asked to perform the tasks continuously from the moment the stopwatch was started until it was completed ten times. After completing the last, tenth item of the task, the stopwatch was stopped and the time taken to complete the test was recorded. The ten-fold fist opening and closing test was performed by the patients with both hands separately.

The MIRS scale was used to determine the severity of the disease. Its scoring was based on the results of the muscle examination during the standard neurological examination, using the standard scoring system of the scale. Based on this, the severity of muscle involvement was graded into grade1-grade 5 groups.

2.3 Results

MMT results show that both the upper and the lower limbs are more severely affected in the distal muscle groups. In the upper limb, finger abduction (5.5±1.52) and adduction (5.5±1.54) were the most affected, while in the lower limb, dorsiflexion (6.7±2.54) and plantarflexion (7.1±2.36) were the most affected. In addition, it can be seen that the values obtained for the proximal muscles (shoulder and hip) on the ten-point scale are at or above eight, which also means that there is no significant paresis in these muscle groups. Also noteworthy is the weakness of the cervical flexors, which severity is between the proximal and distal muscles (8.0±2.0). Overall, both proximal and distal muscle involvement is seen, consistent with the long duration of the disease in our patients, so that involvement of several muscle groups is already evident.

According to the MIRS scale, only a few of our patients were classified as Grade 1 (6%), most of them were classified as Grade 2 (26%) with marked distal muscle involvement and Grade 3 (26%) with moderate clinical presentation. The MIRS result is in line with the MMT test results, as the Grade 3 classification is based on proximal muscle involvement. Consistent with the relatively long duration of the disease in the patient population, almost half of the patients (42%) were in the Grade 4 (19%) and Grade 5 (23%) groups with a severe clinical picture.

Since, in agreement with the literature, our MMT also showed a more severe involvement of the distal muscles, we also performed a dynamometric measurement of these muscles using a qualified instrument (a grip strength meter). The grip strength was measured in kg. All our patients were right-handed. In the patient population, the mean grip strength of the subdominant left hand was 10.4±6.16 kg, whereas the mean grip strength of the dominant right hand was 10.8±6.07 kg. Both are significantly lower than the mean values in the healthy population, where the grip strengths of 25-30 kg are usually measured for women and 35-40 kg for men. There was no significant difference between the dominant and subdominant sides.

During the 6MWT, the average value for women was 336.1±165 m, for men it was 360.3±185.7 m. Both are significantly below the mean values reported in the literature for the healthy population. The difference between the sexes is similar to that observed in the survey of healthy individuals cited as a reference.

The relaxation tests used to test myotonia show the longest time for clenching and releasing the hand, consistent with the fact that these muscle groups are mostly affected by myotonia. Values of 10.4±3.4 s were obtained for 10 tongue stitches, 15.4±11.2 s for 10 fist openings and 11.2±3.7 s for 10 eye openings. There was no difference between the sexes.

In the NHPT test for fine motor control of the small hand muscles, significantly higher values were measured compared to the standard values for both sexes and for both dominant

and subdominant hands. Female patients completed the task in 26.1±5.47 s for the dominant (right) hand and 26.5±5.48 s for the subdominant (left) hand, while male patients performed in 31.2±9.68 s for the dominant (right) hand and 35.1±19.9 s for the subdominant (left) hand. No significant difference was found between the subdominant and dominant hand.
There is no significant difference between the dominant and subdominant sides by gender. However, it is observed that the side difference is not present at all for females, while males perform worse with the sub-dominant hand. Men generally performed worse with both hands.

2.4. Discussion

Our results showed a predominance of distal muscle group involvement in both the upper and lower limbs, in line with international literature. Distal muscle involvement was found to be more severe in the upper limb than in the lower limb. When measuring grip strength, patients performed well below normal for both dominant and subdominant limbs. No significant difference was seen between the two sides, with patients - both men and women - producing slightly higher grip strength with the right hand. It should be added that all our patients were right-handed, i.e. the visible difference can be considered physiological, as the grip strength of the dominant hand is slightly higher in all humans.

