Laboratory assessment of mitochondrial dysfunction in patients with multiple sclerosis

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Abstract: Background: Multiple sclerosis (MS) is an inflammatory demyelinating disease affecting more than 2 million people worldwide and considered a leading cause of non-traumatic disability in young adults in many countries. **Objective:** To evaluate the mitochondrial dysfunction in patients with multiple sclerosis (MS) by investigating serum levels of lactate and uric acid (UA) in MS patients and to explore their potential role in pathogenesis of MS as biological markers for monitoring disease activity and progression. **Methods:** This case-control study was conducted on 52 Egyptian subjects (32 multiple sclerosis patients and 20 normal healthy individuals as control group. Patients were subjected to thorough history taking, detailed neurological examination and clinical assessment of the severity of the disease using Expanded Disability Status Scale (EDSS) and fatigue using Fatigue severity scale (FSS). Serum level of lactate and uric acid were measured in both groups. **Results:** In comparison to the control group, subjects with multiple sclerosis had statistically significant higher serum level of lactate (p= 0.001), with no statistically significant difference in serum levels of UA (p= 0.337). There was statically significant negative correlation between serum lactate levels and EDSS-FSS, but no statistically significant correlation between serum UA levels and EDSS or FSS. **Conclusion:** MS patients have significantly higher serum lactate level. This can support the hypothesis that mitochondrial dysfunction has an important role in the underlying pathogenic mechanism of the disease. However, the potential value of serum lactate as a marker for monitoring disease activity and progression is questionable.

[Kamel Hewedi, Sabry Mohammed, Ahmed Fathy, Ahmed Essmat and Mohammed Saeed. **Laboratory assessment of mitochondrial dysfunction in patients with multiple sclerosis.** *Nat Sci* 2018;16(2):40-44]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature. 6. doi:10.7537/marsnsj160218.06.

Key Words: Multiple Sclerosis, Serum Lactate, Uric Acid, Mitochondrial dysfunction, Disability, Fatigue.

1. Introduction

Multiple sclerosis is an inflammatory demyelinating disease of the CNS in which activated immune cells invade the central nervous system and cause inflammation, neurodegeneration and tissue damage[1]. MS is characterized by multiple focal demyelinating lesions affecting the white matter, which is not infrequently associated with cortical demyelination and which may be preceded by destruction of oligodendrocytes and apoptosis[2]. Alterations inmitochondrial DNA, abnormal mitochondrial enzyme activities, and increased production of free radicals have been reported to increase in MS patients and animal models[3]. Impaired mitochondria can promote neurodegeneration and cause increased anaerobic metabolism in MS[4]. Measurement of serum lactate in MS patients might be relatively an inexpensive test for monitoring of the assumed hypoxia in MS and its role in pathogenesis of the disease [4]. There are an increasing number of reports on anti-oxidant substances such as coenzyme Q10 used to treat many of symptoms in patients with MS[5-8]. Several studies had showed decreased UA levels in MS patients, and possible relationships between level of UA and disease outcomes, with lower levels of UA in patients presenting with higher disease activity, higher relapse rate and disability scores [5,9-11]. The aim of the present study was to evaluate the mitochondrial dysfunction in patients with multiple sclerosis (MS) through determining levels of serum lactate and uric acid and its potential role in pathogenesis and their role as biomarkers for monitoring disease activity and progression.

2. Subjects and Method

Study design and subjects

This case-control, cross sectional study was conducted on 52 Egyptian subjects (32 multiple sclerosis patients (patients group = group I) and 20 normal healthy individuals (control group = group II).

Inclusion criteria:

We included patients with multiple sclerosis (RRMS and SPMS) (according to the revised McDonald's criteria) [12] from both sexes whose age ranged18-50 years.

Exclusion criteria:

We had excluded patients with any other medical conditions that may affect the serum lactate level (such as muscle diseases, renal dysfunction, epilepsy, hepatic failure, uncontrolled diabetes mellitus or malignancy, History of being an athlete or undergoing body-building exercises, history of intake of any

mytochondriotoxic drugs or substances). Also excluded patients with any other medical conditions that may affectthe serum UA level (such as Patients with history of Hypothyroidism, Wilson's disease, Obesity or Psoriasis and Patients on diuretic medications or uricosurics).

