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RESEARCH ARTICLE

Normal Appearing White Matter N-Acetylaspartate Changes Impact on Fatigue in Multiple Sclerosis

Vasilena Petrova, Latchesar Traykov

Military Medical Academy, Clinic of Nerve Diseases, Sofia, Bulgaria
University Hospital Aleksandrovska, Clinic of Neurology, Sofia, Bulgaria

***Corresponding author:** petrovadata@gmail.com

ABSTRACT

***Background*:** Fatigue is one of the most frequent complaints presented by multiple sclerosis patients. Fatigue may be multifactorial. Proton magnetic resonance spectroscopy studies have shown significant reductions in N-acetylaspartate/creatine ratios in multiple brain regions among fatigued multiple sclerosis patients in comparison to non-fatigued multiple sclerosis patients, suggesting axonal loss as a contributing factor. Females are twice as likely to develop the disease.

Aim: To evaluate gender variability in fatigue scores in relapsing-remitting multiple sclerosis patients. To explore potential gender differences in metabolite profiles of normal appearing white matter. To correlate metabolite changes distribution with fatigue severity and to evaluate the gender impact.

***Methods*:** We enrolled 50 relapsing-remitting multiple sclerosis patients on disease modifying treatment and 28 healthy controls. All participants underwent proton magnetic resonance spectroscopy of normal appearing white matter corresponding regions and fatigue severity evaluation.

Results: We found higher fatigue scores in the multiple sclerosis group, due to greater severity in female subjects. We found a significant decrease of N-acetylaspartate/creatine ratio with increase in N-acetylaspartate, choline, and creatine levels in multiple sclerosis subjects. N-acetylaspartate and choline levels were significantly higher in the multiple sclerosis males. Female multiple sclerosis patients presented with lower N-acetylaspartate levels than healthy controls and greater increases in Fatigue Severity Scale score. Regression analysis revealed metabolite specific relationships between fatigue against metabolite variables.

Conclusion: Proton magnetic resonance spectroscopy registered differences in metabolite profiles in normal appearing white matter male and female multiple sclerosis subjects. We might presume gender dependent specifiers in metabolite profiles in relapsing-remitting multiple sclerosis. They impact fatigue severity. N-acetyaspartate might be crucial contributor in central fatigue in multiple sclerosis. Bioenergetic role of N-acetylaspartate needs further collaborative research on genetics and electrical properties of neurons to reveal the underlying mechanism of fatigue and conductivity deterioration.

Keywords: fatigue, spectroscopy, metabolite, multiple sclerosis, gender

Introduction

Fatigue is one of the most frequent complaints presented by multiple sclerosis (MS) patients Fatigue is a subjective symptom. There is neither a unified definition, nor gold standard by which to measure fatigue. Patients describe fatigue as lack of physical and/or mental energy. We assess fatigue through patient interview and questionnaires[1,2]. Fatigue in MS patients may be multifactorial[3]. It is defined as central and peripheral in consideration of pathogenesis. Muscles and related tissues performance insufficiency cause peripheral type of fatigue. Central fatigue develops in the central nervous system. Central fatigue is described as decline in cognitive task performance, changes in motivation, effects of fatigue on central nervous system (CNS) function, or CNS causes of fatigability[4]. Physical fatigue presents as physical exhaustion. Muscle weakness could cause a decline in motor performance. Cognitive fatigue starts independently from the physical disability in the initial stages of MS. It might manifest the pre-diagnostic phase of the disease[5,6]. Cognitive fatigue is defined as a decline in performance during cognitive activity. It is characterized with difficulty in concentration, memory deterioration, and emotional instability[2]. Fatigue is considered a sequel from immune system involvement or central nervous system damage. Proinflammatory cytokines, endocrine influences, axonal loss, and altered patterns of cerebral activation are considered specific reasons. Cytokines involved in disease mechanism are considered to be strong mediators of fatigue. The hypothalamic-pituitary-adrenal (HPA) axis and the hormone dehydroepiandrosterone (DHEA) have been studied in multiple autoimmune diseases in which fatigue is a common symptom. Although definitive conclusions cannot be drawn based solely on these studies, these results suggest a possible endocrine contribution to fatigue in MS. A hypothesis postulates the potential of reduced monoaminergic release in the central nervous system to impact motivation, mood, and attention[7]. Depression commonly accompanies MS, with a prevalence of up to 50%. Depression itself can manifest with fatigue and symptoms often mistaken for fatigue (loss of motivation, anhedonia), making this condition difficult to sort out from MS-associated fatigue[3]. Proton magnetic resonance spectroscopy (PrMRS) studies have shown significant reductions in N-acetyaspartate/creatine (NAA/Cr) ratios in multiple brain regions among fatigued MS patients in comparison to non-fatigued MS patients, suggesting axonal loss as a contributing factor. A study on diffuse microstructural white matter damage concluded that fatigue is a consequence of normal appearing white matter (NAWM) damage[8]. We evaluated changes of brain metabolites profile in NAWM and found gender differences[9]. Our findings motivated further research on the relation between metabolites behavior and fatigue.