In the relaxation tests, myotonia of the small hand muscles was found to be the most severe, with the results of the ten-fist-open test being the worst for both men and women. However, the effect of myotonia was less severe but was also clearly seen in the eye-opening-closing and tongue-extension tests in the majority of patients.

For the NHPT test, both male and female patients showed significantly worse results than adult normals. Men also performed worse than women on the dominant and subdominant sides. There was no significant difference between the right and left sides for either sex.

During the 6MWT, both male and female members of the patient group performed below the expected average. Pre- and post-test parameters did not indicate a high degree of cardiopulmonary system stress, suggesting that the much lower score seen may be a consequence of the musculoskeletal manifestation of the disease.

2.5. Conclusions

The performed tests yielded the results expected based on the international literature. They may be suitable as a standard, systematic assessment of the DM patient population. The tests have the advantage of low instrument requirements and low costs, and do not require a specialized center. An additional advantage is that by following these protocols, results can be standardized and well followed up in control studies. The disadvantage is that most of the tests are influenced by a number of conditions, which always must be clarified and the values should be interpreted with caution.

3. Investigation of the role of urinary titin as a biomarker in myotonic dystrophy type 1

3.1. Introduction

Titin is one of the largest proteins in the human body, encoded by the TTN gene. Its molecular weight is 4200 KDa and its length can be up to 1 micrometer. Its structure is extremely complex, containing tens of thousands of amino acids. It plays a role in forming stable connections between muscle fibers, regulating muscle contractility and ensuring muscle elasticity.

Mutations in the TTN gene cause rare, dominantly inherited distal myopathy (TMD, tibial muscular dystrophy), with some variants being identified in a large number of genetically defined cases of dilated cardiomyopathy. Recently, urinary titin fragments have come to the fore as a new biomarker of muscle disease in both muscular dystrophies and cardiomyopathies. From previous studies, urinary titin concentrations in muscular dystrophies appear to be significantly higher compared to the healthy population, and other data suggest that they may be associated with the severity of the muscular involvment. Higher concentrations have also been observed in patients with motor neurone disease and in healthy individuals after significant physical exertion.

3.2. Aims

In DM, as in other neuromuscular diseases, there are no clinically useful biomarkers of disease activity and severity. The commonly used CK value may be influenced by a number of other factors unrelated to the disease and, as a chronic muscle disease with slow progression, high CK levels after a certain period of time are no longer measurable and informative.

The aim of our study was to determine a laboratory-quantifiable biomarker of disease severity in patients with myotonic dystrophy type 1.

3.3. Patients and methods

The study was approved by the regional ethics committee and included 29 patients with myotonic dystrophy type 1. The male/female ratio was 10/19 and the mean age of the patients was 44.36 ± 14.24 years. The majority of patients had a long disease duration, with a mean duration of 18.1±13.9 years.

Blood and urine samples were taken from the patients during fasting blood sampling on arrival. Urine was stored in a dry and dark room for 12 hours (from 7 pm to 7 am) for patients and control subjects. Routine laboratory tests (electrolytes, liver, kidney function, thyroid-stimulating hormone (TSH), creatinine kinase (CK)) were performed, and urinary creatinine was determined from the collected urine. Additional urine samples were frozen and stored at -80°C. After collection of all samples, urinary titin N-terminal fragment (UNT) was determined using a commercially available ELISA kit according to the manufacturer's instructions (Immuno-Biological Laboratories, Japan). Titin concentration was normalized to urinary creatinine (Cr) concentration, resulting in a pmol/mgCr ratio.

