Original article

Serum ferritin status and mitochondrial function in children

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Abstract:

objective: Iron is the most abundant metal within the mitochondrial matrix . Ferritin reflects bodyironstores. This study aimed to assess the association between serum ferritin levels and mitochondrial function in children. ***Methods:*** Two hundred and forty-three children from four governmental primary schools in Mosul city in Iraq were randomly selected by a multistage random sampling method. Their age range was 6-12 years. Mitochondrial function tests involved measurement of serum lactate, serum pyruvate, and L-Carnitine were analyzed in all selected children along with serum ferritin. ***Results:*** Lower levels of serum ferritin weresignificantly associated with higher levels of serum lactate, serum lactate/serum pyruvate ratio. There was a significant negative correlation (p = 0.000, - 0.296,- 0.286) between mean serum ferritin level and mean serum lactate, serum lactate / serum pyruvate ratio respectively.There were significant differences (p= 0.000) in mean serum level of mitochondrial function tests (Lactate, L- Carnation, and L/P ratio) in comparing children possessing a normal level of serum ferritin versus those with a low serum ferritin level of < 12 ng/ml. ROC analysis showed that when the area under the curve (AUC) was 0.761 ± SE 0.37, a cut off value 4.79 ng/ml of serum ferritin was significantly (p=0 .000) associated with sensitivity of 100 % and 1- specificity of 0.861 value with presence of L: P molar ratio of ≤ equal 20.***Conclusion:***Serum ferritin < 12 ng/ml represntsmitochondrial deficient level. Serum ferritin < 4.79 ng/ml was the critical value at which only 8.6% of children will have a healthy mitochondrial function

Keywords: Serum ferritin; mitochondrial function**.**

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Introduction:

Serum Ferritin is that protein that reflects the main iron storage in the body[1]. Higher concentrations found in many organs like liver, spleen, and bone marrow. A small fraction of ferritin circulating in the plasma with a normal range from 12 to 280 ng/ml[2],[3]. Iron is widely distributed in the body as it serves a broad spectrum of physiological activities as oxygen transport, energy production, electron-transport, regulation of cell cycle, and participation in DNA synthesis[4]. Ferritin inside the cell distributed in the cytoplasm andmitochondrial as mitochondrial ferritin play a vital role in providing the iron for many reactions that take place inside mitochondria, detoxify cellular iron and Reactive Oxygen Species (ROS) to prevent ferroptosis[5], [6]. The generation of ROS takes place due to converts low stability ferrous

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ions into more stable ferric the reaction known as Fenton reaction. The byproducts of this reaction are hydroxyl radical (•OH), which is considered as the most reactive species at the cellular level that can induce serious intracellular oxidative damage[7]. The low iron status was associated with cellular and tissue hypoxia that intern associated with elevation of Lactate/pyruvate ratio due to hyperlactatemia as described by Neill et al, 2012[8]. Hypoxia activates hypoxia-inducible factor that shifts metabolism to glycolysis on a genetic level by regulating PGC-1α that intern downregulates LDH mRNA and the enzymatic activity of pyruvate to lactate [9], [10]. The changes in lactate and pyruvate will cause changes in the Acetyl Co-A input to the citric acid cycle that will impair mitochondrial function[11], [12]. Low Iron store -that reflected as low ferritin- leads to a secondary reduction in synthesis of carnitine as Iron acts as a co-factor for the synthesis of carnitine [13].

Serum lactate and pyruvate are good indicators for mitochondrial function [14]. lactate/pyruvate molar ratio (L:P) signifies better scanning index for mitochondrial function as it assesses the changes in lactate dehydrogenase substrate and product which display the indirect image about redox state. Any alteration in cellular respiration is reflected by changes in both serum lactate and serum pyruvate[14]. Under normal cellular respiration L: P molar ratio value should notexcessed 20, while value more than 25 is highly suggestive of respiratory chain defective12. L-Carnitine is an important component of the inner mitochondrial membrane,moreover, it maintains energy homeostasis as serum level reflects the dietary status of body[12].