Materials and methods

1. STUDY DESIGN AND POPULATION

We recruited subjects (age range 20-40 years) specified with Relapsing Remitting Multiple Sclerosis (RRMS) diagnosis, according to the revised criteria of McDonald. Patients with a history of the disease shorter than 10 years were selected. The group of the patients studied was compared to a group of healthy individuals, selected according to the corresponding demographic criteria. All participants signed written informed consent for study enrollment. We performed all the physical, neurological and PrMRS studies on a single day and at a similar time for each patient.

2. NEUROIMAGING

Proton Magnetic Resonance Spectroscopy

We applied proton magnetic resonance spectroscopy for measuring choline (Cho), creatine (Cr) and N-acetylaspartate (NAA) in parts per million (ppm). Protocol for PrMRS was conducted, used in the Imaging Diagnostics Department of Sv. Ivan Rilski University Multiprofile Hospital for Active Treatment, We examined frontoparietal zones of NAWM, free of lesions of demyelination on conventional magnetic resonance T2 normal images in the MS group. We selected corresponding subcortical zones of interest in the healthy controls group.

3. FATIGUE SEVERITY SCALE

Fatigue Severity Scale (FSS) was applied for self-measuring fatigue. The scale comprises nine questions of self-evaluation of fatigue severity and its impact on daily routine activity for the week, preceding the day of evaluation. It estimates symptoms by 7 points Likert scale (1- strongly disagree to 7- strongly agree). The result varies between 9 and 63 points (pts). The higher the result the more severe is the fatigue. Another way of scoring is to find the mean value of all the scores with minimum score being one and maximum score being seven. MS patients usually estimate fatigue severity up to 6,5 pts.

3. BECK'S DEPRESSION INVENTORY

Beck's Depression Inventory (BDI) is a questionnaire designed by Aaron T. Beck for depression screening in 1961 and revised in 1971 (BDI IA). It consists of questions for multiple- choice self- evaluation and includes both cognitive and somatic symptoms of depression. The subjects estimate the relevance of

each statement based on the previous week including today. The severity of symptoms ranges from the absence of a symptom to an intense level. BDI is one of the most widely used self-report inventories to assess depressive symptom severity in adolescent and adult populations[10]. Items are rated on a 4-point (0 to 3) scale, with total scores obtained by summing the ratings for all items. BDI-II (1996) has the same number of items and four response choices. The subjects indicate the one which best describes how they felt during the past two weeks including today. Scores ranging between 0 and 13 are indicative of none or minimal depression; scores that fall between 14 and 19 are considered to reflect a mild level of depression; scores of 20 to 28 are considered moderate depression; and a score ranging from 29 to 63 is labeled severe.

STATISTICAL ANALYSIS

We analyzed data with the Independent Samples T-test and The Mann-Whitney test. Pearson correlation analysis was used to evaluate the relationships between the metric variables, which comprise Cho, Cr and NAA levels, FSS and BDI scores. Statistical analysis used statistical software IBM SPSS Statistics v23. We considered a p value less than 0.05 statistically significant.

Results

We recruited 50 patients with relapsing remitting multiple sclerosis (RRMS) and compared them to 28 healthy controls (HC). The mean age was 33±5 and 33±7, respectively. Table 1. presents the characteristics of the studied groups.

Table 1. Study population

▪ DEMOGRAFIC DATA	HC	RRMS	P-value Patients
with clinically proven RRMS	N/A	+	
▪ age between 20-40 years	33±5	33±7	1.000
▪ time elapsed since specifying **the diagnose up to 10 years**	**N/A**	**4,85±2,68**	
▪ Interferon beta- 1a treatment	N/A	2.40±2.21	
▪ evidence of relapse **over the last 6 months**	**N/A**	**–**	
▪ Corticosteroid administration **at least 2 months prior the study**	**N/A**	**–**	
▪ other autoimmune disease	–	–	
▪ history of specified diagnosis depression, **severe depressive episode and/or treatment with antidepressants for symptoms of depression found**	**–**	**–**	
▪ active inflammatory process	–	–	
▪ treatment with another disease-modifying therapy	–	–	
▪ motor deficit, visual or auditory disorders	–	–	
▪ alcohol or psychotropic medications **and substances abuse**	**–**	**–**	
▪ clinically healthy individuals	+	+	
▪ **Participants**	**28**	**50**	**0.502**
male	14	21	
female	14	29	
▪ EDSS up to 3.5	N/A	+	
▪ **Education**			
higher	21	30	0.182
secondary	7	20	
▪ **Working activity**			
normal WH	28	44	0.728
flexible WH	–	6	

HC- healthy controls; RRMS- patients with relapsing- remitting multiple sclerosis; EDSS- Expanded Disability Status Scale, WH- working hours.