Participants in the present study were collected among patients following up at Neurology department - Dar Elshefaa hospital from January 2017 to July 2017.

Methods

All patients in group (I) were subjected to:

1. Clinical assessment including detailed medical history as age, sex, past history, family history, age of onset of MS and Disease modifying drugs (DMDs).
2. General examination
3. Full neurological examination to assess patients' disability and fatigue using:

A) Expanded disability status scale (EDSS)[13]

The degree of disability for all patients was rated according to the EDSS, that provides overall rating of disabilities based on a (0) (normal neurological examination) to (10) death due to MS.

B) Fatigue severity scale (FSS).[14]

FSS total score is given by the sum of nine items ranging from one (complete disagreement) to seven (complete agreement), with higher scores indicating more severe symptoms.

A written consent was taken from patient and control groups before start of the study.

The study was approved by the ethics committee of faculty of medicine, Al-Azhar University, Cairo, Egypt.

All participants in both groups were subjected to:

Measurement of serum lactate and uric acid:

A) Collection of serum samples:

3ml of venous blood were collected from each participant in the study after written consent by venipuncture under complete aseptic technique in to a plain tube vacutainer (red cap) then left for 15 minutes to be clotted then centrifuged for 5 minutes at 4000 rpm then the separated serum was stored at -20 centigrade until used for assay of uric acid and lactate[15].

B) Biochemical testing:

Serum lactate was measured using a commercially available L-LACTATE TRINDER liquid (science and technology center BEN – BIOCHEMICAL Milano – Italy). Uric acid was measured using a commercially available uric acid liquid stable (EGY CHEM for Lab Technology Biomed Diagnostics Hannover Germany). Both were analyzed using Semiautomated chemists analyzer 5010Roch Diagnostics.[15]

Statistical analysis:

Data were statistically described in terms of mean +/- standard deviation and compared using Student t test for independent samples. Correlation between various variables was done using Pearson moment correlation equation for linear relation in normally distributed variables and Spearman rank correlation equation for non-normal variables/non-linear monotonic relation. p values less than 0.05 was considered statistically significant. All statistical calculations were done using computer program SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 20 for Microsoft Windows (2010).

3. Results

Characteristics of the study population:

This study included 32 patients with MS and 20 age and sex-matched healthy controls. The mean age of the patients group was 33.5± 8.7 while it was 30.9 ± 4.5 years in the control group. In the patients group, the frequency of males and females was 53.2 % (n= 17) and 46.8 % (n= 15) respectively, while in the control group, there were 10females (50%) and 10 males (50%). The mean duration of the disease in patients was 5.04 ± 3.6 years. The mean of the total number of the attacks was 4.09±3.4. The mean EDSS score was 2.8±1.9.

The mean FSS score was 4.2±1.4.. The most frequent MS type was relapsing remitting MS (27 patients =84.4%), followed by the secondary progressive MS (5 patients =15.6%).

Table (1): Demographic data of study groups

	Group (1) MS N = 32	**Group (2) Control N=20**	**P value**
Age	33.5 ± 8.7	30.9 ± (4.5)	0.22
Sex	**N %**	**N %**	0.83
Female	15 46.8	10 50	
Male	17 53.2	10 50	

Results of the serum lactate and uric acid:

A) Lactate:

In patient group, serum lactate levels ranged from 12.3 – 74.6 mg/dl with mean score 40.7±18.1 while in control group, levels ranged from 10.4- 32 mg/dl with mean score 20.1±5.8. Comparing patient and control groups regarding serum lactate showed that serum lactate levels were significantly higher in patient group in comparison to control group ($p< 0.001$).

B) Uric acid:

In patient group, serum uric acid levels ranged from 3.1 – 7.3 mg/dl with mean score 4.9±1.1. while in control group, levels ranged from 3 – 8.1 mg/dl with mean score 5.3±1.2.

Comparing patient and control groups regarding serum uric acid showed that serum uric acid levels demonstrated no significant difference between both groups (p= 0.337).