This study is designed to assess the association between serum ferritin level and mitochondrial function in children

Methods:

Multistage random sampling method is applied, the first stage involved a geographical stratification into left and right side of Mosul city. The second stage comprises gender stratification, to select male schools and female schools. Lot randomization to choose schools in each side. Grading stratification, to select a cluster of the second class of each grade in the school, in school with one class per grade; we select the available class. Finally, every fourth pupil in the student list of the chosen classes will be enrolled in the study. According to this, four governmental primary schools were selected; two on each bank of Mosul city. The study period extended from April to June 2019.

Two hundred and forty-three children selected. Their age range was 6-12 years with a mean of 10.4 ± 0.72 with male to female percent 52, 48 respectively. Serum ferritin estimation was analyzed for each studied child, using VIDAS®Ferritin-Biomerieux (France) kit which is used for the determination of serum ferritin, by enzyme-linked fluorescent assay) ELFA technique[15].

Mitochondrial function analysis involved measurement of serum lactate and serum pyruvate by fluorescence-based methods of Cayman chemicals (700510 and 700470 respectively) 16, 17 and L-Carnitine was assayed using colorimetric/ fluorometric MyBioSource (MBS841446) after standard curve was established. The entire sample was evaluated using Synergy HT-Multi-Detection Micro-plate Reader (BioTek-Instruments) at a different wavelengths as specified by manufacturer[16],[17].

Written informed consents were taken from parents of all children before initiating the study. The ethical committee approved this study. Independent sample

Table 1: Pearson correlation analysis betweenmean serumferritin level and mean serum levels of mitochondrialfunction testsamong 243 primary school children

Serum level (Mean ± SD)		**Pearson Correlation to Mean ± SD of serum ferritin 16.32 ± 14.84 ng /ml**	**Significance (2-tailed)**
Lactate (µM)	1510.16 ± 277.62	- 0.296**	0.000
pyruvate (µM)	69.01 ± 7.78	0.013	0.839
L/P	22.04 ± 3.86	- 0.286**	0.000
L-Carnitine(µM)	18.16± 13.18	0.224**	0.000
****. Correlation is significant at the 0.01 level (2-tailed).**			

t-test was used to evaluate differences between means of continuous variables, p-value ≤ 0.05 was considered statistically significant. Receiver operating characteristic (ROC), was used to obtain serum ferritin cut off value predictive of normal mitochondrial function results. Pearson correlation test was performed to explore the correlationbetween numerical variables. Data analysis was executed using the version 17 SPSS program.

Results:

Lower levels of serum ferritin weresignificantly associated with higher levels of serum lactate, serum lactate/serum pyruvate ratio. There was a significantnegative correlation between mean serum ferritin level and mean serum lactate, serum lactate/ serum pyruvate ratio Table (1).

Table 2 demonstrated a significant difference in mean serum level of mitochondrial function tests (Lactate, L- Carnitine, and L/P ratio) in comparing children possessing a normal levels of serum ferritin versus those with a low serum ferritin level of < 12 ng/ml.

Table 2: Comparison between mean serum levels of mitochondrial function tests (Mean ± SD) in relation to serum ferritin groups among 243 primary school children

Parameter	Low Serum ferritin group (< 12 ng/ml) (n = 136)	Normal serum ferritin group (≥ 12 ng/ml) (n =107)	*p*-value		
				Lower	Upper
Lactate(µM)	1604.25 ± 167.29	1390.56 ± 338.40	0.000	148.27	279.18
Pyruvate(µM)	68.85 ± 4.582	69.21 ± 10.55	0.786	-2.35	1.61
L/P Ratio	23.30 ± 1.93	20.44 ± 4.96	0.000	1.94	3.77
L-Carnitine(µM)	14.67 ± 6.13	22.59 ± 17.70	0.000	-11.12	-4.70

Receiver operating characteristic (ROC) analysis showed that when the area under the curve (AUC) was 0.761 ± SE 0.37, a cut off value 4.79 ng/ml of serum ferritin was significantly (p=0 .000) associated with sensitivity of 100 % and 1- specificity of 0.861 value with presence of L:P molar ratio of ≤ equal 20 (Figure 1).