We evaluated the differences between the mean values of the studied variables (Table 2). The RRMS group had higher FSS scores in comparison to healthy controls (HC), but with no significant difference (x= 26,298± 15,746 and x= 23,964± 10,922 respectively, p=0.700, α=0.05). Female subjects reported greater fatigue in comparison to males in the RRMS group. It was the opposite in the HC group. We found mean BDI-II scores in RRMS and HC groups compatible with absent or minimal depression. RRMS subjects and the HC had mean BDI-II scores lower than 13pts. There was no significant difference between either group. All HC reported lack of or minimal depression. Female

RRMS and HC subjects had higher BDI scores in comparison to male subjects, but with no significant difference. Correlative analysis found significant correlation (r= +0.678, p<0.01) between FSS and BDI. The correlation is stronger in RRMS females (r=+0.693, p< 0.01) than in RRMS males (r=+0.638, p<0.01). We did not find a significant difference in demographic criteria between RRMS patients and the healthy controls. Both groups were similar in terms of gender, age, education, working activity and disability, and the latter did not impact the analysis results. Female subjects' disability assessment presented mean Expanded Disability Status Scale (EDSS) score x= 1.793±0.662 and male subjects had mean EDSS score x= 1.690±0.715 (p= 0.6033, α=0.05).

Table 2. Statistical comparison between RRMS and HC with gender analysis.

	RRMS (N = 50)	HC (N = 28)	P-value
M/F	21/29	14/14	0,502
DD (SD) M F	4,943 (2,604) 4,655 (2,594) 5,152 (2,637)	NA	NA
TD (SD) M F	2,418 (2,247) 2,790 (2,389) 2,149 (2,140)	NA	NA
Mean EDSS (SD) M F	1,750 (,680) 1,690 (0,715) 1,793 (0,662)	NA	NA
Mean FSS (SD) M F	26,298 (15,746) 24,762 (9,389) 28,586 (17,332)	23,964 (10,922) 25,143 (11,967) 22,786 (10.078)	0.700 0,735 0,517
Mean BDI- II (SD) M F	6,640 (7.303) 4,143 (5,868) 8,448 (7,790)	3,857 (3,875) 2,000 (2,287) 5,714 (4,304)	0.210 0,458 0,370
Mean Cr (SD) M F	70227,980 (17326,469) 72892, 857 (17461,813) 68298,241 (17273,737)	61056,964 (14334,289) 61668,357 (16290,240) 60445,571 (12671,708)	**0,025** 0.0645 0.1382
Mean Cho (SD) M F	85682,920 (20018,070) 89734,619 (21180,987) 82748,931 (18962,715)	78196,429 (15798,724) 74910,786 (19110,580) 81482,071 (11398,829)	0.122 **0.0428** 0.8193
Mean NAA (SD) M F	160673,880 (28647,211) 165148,857 (24383,651) 157433,279 (31393,905)	153609,429 (23048,165) 145229,143 (22076,741) 161989,714 (21555,821)	0,151 **0.0195** 0.6276
Mean NAA/Cre (SD) M F	2,362 (,454) 2,343 (0,467) 2,376 (0,451)	2,592 (,458) 2,447 (0,490) 2,738 (0,387)	**0,035** 0.530 **0,014**
Mean Cre/NAA (SD) M F	,439 (,083) ,443 (0,086) ,436 (0,082)	,398 (,076) ,423 (,083) ,373 (0,062)	**0,041** 0,505 **0,005**

Data are means ± SD. RRMS- Relapsing Remitting Multiple Sclerosis, HC-healthy Controls, M- male, F-female, SD- Standard Deviation, TD- Treatment Duration, DD- Disease Duration, EDSS- Expanded Disability Status Score, FSS- fatigue severity scale, BDI-II- Beck's depression inventory II, NAA- N-acetylaspartate (ppm), Cho- Choline, (ppm), Cr- Creatine (ppm), p- value less than 0.05 statistically significant. Statistically significant differences between groups are in boldface values.

PrMRS metabolite data indicated a distinct change in metabolite peaks. There was a significant difference in the mean levels of Cr (p =0.025, α=0.05). The evaluation for sex related differences found higher concentrations of Cho, Cr and NAA in RRMS male subjects. Female RRMS subjects had higher Cho and Cr levels, and lower NAA levels in comparison to HC female subjects. We found statistically significant increase of NAA (p=0.0195, α=0.05) and of Cho (p=0.0428, α=0.05) in male RRMS group.

We examined the relation between the metabolites in both groups. Corelative analysis found moderate relation between NAA and Cho (r=+0.472, p<0.05) and significant relation between NAA and Cr (r=+0.563, p<0.05) in the HC group. We found significant relation between NAA and Cr also in the RRMS group (r=+0.665, p<0.001).

We checked for correlation between the studied variables according to gender. Table 3 summarizes the analyzed data in male RRMS group. Significant correlation (r=0,665, p<0,01) was found in RRMS group between NAA and Cr. For males and females, it was as follows: significant in males (r=0,566, p<0,01) and strong in females (r=0,720, p< 0,05). No correlation was found between Cho and NAA, or Cr either.

The relation between FSS and BDI-II was significant in male and female RRMS subjects (r=0,638 and r=0,693 respectively, p<0.01).

Table 3. Correlative analysis of the variables in RRMS male group.

