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ORIGINAL ARTICLE

SERUM LACTATE AS A BIOMARKER IN MULTIPLE SCLEROSIS

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ABSTRACT

Background: Multiple sclerosis (MS) is an inflammatory demyelinating disease of central nervous system with secondary progressive neurodegenerative changes lead to accumulation of disability. The important role of mitochondrial dysfunction has been reported in the pathogenesis of MS.

Objective: To investigate the serum lactate level in MS and to explore the prognostic value of lactate regarding disability and progression

Methods: This case-control study was conducted on 80 Egyptian subjects; 40 MS patients and 40 normal healthy individuals. Patients were subjected to detailed history taking, thorough neurological examination and clinical assessment of the disability using Expanded Disability Status Scale (EDSS).Serum level of lactate was measured in both groups.

Results: In comparison to control, serum lactate level was significantly higher among MS patients. There was no significant correlation between serum lactate level and any clinical variants apart from age and age of onset of MS.

Conclusion: Measurement of serum lactate may be helpful in MS and this support the hypothesis of the critical role of mitochondrial dysfunction and axonal damage in MS. Our study recommend future researches on large cohort of MS patients to assess long term prognosis in patients with elevated lactate levels .In addition , we recommend measurement of lactate in cerebrospinal fluid of MS patients to evaluate its clinical significance as a biomarker in MS..

Keywords: Multiple sclerosis, Lactate, EDSS, Mitochondrial dysfunction.

INTRODUCTION

Multiple sclerosis (MS) is a primary inflammatory demyelinating disease with secondary degenerative changes and disability increasing to the patients [1]. The main mechanism of MS is demyelination causing defect in nerve conduction which would augment the energy increase of a neuron and thus encounter the mitochondrial work [2] .The transport velocity in the axons increases in remaining neurons and the load of abnormal mitochondria from motor cortex of MS patients with reduced complexes I and III with over production of pyruvate occur [3]. As ATP supply caused by altered mitochondrial functions diminished, the cerebral tissue would increase

the glycolytic pathway to recompense the energy defect. As a consequence of an increased glycolytic rate and pyruvate accumulation, predictable increase in lactate production occurred [4]. Measurement of serum lactate in MS patients might be relatively an inexpensive test for monitoring of the assumed hypoxia in MS [5].

The aim of our study was to determine whether MS patients had values of circulating lactate different from those of controls and to detect the potential role of lactate as prognostic marker for disability and progression.

Subjects and methods

This case-control study was conducted on 80 Egyptian subjects, 40 MS patients (patients group = group I) and 40 normal healthy control (control group = group II).

Inclusion criteria:

We included patients with MS according to the revised McDonald's criteria 2017[6]. All patients, whatever the clinical course, were included (relapsing remitting multiple sclerosis, secondary progressive multiple sclerosis, primary progressive multiple sclerosis, clinically isolated Syndrome) [6].

Exclusion criteria:

We had excluded patients with clinical relapse or receiving adrenocorticotropic hormones (ACTH) or steroids one month prior to sample withdrawal. Patients suffering from any acute or chronic systemic disease which might influence the lactate levels were also excluded e.g chronic obstructive pulmonary disease, rheumatoid arthritis , sepsis and multi-organ failure , muscle diseases, renal dysfunction, epilepsy, hepatic failure, uncontrolled diabetes mellitus, malignancy , undergoing body building exercise

Our patients enrolled from Neurology Department and Outpatient Clinic of Zagazig University Hospital in the period from May 2017 to May 2019.

Methods

All patients were subjected to:

1- Detailed history taking including age, age of onset, disease duration, course and type of treatment (immunosuppressive or disease modifying).

2- General medical examination

3- Thorough neurological examination according to the multiple sclerosis sheet officially used in MS unit, Neurology Department, Zagazig University.

4- Assessment of neurological disability for all patients by Expanded disability status scale (EDSS) .The scale which rates eight different neurological domains and ranging from 0 (normal neurological examination) to 10 (Death from MS) [7].

5- Magnetic resonance imaging (MRI) of brain and spinal cord was done at Radiology Department, Zagazig University Hospitals to all patients at the time of recruitment.

6- Routine laboratory investigations

7- Measurement of serum lactate levels in both cases and control group at Clinical Pathology Department, Zagazig University Hospitals.

Plasma sample for Lactate:

Peripheral venous blood samples were collected after at least 15min of complete rest, using the standard tourniquet procedure, from the antecubital vein .After 30 min at room temperature, Plasma on Na-fluoride/ k-oxalate and Na-fluoride / Na-heparin were obtained, centrifuged within 15 minutes of collecting the specimen and the resulting samples were analyzed.