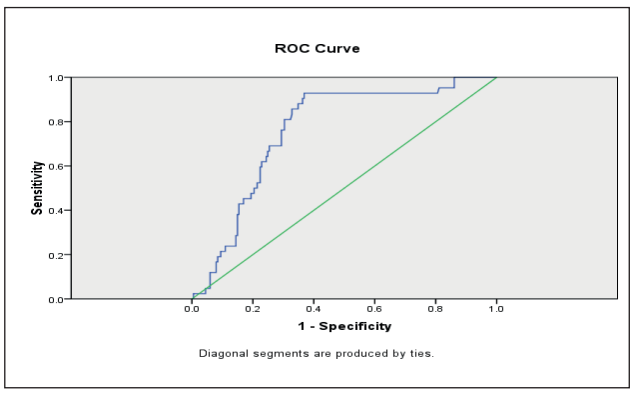

Figure 1. ROC Analysis (sensitivity vs 1-specificity)

Discussion:

The low iron status that reflects as low ferritin is associated with reduction of iron-containing enzymes activities, impaired oxygen metabolism and reduction in cytochrome concentration and activities by reducing iron-sulfur cluster in complexes I, II and III, but not complexes IV and V leading to reduced ATP production ofup to 70% of cellular ATP[18].

In addition to low gluconeogenesis in many vital tissues due to downregulation of both acetyl-CoA carboxylase-1 & 2, ATP citrate lyase with upregulation in glycolysis genes pyruvate kinase, hexokinase II and lactate dehydrogenase A [19, 20] and this is in accordance with results of this study that showed that low levels of serum ferritin had a significant inverse correlation with the level of serum lactate, serum lactate/serum pyruvate ratio which is an indirect indicator for mitochondrial function; Table (1), hence the lower the level of serum ferritin the more mitochondrial dysfunction would be present.

Mitochondria were long considered the powerhouses of the cell; however, over the last decade, it has become increasingly clear that mitochondria have a plethora of other biological functions inside the

cell. Importantly, mitochondria are the sole site of heme synthesis and the primary generator of iron-sulfur (Fe-S) clusters, both of which are present in mitochondria and the cytosol and required for several vital cellular processes [21].

Exhausted iron stores (serum ferritin < 12 ng/ml) was significantly associated with abnormal mitochondrial function test (Table 2), this support that the defineddeficient ferritin value is also representing a deficient ferritinvalue atthe mitochondrial level.On the other hand,depending on the analyzed ROC findings, which displayedthat a cut off value of serum ferritin ≥ 4.79 ng/ml has 100% sensitivity in its association with normal healthy mitochondrial function; signifying that 100 % of all studied children who have a level of serum ferritin ≥ 4.79 ng/ml would be correctly identified as having normal mitochondrial function by the finding of L/P ratio of ≤ equal 20. Furthermore,the analyzed ROC findings, displayed that 1- specificity of 0.861 value was associated with the presence of L: P molar ratio of ≤equal 20indicating that only 8.6 % of thosewith serum ferritin< 4.79 ng /ml would be wrongly identified as having a normal mitochondrial function.It seems that although serum ferritin < 12 micrograms/L was significantly associated with mitochondrial dysfunction; the serum ferritin <4.79 ng/ml was the critical value at which only 8.6% of children will have a healthy mitochondrialfunction. Iron played a critical role in mitochondrial function [22, 23].

Mitochondria are major hubs of iron utilization and accumulation. Over and above, iron is the most ubiquitous metal within the mitochondrial matrix and it acts to support the complex redox chemistry of the electron transport chain, hence it is not surprising that mitochondrial iron levels and mitochondrial energy metabolism are tightly linked[22, 23].

In conclusion,there was a significant association between low serum ferritin level and mitochondrial dysfunction in studied children.

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Authors' contribution:

Data gathering and idea owner of this study: All authors conceptualized the research aims and gathered the data of the study.

Study design: All authors designed the study

Writing and submitting manuscript: All authors, wrote, edited and revised the manuscript.

Nashwan M. Al-Hafidh : submitted the manuscript.

Editing and approval of final draft: All authors

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