		DD	FSS	Beck	TD	EDSS	Cho	Cre	NAA
DD	Pearson Correlation	1	.154	-.257	,532**	.091	.001	-.125	-.203
	Sig. (1-tailed)		.253	.130	.007	.347	.499	.295	.188
	N	21	21	21	21	21	21	21	21
FSS	Pearson Correlation	.154	1	**,638****	.105	.222	,373*	,383*	-.066
	Sig. (1-tailed)	.253		.001	.325	.166	.048	.043	.388
	N	21	21	21	21	21	21	21	21
Beck	Pearson Correlation	-.257	**,638****	1	-.298	.279	.135	**,627****	.332
	Sig. (1-tailed)	.130	.001		.095	.110	.280	.001	.071
	N	21	21	21	21	21	21	21	21
TD	Pearson Correlation	**,532****	.105	-.298	1	-,401*	.103	.025	-.063
	Sig. (1-tailed)	.007	.325	.095		.036	.329	.457	.393
	N	21	21	21	21	21	21	21	21
EDSS	Pearson Correlation	.091	.222	.279	-,401*	1	-.166	.213	-.251
	Sig. (1-tailed)	.347	.166	.110	.036		.236	.177	.136
	N	21	21	21	21	21	21	21	21
Cho	Pearson Correlation	.001	,373*	.135	.103	-.166	1	-.028	.165
	Sig. (1-tailed)	.499	.048	.280	.329	.236		.452	.237
	N	21	21	21	21	21	21	21	21
Cre	Pearson Correlation	-.125	,383*	**,627****	.025	.213	-.028	1	**,566****
	Sig. (1-tailed)	.295	.043	.001	.457	.177	.452		.004
	N	21	21	21	21	21	21	21	21
NAA	Pearson Correlation	-.203	-.066	.332	-.063	-.251	.165	**,566****	1
	Sig. (1-tailed)	.188	.388	.071	.393	.136	.237	.004	
	N	21	21	21	21	21	21	21	21

**. Correlation is significant at the 0.01 level (1-tailed). Significant correlations between groups are in boldface values.

*. Correlation is significant at the 0.05 level (1-tailed).

Significant correlation (r=0,665, p<0,01) was found in RRMS group between NAA and Cr. For males and females, it was as follows: significant in males (r=0,566, p<0,01) and strong in females (r=0,720, p< 0,05). No correlation was found between Cho and NAA, or Cr either.

The relation between FSS and BDI-II was significant in male and female RRMS subjects (r=0,638 and r=0,693 respectively, p<0.01). Table 4 summarizes the analyzed data in female RRMS group.

Table 4. Correlative analysis of the variables in RRMS female group.

		DD	FSS	Beck	TD	EDSS	Cho	Cre	NAA
DD	Pearson Correlation	1	.084	.010	.281	,359*	.246	.196	.303
	Sig. (1-tailed)		.333	.480	.070	.028	.099	.154	.055
	N	29	29	29	29	29	29	29	29
FSS	Pearson Correlation	.084	1	**,693****	.077	.310	-.025	.172	.286
	Sig. (1-tailed)	.333		.000	.346	.051	.450	.186	.066
	N	29	29	29	29	29	29	29	29
Beck	Pearson Correlation	.010	**,693****	1	.114	.278	.152	**,434****	.292
	Sig. (1-tailed)	.480	.000		.277	.072	.215	.009	.062
	N	29	29	29	29	29	29	29	29
TD	Pearson Correlation	.281	.077	.114	1	-,331*	.073	.004	.135
	Sig. (1-tailed)	.070	.346	.277		.040	.353	.492	.243
	N	29	29	29	29	29	29	29	29
EDSS	Pearson Correlation	,359*	.310	.278	-,331*	1	-.036	.243	.189
	Sig. (1-tailed)	.028	.051	.072	.040		.426	.102	.163
	N	29	29	29	29	29	29	29	29
Cho	Pearson Correlation	.246	-.025	.152	.073	-.036	1	,339*	.287
	Sig. (1-tailed)	.099	.450	.215	.353	.426		.036	.066
	N	29	29	29	29	29	29	29	29
Cre	Pearson Correlation	.196	.172	**,434****	.004	.243	,339*	1	**,720****
	Sig. (1-tailed)	.154	.186	.009	.492	.102	.036		.000
	N	29	29	29	29	29	29	29	29
NAA	Pearson Correlation	.303	.286	.292	.135	.189	.287	**,720****	1
	Sig. (1-tailed)	.055	.066	.062	.243	.163	.066	.000	
	N	29	29	29	29	29	29	29	29

*. Correlation is significant at the 0.05 level (1-tailed). Significant correlations between groups are in boldface values

**. Correlation is significant at the 0.01 level (1-tailed).

It is considered that total Cr concentration (creatine + phosphocreatine) is stable and constant[11]. It is used as a comparison marker in analyzing changes in other brain metabolites. We performed regression analysis and assessed the relationship between NAA against Cr (Fig. 1).