Test was performed on Cobas 6000 Autoanalyser (Roche Diagnostic, Germany).

Test Principle: Colorimetric assay

L-Lactate is oxidized to pyruvate by a specific enzyme lactate oxidase (LOD).Peroxidase (POD) is used to generate a colored dye using the hydrogen peroxide generated in the first reaction[8]. Color is measured colorimetry .Serum lactate level is measured in mmol/L

Ethical considerations:

-Written consents were taken from patient and control groups before start of the study.

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

-The work has been carried out in accordance with The Code of Ethics of the World Medical

Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

Data were statistically analyzed using Statistical Package for the Social Sciences (SPSS version 20.0) [9]. Data were statistically described in terms of mean ± standard deviation (± SD) 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.

RESULTS

Characteristics of the studied population:

As shown in table (1), this study included 40 patients with MS and 40 age and sex-matched healthy controls. As shown in table (1).The mean age of the patients group was 29.52±0.65while it was 26.45±5.49 years in the control group. In the patients group, the frequency of males and females was 67.5% (n=27) and 32.5% (n= 13) respectively, while in the control group, 28 females (70%) and 12 males (30%) were included. The mean age of onset was 25.07±7.14. The mean duration of the disease in patients was 4.51±1.47years. The most frequent MS subtype was relapsing remitting MS (22patients = 55%) followed by the secondary progressive MS (9 patients =22.5). The primary progressive MS were 3 patients (6.5%), while 6 patients of clinical isolated syndromes were included in our study (15%).

Results of serum lactate

As shown in Table (2) and Figure (1), the mean serum lactate level in the patients group was 1.82± 0.65, while in the control group, it was 1.4±0.36. This difference was highly statistically significant (p=0.001).

Correlation between serum levels and clinical variables:

Table (3) presented the correlations between levels of serum lactate in the MS group and the clinical variants like age of patients, age of onset of the disease, the duration of the illness, the number of attacks and the clinical scale of disability; Expanded Disability Status Scale (EDSS)

There was significant positive correlation between the level of serum lactate in MS patients with age (Figure 2) and age of onset while no significant correlation between serum lactate level and other clinical parameters (Figure 3).

Table (1): Clinical characteristics of multiple sclerosis patients (N=40):

Clinical characteristics	
Age Mean ± SD	 29.52±0.65
Age of onset Mean ± SD Median (range)	 25.07±7.14 26.0 (6-42)
Disease duration Mean ± SD Median (range)	 4.51±1.47 4.0 (3m-14y)
Clinical Subtypes: Number of patients (%) RRMS SPMS PPMS CIS	 22 (55%) 9 (22.5%) 3 (7.5%) 6 (15%)
Type of treatment: Number of patients	

Clinical characteristics	
No	16
Interferon B	18
Finglimod	5
Cyclophosphamide	1

SD: Standard Deviation.

Table (2): Comparison of serum lactate between cases and control

	Case	Control	t-test	P
Serum Lactate (Mean ± SD)	1.82±0.65	1.4±0.36	**3.51**	**0.001****

SD: Standard Deviation.
*****Highly Statistical significant***

Table (3): Correlation between serum Lactate and different clinical variants of the patient group

		Serum Lactate
Age	*r*	**.378***
	P	**.021**
Age of onset	*r*	**.389***
	P	**.017**
Disease Duration	*r*	.026
	P	.877
Number of attacks	*r*	-.027-
	P	.904
EDSS	r	.244
	P	.145

****Statistical significance***

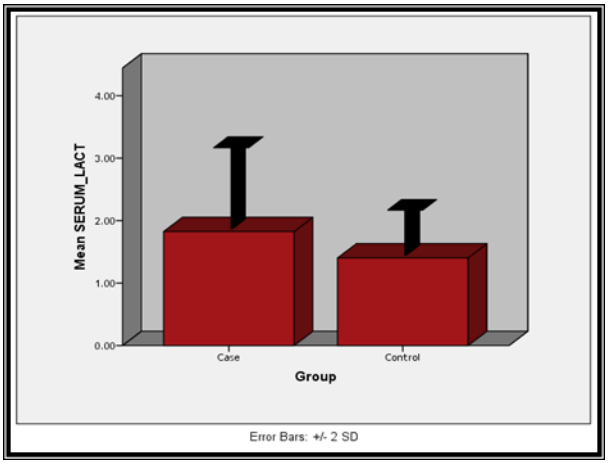

Figure (1): Serum lactate distribution between cases and control.