Additional clinical parameters were also collected. Both proximal and distal muscle strength were measured using manual muscle testing (MMT) and patient’s muscular symptoms were assessed using the Muscle Research Council (MRC) scale. Grip strength was measured

with a commercial dynamometer (Kern und Sons) (for better objectivity (given that it cannot be adequately assessed on the MRC scale)). The DM1-activ questionnaire was used to estimate the impact of the disease on quality of life and patient's daily routine. This is a 20-item scale, where a lower score indicates a more severe condition. The possible responses for each item are: 0: impossible to perform, 1: difficult to perform, 2: easy to perform. The Muscle Impairment Rating Scale (MIRS) was used to score patients according to the severity of muscle impairment. I have previously written more about the construction and use of the scale. The MIRS is a 5 point scale that essentially models this type of progression to some degree. A grade 1 is defined as no symptoms of muscle involvement. Grade 2 includes weakness of the facial muscles, neck muscles, finger flexors affected at an early stage and weakness of the finger flexors. If the distal limb muscles and the elbow extensors of the proximal muscles are also affected, we can speak of grade 3. Grade 4 is when mild to moderate weakness of the proximal muscles is present. In grade 5, the proximal muscles are already severely affected, with a muscle strength of 3/5 or less. The grades are determined by manual muscle strength testing (MMT) and the grading provides information on the stage of the disease.

Since the Titin/Cr ratio was not normally distributed, a Mann-Whitney U test was performed. Vargha and Delaney A test was calculated to measure the effect size. Correlation was determined by calculating Kendall's τ correlation coefficients. A strong or very strong correlation was defined as 0.70-1.00, a moderate correlation as 0.40-0.69 and a weak correlation as 0.10-0.39. A significance level of $P = 0.05$ was chosen, p values below this level were considered significant. Kendall's τ correlation coefficients were calculated to test the correlations between urinary titin/Cr ratio and the following measures: MIRS scale score, age at time of measurement, disease duration, right and left hand grip strength, DM1-active score, and CK value. The calculated p-values were corrected for multiple comparisons using Holm-Bonferroni correction.

3.4. Results

According to the MIRS scale, our patients were at a moderately advanced stage (MIRS 2-4). The DM1 activity questionnaire scoring quality of life indicated moderate functional impairment (DM activity between 16 and 40 out of 40).

The urinary Titin/Cr ratio was significantly higher in samples from the DM1 patient group compared to controls (median±MAD: patient: 39.313±26.546 pmol/mgCr and control: 6.768±5.245 pmol/mgCr, $p<0.0001$). Vargha and Delaney A value $A = 0.011$, indicating a large difference between the control and patient groups. Sensitivity and specificity of the measurement at a cut-off value of 12.267 pmol/mgCr were 0.966 and 0.967, respectively, with $AUC = 0.989$.

Following the above, further data analysis was carried out. Within the patient group, patients were divided into groups according to their score on the MIRS scale, Grade 1 to Grade 5 severity. There were no patients in the patient group classified as Grade 1 at the time of the present study. The urine Titin/Cr ratio showed a significant difference between the MIRS scale subgroups of the patient group ($r = 0.503$; $p = 0.038$). More severe disease was associated with a higher urine Titin/Cr ratio.

The Titin/Cr ratio was also compared with various other clinical parameters. Significant positive correlation was found only between Titin/Cr ratio and MIRS, no correlation was found for other parameters.

3.5. Discussion

There is a significant amount of research to identify biomarkers in neuromuscular diseases, and the importance of this is underlined by the fact that an increasing number of therapeutic options for diseases such as DM are now in the experimental phase, and we can expect to have the possibility to treat patients in the future. For this reason, it is particularly important to be able to monitor the condition of our patients, the severity of their disease, its activity and the degree of progression.

In our study, we attempted to identify the biomarker corresponding to the above. We chose the molecule titin, which was measured in urine, standardized as Titin/Cr ratio as seen in recently published studies.

Our results are in line with the international literature, where it has been found that muscular patients have higher Titin/Cr levels compared to healthy individuals. We could confirm that a significantly higher Titin/Cr ratio was measured in the DM1 patient population compared to healthy control subjects. It can also be shown that the Titin/Cr ratio shows a significant positive correlation with the disease severity represented by the MIRS scale, i.e. a higher MIRS score (more severe disease) is associated with a higher Titin/Cr ratio. However, the lack of correlation between the Titin/Cr ratio and serum CK value may confirm our previous observation that CK does not correlate with the clinical picture and it is not a good predictor of disease severity and progression.