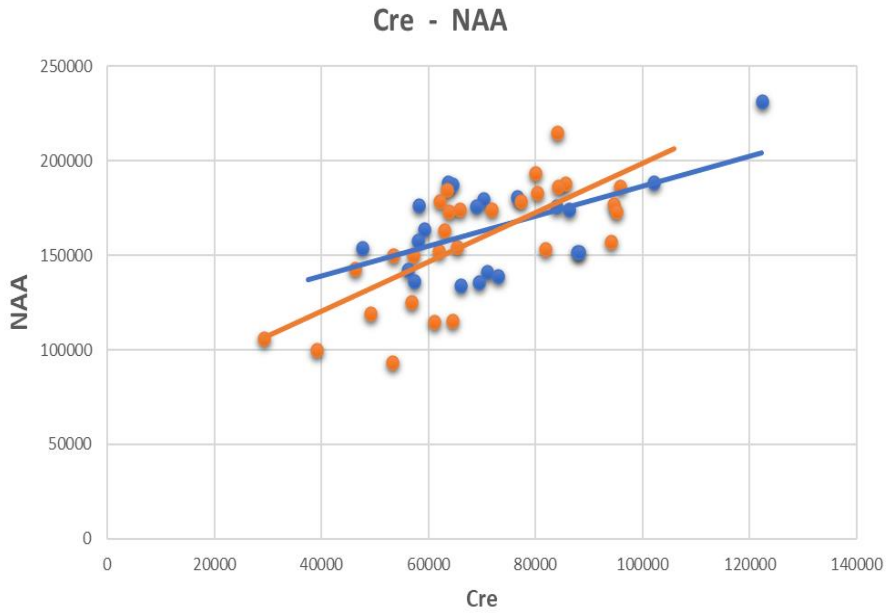

Figure 1: Regression analysis of the relationship between NAA against Cr in RRMS subjects. NAA- N-acetylaspartate, Cre- Creatine.
Blue dots and line- RRMS male subjects
Orange dots and line- female RRMS subjects

The content of Cr and phosphocreatine reflects the energy balance of nerve tissue. Fatigue is described by the patients as "lack of energy". The analysis revealed a significant elevation of the mean levels of Cr in the RRMS group ($p = 0.025$, $\alpha = 0.05$). There was no significant difference in FSS scores between both groups. We performed regression analysis and assessed the relationship between FSS against Creatine (Fig. 2).

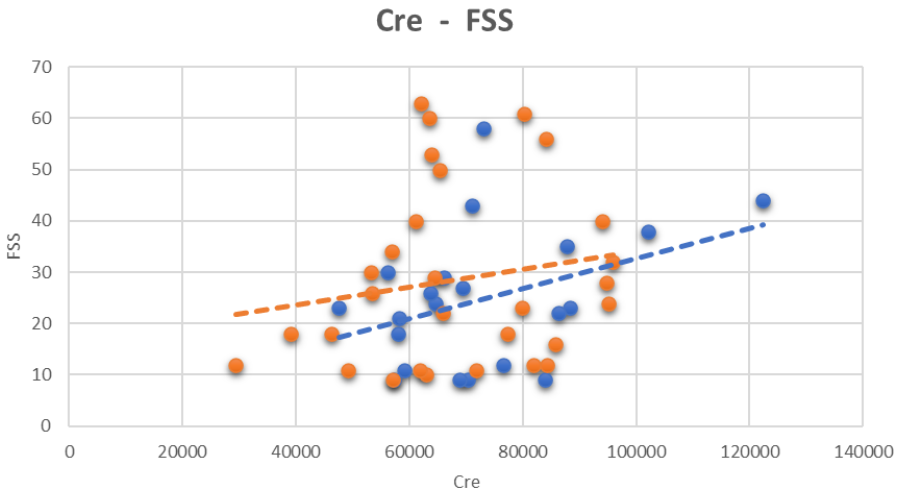

Figure 2: Regression analysis of the relationship between FSS against Cr in RRMS subjects. FSS- Fatigue Severity Scale, Cre- Creatine.
Blue dots and line- RRMS male subjects
Orange dots and line- female RRMS subjects

There was no significant difference in FSS scores between RRMS subjects and HC. There was no significant difference in NAA levels neither ($p = 0.151$, $\alpha = 0.05$). Both variables had higher values in the RRMS group. We performed regression analysis and assessed the relationship between FSS against NAA (Fig. 3).