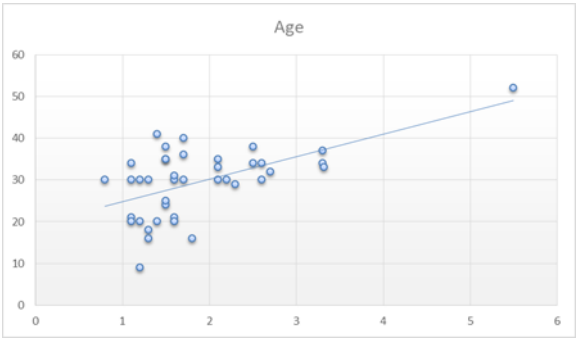

Figure (2) : Correlation between Serum lactate and age

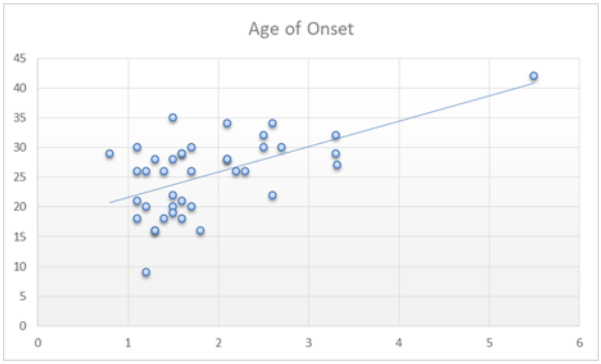

Figure (3): Correlation between serum lactate and age of onset

DISCUSSION

Several studies underlined the critical role of mitochondrial dysfunction during the pathogenesis of MS and its particular association to the neurodegenerative process of MS [10] [11].There are multi-mitochondrial aberrations noted during the development and progression of MS as variations in mtDNA, anomalous mitochondrial protein functions, oxidative injury ,increased free radical production, cellular ionic imbalance, apoptosis and cellular clearance mechanisms [12].

The present study demonstrated increased serum level of lactate in the Egyptian MS patients as compared to controls. This finding may support the hypothesis that that an energy deficit in MS would lead to higher lactate levels.

Data referring to possible changes in lactate in MS patients are debatable and started years ago. Similar to our results , previous studies were conducted on 613 and 518 MS patients showed elevated serum lactate levels of MS group three times to control group and reported that serum lactate might be biomarker of MS [5] [13] . In contrast, other studies reported decrease or no change in lactate levels between MS patient and their control group [14] [15] [16], but these studies obtained their data on relatively restricted number of patients with high variability in their sample collection.

Increasing evidence suggest that mitochondria is the main source of neuronal ATPs, which are depleted in MS patients due to the loss of ATPase activity[4].Different studies point to discrepancy between energy production and consumption in MS; characterized by a defect in the mitochondria ability to supply adequate ATP necessary for survival of neurons[17] .These energy metabolism impairment and mitochondrial malfunctioning in MS patients are demonstrated by higher levels of compounds derived from ATP catabolism. Indeed, during glycolysis in the Krebs cycle, glucose breaks down to pyruvate which is subsequently reversibly converted to lactate by the enzyme lactate dehydrogenase. Lactate is the final cytoplasmic product of the glycolysis pathway [18].

On studying correlation of demographic data of MS patients and lactate, we found significant positive correlation between serum lactate in MS with age and age of onset. Higher concentrations in older individuals, was previously demonstrated by a very large study on 7614 CSF samples [19] and was confirmed by another study in which CSF lactate concentrations significantly correlated with age in both patients and controls [20] ,indicating an age-related increase in CSF lactate apart from MS pathology[21].Hassan and Mehany found no statistically significant correlation between serum lactate level on one hand and age, age of onset on the other hand [22].

No significant correlation between serum lactate, duration of illness and total number of relapses was demonstrated in our study .This goes in hand with previous studies reported that lactate concentration has no correlation with total no of relapse [20] [23] [24] . In contrast , **Albanese et al.** and **Haarmann et al.** reported that lactate negatively correlated with clinical disease duration, however last two studies were conducted on CSF lactate [20] [24]

In this work, we studied the correlation between serum lactate level and neurological disability. We found no significant correlation between serum lactate level and EDSS . Similar to our results , An Egyptian study found no correlation between serum lactate and EDSS [22] .Also Albanese and Haarmann , reported no significant correlation between CSF lactate and EDSS in their studies [20] [24] .In contrast , study by Amorini found that elevated levels of circulating lactate in MS patients are closely correlated with the EDSS scores [5]

CONCLUSION AND RECOMMENDATIONS

Serum lactate had higher values in MS patients; however its potential use as biomarkers for monitoring disability and progression is doubtful. We recommend future researches on large cohort of MS patients to assess long term prognosis in patients with elevated lactate levels. .In addition, we recommend measurement of lactate in cerebrospinal fluid of MS patients to evaluate its clinical significance as a biomarker of disability and progression in MS.

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