Table (2): comparison between serum lactate and uric acid between the study groups.

	Group (1) MS patients N = 32	Group (2) Controls N=20	P value
Serum lactate	**X/ ± (SD)** 40.7 ± (18.1)	**X/ ± (SD)** 20. 1 ± (5.8)	**0.00 1**
Serum uric acid	**X/ ± (SD) 4.9** ± (1.1)	**X/ ± (SD)** 5. 3 ± (1.2)	0.337

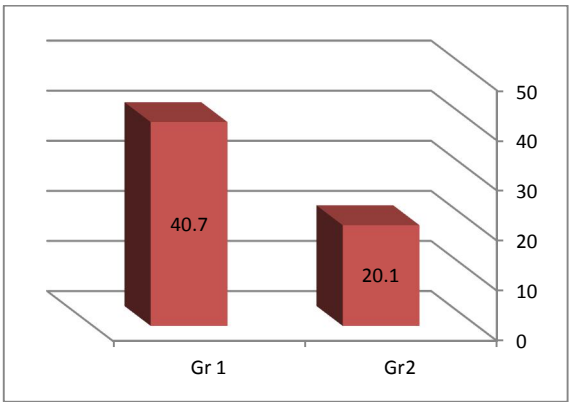

Figure (1) Serum lactate difference between the two study groups

Comparison between relapsing- remitting (RR) and secondary progressive (SP) MS patients regarding serum lactate and uric acid:

1- Lactate:

In RR patients, serum lactate levels mean score was 40.1±18.6. while in SP patients, levels mean score was 44.1±17.

No statistically significant difference in serum lactate level between RR and SP in spite mean is higher among SP group.

Table (3): comparison between serum lactate and MS type

	Group (1) RR N = 27	Group (2) SP N=5	P value
Serum lactate	**X/ ± (SD)** 40.1 ± (18.6)	**X/ ± (SD)** 44 ± (17)	0.623

2- Uric acid:

In RR patients, serum uric acid levels mean score was 4.9 ± 1.1. while in SP patients, levels mean score was 5.3±1.5.

No statistically significant difference in serum uric acid level between RR and SP patients.

Table (4): comparison between serum uric acid and MS type.

	Group (1) RR N = 27	Group (2) SP N=5	*p*-value
Serum uric acid levels	**X/ ± (SD)** 4.9±1.1	**X/ ± (SD)** 5.3 ±1.5	0. 475

Correlation between serum lactate levels and EDSS – FSS in patient group

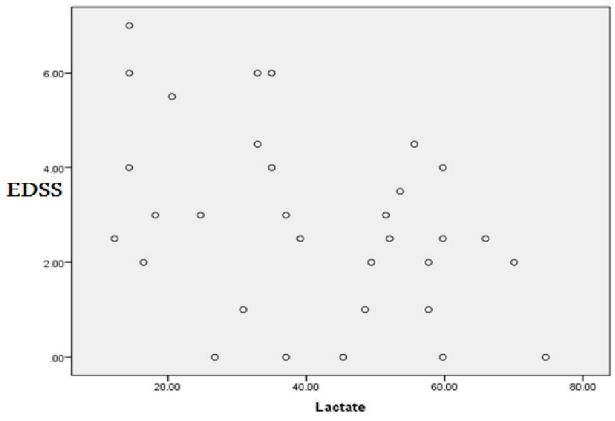

Figure (2) Correlation between serum lactate and EDSS.

There is moderate negative correlation between serum lactate and EDSS- FSS in patient group and difference is statistically significant as showed in figure (2, 3).

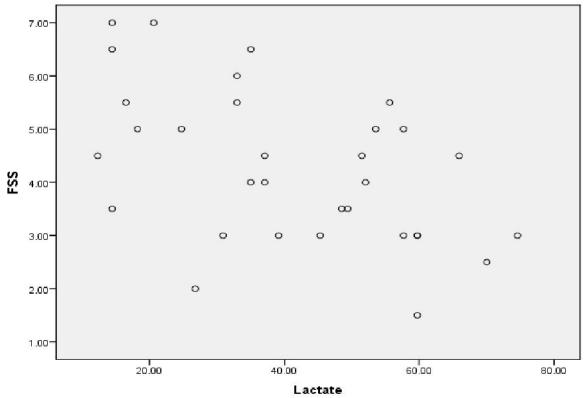

Figure (3) Correlation between serum lactate and FSS.