In the context of the results of our study, we must mention the limiting factors. This was a cross-sectional study with a small number of patients. Based on these results, we cannot assess the role of the urinary titin on disease activity and progression. Longitudinal follow-up is needed to investigate how the urinary titin/Cr ratio changes in the early and advanced stages of DM1. Further studies are also needed, including a large population of patients, age-matched, to clarify the extent to which physiological sarcopenia influences the results.

3.6. Conclusions

Based on our results, titin as a biomarker molecule appears to be suitable for assessing disease severity in myotonic dystrophy type 1. However, its value is influenced by several factors (age, increased muscle stress, e.g. exercise, trauma, etc.), and the exact effect of these factors is subject to further investigation. Further longitudinal studies are also needed to assess the usefulness of titin in monitoring disease progression and activity. These studies are currently ongoing in our working group.

4. Risk factors for ischaemic stroke in patients with myotonic dystrophy type 1

4.1. Introduction

Stroke is the leading cause of disability and incapacity for work worldwide. Basically, two forms are distinguished, such as ischaemic and haemorrhagic stroke. Ischemic stroke accounts for approximately 85 percent of all strokes, while haemorrhagic stroke is much less common, accounting for only about 15 percent of all strokes. Among the main risk factors for ischaemic stroke are hypertension, type 2 diabetes mellitus, obesity, athero-, and arteriosclerosis and various coagulation disorders that increase the chance of clot formation. The most important risk factor for haemorrhagic stroke is the (untreated) hypertension. The etiology of ischaemic stroke can be divided into 6 groups: (1) cardiogenic embolism, (2) large vessel atherosclerosis, (3) arteriolosclerosis, (4) immunological origin, (5) inherited coagulation disorders with increased clot formation and (6) cryptogenic stroke.

In the myotonic dystrophy type 1 patient population, almost all risk factors for ischaemic stroke are present, such as supraventricular arrhythmias, dyslipidemia and type 2 diabetes mellitus. Biller and his colleagues in the UK studied the risk of cerebral infarction in 131 patients with neuromuscular disease (52 Duchenne muscular dystrophy, 61 myotonic dystrophy, 14 Becker muscular dystrophy, 4 Friedreich's ataxia). Atrial fibrillation was identified in 3 patients and definite cerebral infarction was described in 2 patients (1 myotonic dystrophy, 1 Friedreich's ataxia). Supraventricular arrhythmia was detected in both patients with stroke. The results reported by Wahbi et al. showed an 8.5% incidence of atrial flutter in patients with myotonic dystrophy type 1, while the incidence of ischemic stroke in the same population was 3.3%. In another study published by Yoshida et al. in 2018, 72 patients out of 108 patients with myotonic dystrophy type 1 had a cranial MRI, which confirmed ischaemic lesions in 4 cases. One case was due to lacunar infarction, 1 case to cardiogenic embolization, and 2 cases were of unknown etiology. Routine electrocardiography in 3 patients and 24-h Holter ECG in two others demonstrated supraventricular arrhythmia, atrial fibrillation and atrial flutter. For 70 patients, the CHA_2DS_2 and CHA_2DS_2 -Vasc score was calculated and found that for CHA_2DS_2 , 13 patients scored 1, 10 patients scored 2, and 1 patient scored 3, whereas for CHA_2DS_2 -Vasc, 36 patients scored 1, 11 patients scored 2, 8 patients scored 3, and 1 patient scored 4. Based on their results, they conclude that the supraventricular arrhythmia may be a significant risk factor for ischemic stroke in patients with myotonic dystrophy type 1.

4.2. Aims

The quality of life of patients can be significantly impaired by possible cerebrovascular events, exacerbating the disability resulting from the muscular disease. Our study assessed the presence of risk factors for ischaemic stroke in patients with myotonic dystrophy type 1.