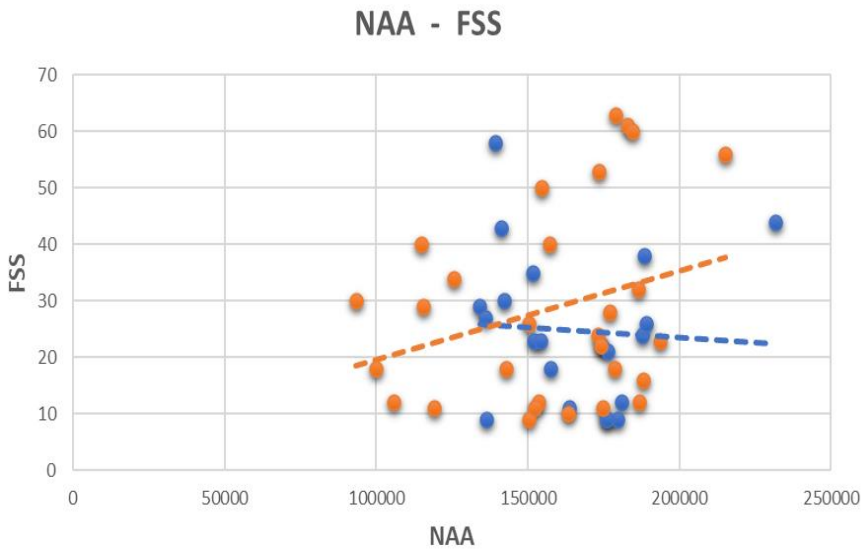

Figure 3: Regression analysis of the relationship between FSS against NAA in RRMS subjects. FSS- Fatigue Severity Scale, NAA- N-acetylaspartate.
Blue dots and line- RRMS male subjects
Orange dots and line- female RRMS subjects

Discussion

The aim of our study was to explore the gender differences in fatigue profiles and metabolite pattern of NAWM in RRMS patients with short disease course in comparison with healthy controls. The second aim was to correlate metabolite changes distribution with fatigue severity and to evaluate the gender impact. MS is an autoimmune chronic inflammatory disease of the central nervous system that results in myelin destruction and axonal degeneration in the brain and spinal cord[12,13]. The disease affects people aged between 30-34 years, predominantly[13]. Harbo et al state that autoimmune diseases (cell or antibody mediated), including MS, occur more frequently in women[14]. Fatigue is one of the most common symptoms. At least 75% of MS patients complain of fatigue at some point in the disease course[3]. According to Braley and Chervin up to 90% of the patients report fatigue as the most debilitating symptom, which impairs quality of life[3]. Fatigue tends to get worse during the day. Heat and humidity aggravate fatigue[12]. All enrolled RRMS subjects and HC underwent evaluation on a single day and at a similar time for each patient. We found low FSS scores in RRMS group. The RRMS group had higher FSS scores in comparison to HC, but with no significant difference. Female subjects reported greater fatigue in comparison to males in the RRMS group.

Multiple sclerosis is associated with an increased prevalence of other conditions that contribute to fatigue, including depression and sleep disorders[3]. About 50% of people with MS are diagnosed with depression[11]. It can manifest with fatigue. MS patients might misperceive symptoms of depression for fatigue. Recent studies have identified a strong correlation between fatigue and depression[2,15]. Various rating scales including the Beck Depression Inventory correlate with the Fatigue Severity Scale[3]. The RRMS subjects and the HC had mean BDI-II scores lower than 13pts, which is absent or minimal depression. The RRMS subjects evaluated mood level as follows: 82% lack or have minimal depression, 12% reported mild, 6% reported moderate and no one considered severe depression. All healthy controls reported lack of or minimal depression. Female subjects had higher BDI-II scores. Correlative analysis found significant correlation between FSS and BDI-II. The correlation is stronger in RRMS females than in RRMS males. A study on RRMS and progressive type of disease found significant correlation between FSS and age, disease duration, BDI and EDSS. Male subjects had higher EDSS, but similar FSS and BDI scores with female subjects[5]. We found relation between disability, fatigue, and depression respectively lower than 0.3 ($p<0.01$), which stands for no influence of disability on fatigue and mood changes. We did not find a significant difference in demographic criteria between RRMS patients and the healthy controls. Both groups were similar in terms of gender, age, education, working activity and disability, and the latter did not impact the analysis results. There was no impact of EDSS differences on the analysis results. The multiple sclerosis participants were fully ambulatory. Neurological evaluation registered pyramidal and cerebellar systems signs without disability (Kurtzke