4. Discussion

Several studies have underlined the important contribution of mitochondria during the establishment of MS[16,17]. There are multi-mitochondrial abnormalities observed during the development and progression of MS as alterations in mtDNA and anomalous mitochondrial protein functions, increased free radical production and oxidative damage, cellular ionic imbalance, apoptosis and cellular clearance mechanisms [18].

The present study demonstrated the increased serum levels of lactate in the Egyptian MS patients as compared to controls. This finding may support the hypothesis that mitochondrial dysfunction has an important role in the pathogenesis of MS and of its particular relevance to the neurodegenerative component of the illness.

These findings are supported by the results of a recent study that has been published in 2015 in Egyptian journal of neurology, psychiatry and neurosurgery in which 89 Egyptian subjects 55 multiple sclerosis patients (Group 1) and 34 normal healthy individuals (Control group = Group 2). Group1 patients were subjected to history taking; detailed neurological examination and clinical assessment of the severity of the disease using Expanded Disability Status Scale (EDSS). Serum level of lactate and uric acid were measured in both groups. In comparison to the control group, subjects with multiple sclerosis had statistically significant higher serum level of lactate (P=0.005) along with lower serum levels of UA (P=0.001), there was no statistically significant correlation between their levels and duration of illness, EDSS scores or number of attacks.[19]

And another multicenter study that has been published in 2014 in which 613 MS patients were recruited, assessed for the clinical disability and serum lactate level. They found that levels of serum lactate in patients with MS was three times higher than that of healthy controls group along with higher levels in cases with a progressive than with a relapsing-remitting disease course. [4]

However, in contrast to our results, where we found a significant negative correlation between lactate and clinical disability - fatigue, they found a linear correlation between serum lactate levels and the expanded disability scale (EDSS).

This difference may be explained by that increased disability and fatigue cause decrease daily activities performed by MS patients leading to lower lactate levels and vice versa, decrease disability and fatigue enable MS patients to perform their oridinary daily activities, more energy needed in presence of mitochondrial dysfunction leads to accumulation of lactate.

The present study demonstrated no statistically significant difference in serum levels of UA in the MS patients as compared to healthy controls.

These finding Supported by Study published in 2017 by Gosch et al, Brigham Young University in which 499 MS patients were recruited compare to 276 health controls according to serum uric acid levels they found no significant difference in UA levels between MS patients and controls (p=0.0858). Also UA levels showed no significant difference between MS subtypes (p = 0.628) their study failed to support previous evidence of low UA levels associated with MS patients. Gender and age were identified as contributing factors to UA level. There was no significant difference found between UA levels of different subtypes of MS.[20]

And another study published by Kohansal et al,2016 in the journal of Shahrekord University of medical science in which uric acid was measured in 52 patients with MS and compared with 52 healthy subjects. And showed The mean concentration of uric acid in the patient group was 4.66 ± 0.99 and in the healthy group was 64.4 ± 1.06 mg / dL. there was no significant difference between the patient and the control group.[21]

In contrast to our study Fereshteh et al 2013, found a significant difference between mean UA concentration in patients with relapsing MS and controls (p = 0.002).[22]

And In 2012, a multicenter study found the serum UA levels were lower in patients with MS than in healthy controls. This finding is also matching with many other studies that reported the significantly lower serum level of UA in MS patients[9-11].

On the other hand a multicenter study published in 2009 found elevation of all purine compounds, including uric acid, in biological fluids of MS patients. They suggested that sustained purine catabolism may be the cause[23].

According to the present study and previous studies we need further studies increasing patient numbers as these results cast doubt on the hypothesis that uric acid is depleted in MS due to increased oxidative stress, because generalized increase in purine catabolism act as another force determined its level.

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12/31/2017