4.3. Patients and methods

We investigated the known risk factors for ischemic stroke in patients with myotonic dystrophy type 1, verified by genetic testing, managed by the Department of Neurology of the University of Pécs. 31 patients (10 men, 21 women) were involved in our study.

A detailed medical history was taken of the patients, including alcohol consumption and smoking habits, as well as their history of hypertension and diabetes. Body weight and height were determined in all patients and used to calculate body mass index (BMI). ECG examination and transthoracal echocardiography were also performed as part of the cardiological examination.

Following the above, fasting cholesterol, LDL, HDL cholesterol and triglyceride levels were measured in laboratory tests for fat metabolism, while fasting blood glucose levels, HbA1c and plasma insulin concentrations were determined in addition to fasting blood glucose levels to assess carbohydrate metabolism. The reference values of the Department of Laboratory Medicine of the University of Pécs were used as reference for the evaluation of the results of the laboratory tests.

To assess the vascular status, carotid duplex ultrasound was performed in all patients. This involved longitudinal B-mode measurements of the intima-media thickness on the dorsal wall of the common carotid artery 1 cm below the bifurcationon, on both sides, and averaging the values to calculate the mean intima-media thickness (IMT). As a reference, we used the results of Simone Nascimento dos Santos and colleagues in 2019, which showed that the abnormal intima thickness is defined as 1 mm or above. It should be noted that, according to the principles in our country, an IMT value of 0.7 mm and above is considered as abnormal intima thickness.

Statistical calculations were performed using IBM SPSS v. 28 (IBM Co., Armonk, New York). Statistical analysis included descriptive statistical analysis for both the patient and healthy control groups and Pearson's correlation test to examine the relationship between each parameter.

4.4. Results

The patient population consisted of 31 patients with an average age of 43.71±14.04. The study included 10 males (32.3%) and 21 females (67.7%). Patients had a mean BMI of 25.9±5.3, which is above the accepted BMI of 24.9, 45.2% of our patients was in the overweight category. The mean abdominal skinfold was 21.54±10.41 mm. The patient population was characterized by a mean duration of 13 years of disease.

Out of the 31 patients, 11 patients were smokers, 20 were non-smokers, 11 patients consumed small amounts of alcohol regularly, and 20 patients negated alcohol consumption. Four cases (12.9%) had a known history of type 2 diabetes mellitus, all four of which were treated with oral antidiabetics. Type 1 diabetes mellitus was not present in any of the patients. Five patients (16.1%) received oral antihypertensive treatment.

ECG examination revealed supraventricular arrhythmia in 5 patients (16.1%), atrial fibrillation in 3 (9.7%), atrial flutter in 1 (3.2%) and frequent supraventricular extrasystoles in 1 patient (3.2%). Ventricular arrhythmias were not recorded in any patient.

In laboratory tests (mean±sample), total cholesterol: 5.69±1.12 mmol/L, LDL cholesterol: 3.60±0.94 mmol/L and triglyceride: 2.59±1.34 mmol/L were higher than the upper limit of the normal range for fat metabolism. Of these results, triglyceride is noteworthy, since while the other parameters, although above the upper limit, were close to it (cholesterol 109%, LDL 106%), triglyceride showed an increase of one and a half times (153%) compared to the

normal value. The HgbA1c value of our patients in the context of carbohydrate metabolism was 5.93±0.97, which is also above the upper limit of the accepted normal range.

The mean intima-media thickness was 0.58±0.12 mm in female patients and 0.64±0.11 mm (mean±standard deviation) in male patients. No patient had an intima-media thickness of 1 mm or more, defined as pathological in the above-mentioned reference publication, and only one patient had a measured value exceeding the 0.7 mm limit routinely used in Hungary.

Further statistical analysis revealed that there was no significant correlation between triglyceride and intima-media thickness (0.69; p:0.71) for the whole population; between intima-media thickness and other fat metabolism parameters (total cholesterol, LDL, HDL), and between triglyceride and BMI, triglyceride and abdominal skinfold values. However, it is worth noting that a significant positive correlation was found for BMI and abdominal fat (0.49, p:0.005), suggesting abdominal obesity and fat accumulation, a subclinical symptom of metabolic syndrome, and a change in body composition.