EDSS pyramidal and cerebellar functions score=1). We presume that no peripheral type of fatigue impacts the subjects' FSS scores. The regression analysis showed that the increase in FSS score leads to increase in BDI-II score in both males and females, with higher increase rate in females in comparison to males (0,311 and 0,281 respectively). The differences between the males and females mean values of BDI-II is 4,31, greater than the mean value in males. We could conclude that BDI-II comprises gender-oriented questions and gender indifferent ones in the self -evaluation. The gender dependent questions elevate the regression line in females, while the gender indifferent part provides the parallel course of regression lines. Data regarding disease modifying treatments (DMTs) influence on fatigue symptoms are still controversial. Some DMTs may increase the risk of fatigue and depression. People treated with interferon-b (INF-b) report fatigue and depression more frequently because of side effects like flu-like symptoms resembling sickness behavior[12]. On the contrary, there are studies that have not found any relationship between DMT type and depression. Several publications raise the positive impact of natalizumab, fingolimod, and glatiramer acetate on fatigue and depression. Antifatigue and antidepressive effectiveness of certain DMTs may be related to the suppression of inflammatory pathways leading to depression[2]. Patients recruited in our study received interferon-b 1a treatment. They tolerated the DMT well. There were no flu-like symptoms or injection site reactions complaints. We registered no clinical signs of disease activity for at least 6 months period prior to the study. We found no correlation between FSS score in both men and women and treatment duration. There was no correlation with the disease duration either. According to literature the inflammatory etiology of fatigue and depression in multiple sclerosis was supported by evidence of increased serum and cerebrospinal fluid (CSF) concentration of inflammatory mediators such as Tumor Necrosis Factor (TNF), interleukins (IL-1a, IL-1b, IL-6), interferon gamma (INF gamma), and neopterin[2]. According to our results, if possible, inflammatory etiology is not the only explanation of fatigue. A study by Ana Margarida Novo et al concluded that fatigue is associated with NAWM damage. The researchers proposed diffuse microstructural white matter damage to be the main neural basis of fatigue in MS. The authors found no association of fatigue with lesion load or gray matter atrophy[8]. We registered metabolite levels in NAWM regions and compared data from HC. The events preceding the lesion formation in NAWM according to the literature reference are increase in Cho metabolites levels[12,16]. Choline is essential for structural integrity and signal function of membranes. It is a vital component of the myelin sheath of the nerve fibers[16,17]. Months before the formation of a new plaque of demyelination in NAWM, Proton magnetic resonance spectroscopy can detect a local increase in Cho level. The elevated level is a marker for cell and membrane turnover. Further increase in Cho, Cr and lipids levels and decrease in NAA occurs after lesion formation[12]. Our study found a significant increase in Cho and Cr levels, but also elevated NAA level. Creatine and phosphocreatine participate in the energy metabolism. Creatine is a marker for energy homeostasis. Literature reference hypothesized NAA as a marker of viable neurons[18]. A diminished NAA peak represents neuronal/axonal dysfunction or loss. Elevated Cho peak represents heightened cell-membrane turnover, as seen in demyelination, remyelination, inflammation, or gliosis[6]. The metabolite ratio levels are used for scientific investigations. We found gender differences in previous research work with significant decrease of NAA/Cr and increase of Cr/NAA ratios, respectively. The finding is consistent with published studies so far[9,19]. According to our research it is due to changes in RRMS female subjects. We found elevated levels of all the metabolites evaluated with significant elevation of Cr in the RRMS group. There was elevation of Cho and NAA levels also, but significantly only in RRMS males. Female RRMS patients presented with lower NAA levels than HC and greater increases in FSS score. Our results are not in line with the findings of metabolite changes reported in other studies. We presume the probable reason was the short disease course, absence of clinical activity, and the low EDSS scores. All of this stood for restricted microstructural morphological changes in the NAWM. Elevated Cho levels in both male and female RRMS subjects, though with gender related difference, present microstructural changes in NAWM. Elevated Cr levels might present deviation in energy demands. NAA concentration changes, either as an absolute concentration or as a ratio between NAA versus total creatine (NAA/Cr), have proved diagnostically important[11]. Proton magnetic resonance spectroscopy studies express NAA levels more often as ratios to other brain metabolites, but not as absolute concentrations. Other metabolites may vary independently of NAA. The use of such ratios may confound the quantification of NAA[20]. N-acetylaspartate proton signal is the most prominent in PrMRS. Thus, NAA appears to be one of the most reliable markers for brain MRS studies[11]. We could regard NAA as a PrMRS marker for neural health, viability, and number. N- acetylaspartate enhances mitochondrial energy production from glutamate by conversion of glutamate to alpha ketoglutarate

which can enter the tricarboxylic acid cycle for energy production. Regional NAA levels decreases can also represent reversible neuronal or mitochondrial dysfunction[11]. The metabolite is a vital component of neuronal osmoregulation[18]. It is a direct precursor of the neuron specific dipeptide N-acetylaspartylglutamate (NAAG)[11]. Both metabolites are important for neuron energetics, osmoregulation, and neuron- glia communication. N-acetylaspartate specifically targets oligodendrocytes. N-acetylaspartylglutamate specifically targets astrocytes where it interacts with their metabotropic glutamate receptor 3 (mGluR3) surface receptors[20]. The possible role of neurotransmitter disruptions and circadian rhythm in precipitating fatigue in MS generated considerable interest[21]. Our data revealed significant increase in NAA in RRMS male subjects with FSS score lower than in the HC group. Decrease in NAA levels in RRMS females accompanied by higher FSS score.

Electrical signals underlie information processing in neurons. Charge distribution creates an electrical field. Resting/ equilibrium potential depends on temperature of the solution, concentration of ions inside and outside, valence of ions and work, required to separate charge. Resting potential represents a steady state with no ion's movement, charge balance and water/ osmolarity balance. The brain spends forty percent of the energy for ion carriers[22]. Neurons require energy in the form of adenosine triphosphate (ATP) for repolarization[15]. Ions move through protein pores in the membrane[22]. Membrane health and structural stability are crucial for charges distribution. Oxidation of glucose (Glc) is the main energy source. It is proposed that the NAA–NAAG system works as a neuronal control mechanism. It ensures the timely delivery of adequate energy components and the removal of waste products. For every 400 mol of glucose oxidized in the brain to replenish ATP supplies, ten mol of N-acetylaspartate and one mol of N-acetylaspartylglutamate are synthesized by neurons via a chemometabolic process, wherein glucose carbon is used to synthesize the acetate portion of NAA and then NAAG[15].