4.5. Discussion

In our study, we performed a physical and laboratory examination of 31 patients with myotonic dystrophy type 1 and a detailed review of their medical history, with particular attention to the risk factors for ischaemic stroke. In harmony with the international literature, our results show that abdominal obesity, dyslipidemia (elevated LDL and triglycerides) and moderately elevated HgbA1c, are also present in patients with myotonic dystrophy type 1. Our results suggest that the prevalence of type 2 diabetes mellitus is higher in myotonic dystrophy type 1 patients. While the prevalence of type 2 diabetes in the general population ranges from 8-10% (National Health Interview Survey: 8.5%, Center for Disease Control and Prevention Diabetes Surveillance System: 10.5%), in our patients with DM1 this value is 12.9%.

Although the presence of well-established vascular risk factors underlying ischaemic stroke can be demonstrated, the consequent atherosclerotic lesions that may lead to stroke cannot be detected, and normal intima-media thickness was measured in our patients during standard carotid ultrasound examination. This may be explained by the relatively high number of young individuals among our patients. Despite the low number of patients, we were able to confirm cardiac arrhythmias in 5 cases (16.1%) during routine ECG examinations, which underlines the need for regular screening for cardiac arrhythmias.

These results, although based on a study with a small patient population, support the idea based on literature data that, although a large number of factors contributing to the development of atherosclerotic vascular disease are present in myotonic dystrophy, but the supraventricular arrhythmias (atrial fibrillation and atrial flutter) may be the most important risk factors for ischemic stroke.

4.6. Conclusions

Our results highlight that in the care of patients, cardiological follow-up examinations, periodic ECG and echocardiography examinations are as important as the monitoring of patient's neurological status. The long-term management of detected cardiac arrhythmias is also a complex issue that requires consultation between neurologist and cardiologist colleagues, as secondary stroke prevention in the general population with supraventricular arrhythmias, i.e. anticoagulant therapy, may be associated with an increased risk of bleeding in patients with type 1 DM due to motor deficits and balance disorders. The way forward is probably to screen these patients and, if it is possible, to treat cardiac arrhythmias by electrophysiological methods, which may represent successful stroke prevention without the need for anticoagulation therapy.

It is important to emphasize that regular cardiovascular risk screening of patients is essential, as well as the early intervention in case of detected abnormalities to prevent the long-term complications.

5. Summary of results

We performed detailed clinical and laboratory examinations of patients with myotonic dystrophy type 1 managed by the Department of Neurology of the University of Pécs. In addition to assessing the motor performance of the patients, our aim was to identify laboratory biomarkers reflecting the activity and severity of the disease and to map the risk factors for ischemic stroke in the patient population.

1. We have developed a standardized health assessment system that we have been using for years to assess the patient's current condition at the time of diagnosis and follow up patients.

2. For our biomarker identification studies, we chose to determine titin from urine, using the titin/Cr ratio as the standard measurement. As a new finding, we found that the Titin/Cr ratio in urine samples of patients was significantly higher than in the sex and age-associated control group, i.e. the urinary Titin/Cr ratio was associated with the presence of muscular dystrophy.

3. In addition, there was a significant positive correlation between the MIRS scale of disease severity and the Titin/Cr ratio, i.e. the more severe the patient's musculoskeletal symptoms, the higher the urinary Titin concentration. In the light of these data, we can conclude that, as in other neuromuscular diseases, the role of titin as a potential biomarker representing disease severity is also emerging in patients with myotonic dystrophy type 1. Further studies with larger case numbers are needed to confirm this. To clarify the relationship between disease activity and urinary titin concentration, longitudinal studies are needed and are already underway in our working group.

4. With regard to the risk factors for ischemic stroke, our studies have shown that factors that are part of multisystemic involvement and that are associated with a high vascular risk, such as hypertriglyceridemia, IGT or type 2 diabetes mellitus, and obesity, are present in a high proportion of patients, but atherosclerosis does not seem to be significant. On the other hand, a high rate of cardiac arrhythmias in the patients wiht DM1, manifested as supraventricular arrhythmias, is observed. Thus, the high risk of stroke may be primarily due to cardiogenic embolization rather than to the presence of risk factors leading to atherosclerotic lesions.

6. List of publications

6.1. Publications on which the doctoral thesis is based

1. Varga, D., & Pál, E. (2019). Multiorgan manifestations in myotonic dystrophy type 1. Orvosi Hetilap, 160(37), 1447-1454.
2. Varga, D., Perecz, B., Sípos, A., Jedlicska, D., & Pál, E. (2022). Risk factors for ischemic stroke in myotonic dystrophy type 1. Orvosi Hetilap, 163(49), 1962-1966.
3. Varga, D., Perecz, B., Fülöp, K., Sipos, A., Janszky, J. V., Hajdú, N., & Pál, E. (2023). Urinary titin in myotonic dystrophy type 1. Muscle & Nerve. (DOI: 10.1002/mus.27917)
4. Kupó, P., Fődi, E., Debreceni, D., Pál, E., Faludi, R., Tényi, D., & Simor, T. (2021). Successful termination of ventricular arrhythmias with implantable cardioverter defibrillator in a patient with myotonic dystrophy. Orvosi Hetilap, 162(46), 1856-1858.

6.2. Other publications

1. Makó, T.; Pintér, D.; Varga, D.; Pál, E.; Aschermann, Zs.; Molnár, G. A.; Fülöp, G.; Szalma, K.; Kovács, N., Wittmann, I. (2021). How common is Parkinson's disease in type 2 diabetes mellitus? Diabetologia Hungarica, 29:Suppl.1 pp. 39-40., 2 p.

6.3. Lectures related to the topic of the doctoral thesis

1. Varga, D.; Perecz, B.; Pál, E. (2022) Risk factors for ischemic stroke in dystrophic myotonic type 1. 38th Congress of the Hungarian Neurological Society, Budapest, 16.06.2022-18.06.2022.
2. Varga, D.; Sípos, A.; Pál, E. (2022). Biomarkers in dystrophic myotonic type 1. XIX Congress of the Hungarian Clinical Neurogenetics Society, Kecskemét, 2022.09.09.-2022.09.10.

6.4. Posters related to the topic of the doctoral thesis

1. Varga, D.; Perecz, B.; Sipos, A.; Fülöp, K.; Pál, E. (2022) Urinary Titin as a Biomarker of Myotonic Dystrophy Type 1. International Congress on Neuromuscular Diseases, Belgium, Brüsszel, 2022.07.05.-07.09.

6.5. Other lectures

1. Varga, D.; Makó, T.; Ointér, D.; Pál, E.; Molnár, G.; Fülöp, G.; Szalma, K.; Wittmann, I.; Kovács, N. (2021). Pathological metabolites in Parkinson's disease. Hungarian Scientific Parkinson's Society Online Conference 2021, 2021.05.28.-2021.05.29.
2. Makó, T.; Varga, D.; Pál, E.; Aschermann, Zs.; Molnár, G.; Fülöp, G.; Szalma, K.; Wittmann, I.; Kovács, N.; Pintér, D. (2021). How common is Parkinson's disease in type 2 diabetes? Hungarian Scientific Parkinson's Society Online Conference 2021, 28.05.2021-29.05.2021.
3. Makó, T.; Pintér, D.; Varga, D.; Pál, E.; Aschermann, Zs.; Molnár, G. A.; Fülöp, G.; Szalma, K.; Kovács, N., Wittmann, I. (2021). How common is Parkinson's disease in type 2 diabetes mellitus? XXIX Congress of the Hungarian Diabetes Association, Pécs, 2021.09.02.-2021.09.05.

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