We checked for correlation between the studied variables. Significant correlation was found in RRMS group between NAA and Cr. For males and females, it was as follows: significant in males and strong in females. No correlation was found between Cho and NAA, or Cr either. There was a significant correlation between FSS and BDI-II in the RRMS group for male and female subjects. We found moderate correlation between disease duration and treatment duration. The gender distribution analysis found significant relation in male RRMS subjects. The relation between both variables was weak in female RRMS subjects. We could assume that there is gender dependent correlation.

The analysis of gender distribution of metabolite changes revealed significant increase in NAA in RRMS male subjects and decrease in RRMS females. These changes were accompanied by the opposite results of FSS score. It is presumed that total Cr concentration (creatine + phosphocreatine) is constant[11]. Female subjects had lower levels of Cr in both RRMS and HC groups. The regression analysis studied the relationship between NAA against Cr. The increase in Cr lead to an increase in NAA levels in RRMS subjects. The increase was with greater rate in females. For the females it estimated 1.308. In males it was 0.709. The regression lines cross at the point of Cr= 76 189; NAA= 167 753. Males with lower Cr levels had greater levels of NAA in comparison to females. The increase in Cr above 76 189 ppm corresponds to increase in NAA, but greater in females. The increase in Cr in female RRMS subjects leads to greater increase in NAA, but lower increase in FSS when compared to male RRMS subjects. The relation between FSS against NAA reveals the same tendency. The regression lines cross. The lower NAA level, the higher the FSS score in males. It is the opposite in females. Can we speculate that there is a gender dependent factor with impact on metabolite changes? Multiple sclerosis is found to be more prevalent in women than men, like other autoimmune diseases[14]. Females are twice as likely to develop the disease[13]. The effect of sex on clinical features of MS is not as clear as the effect on MS prevalence, however, there is evidence that women generally have an earlier onset of disease, they have a slightly lower prevalence of primary progressive disease course and show in general less progression of disability than men[14].

Data, supported by histological and anatomic magnetic resonance imaging (MRI) evidence, indicates NAA-recovery with time. It confirms that NAA is responsive to transient neuronal dysfunction[11]. Serles and colleagues found postoperative increases in NAA levels behind the resection and in the contralateral hemisphere in patients with nonlesional temporal lobe epilepsy. The increase may result from recovery of neuronal metabolism, and possibly increased dendritic sprouting, synaptogenesis, and neurogenesis[23]. Multiple sclerosis is an autoimmune inflammatory demyelinating disease of the CNS which links axonal damage to reduced NAA levels in gray and white matter. Clinical studies and a recent metanalysis of the use of PrMRS in multiple sclerosis

show decreased NAA levels associated with the progression of the disease. The degree of loss of whole-brain NAA exceeds the development of atrophy by several fold, encouraging the conclusion that neuronal dysfunction may precede tissue loss in multiple sclerosis[13].

A hypothesis takes place based on the study results. We examined normal appearing white matter. Metabolites levels reflect structural changes (Cho elevation) preceding morphological MRI detectible lesions. Membrane structural changes lead to permeability, and equilibrium state deviations. They interfere with membrane repolarization capacity. Consequently, followed by or coexisting with osmoregulation disturbances and impairment of energy-dependent signaling functions (NAA and Cr levels changes). Thus, limiting the neurons to receive and transmit encoded information. We could consider fatigue a clinical presentation of neuron communication properties deterioration. There is a specific point where a gender specific factor directs the pathological process to a certain development and clinical manifestation. Partial recovery of NAA levels has been reported after treatment of patients with interferon beta-1b[6], glatiramer acetate[4] or fluoxetine[24] suggesting that NAA levels reflect not only neuronal and axonal integrity, but also may reflect improvements in neuronal energetics and possibly remyelination[11].

Modern imaging techniques provide information beyond contemporary knowledge. They have the potential to explore the microstructural mechanisms causing gender differences in multiple sclerosis. Collaborative investigation in the genetic and immunological field could provide substantial clarity on pathogenesis of the disease[14].

The main limitation of our work is the small number of participants and the one- time study of the variables. The demografic characteristics also put some restrictions on data interpretation. However, we should emphasize the strengths of our study. We evaluated fifty MS patients with similar disease phenotype in a stable clinical state with at least six months relapse free history. All of them had disease modifying treatment. Adjustment for confounding factors age, education and working activity excluded impact of these variables on metabolite variations found in RRMS group.

Conclusion

Proton magnetic resonance spectroscopy registered differences in metabolite profiles in NAWM male and female RRMS subjects. We might presume gender dependent specifiers in metabolite profiles in RRMS. They impact fatigue severity. N-acetylaspartate might be crucial contributor in central fatigue in multiple sclerosis. Bioenergetic role of NAA needs further collaborative research on genetics and electrical properties of neurons to reveal the underlying mechanism of fatigue and conductivity deterioration.

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Data availability: The data supporting the study results and analysis are available from the corresponding author upon reasonable request.

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