Vitamin D Status and Mitochondrial Function in Children

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Abstract

Objective: Mitochondrial dysfunction is likely to be determined by vitamin D deficiency. The aim of this study is to assess the association between mitochondrial function and Vitamin D level in children.

Patients and methods: This study was a prospective study conducted in primary school children in Mosul city. The study included 300 children, with an age ranged from 6 to 12 years. Serum 25(OH) D was analyzed and mitochondrial function was evaluated by measurement of serum lactate, pyruvate Lactate and L-Carnitine

Results: Serum lactate, serum pyruvate and the lactate/ pyruvate ratio increased significantly (*p* ranged between .0001- .026) in children with insufficient vitamin D level (12-19 ng/ml) compared to those with sufficient vitamin D values (20-100 ng/ml). There was significant elevation in serum lactate, serum pyruvate and lactate/pyruvate ratio ($p < .000$) with significant reduction in serum L-Carnitine ($p < .000$) in children with deficient vitamin D level (below 12 ng /ml) in comparison to the group with sufficient vitamin D level. In hypervitaminosis D group (> 100 ng /ml), there was significant elevation in serum lactate, lactate/pyruvate ratio ($p < 0.05$) with significant reduction in serum pyruvate and L-Carnitine ($p < .05$) in comparison to child with sufficient serum vitamin D group. Receiver operating characteristic (ROC) analysis showed that with an area under the curve of 0.990 ± SE 0.001, the cut off value of 20.950 ng/ml of 25(OH) D had significant ($p = .000$) association with sensitivity of 100 % and 1-specificity of 0.060 with presence of L:P molar ratio of ≤ 20.

Conclusion: A cut off value of serum 25(OH) D ≥ 20.950 ng/ml should be maintained to insure normal mitochondrial function. Value of serum 25 (OH) D > 100 ng/ml is associated with abnormal mitochondrial function

Keywords: Vitamin D, mitochondrial function, children, Lactate, Pyruvate and L-Carnitine.

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Introduction

Whatever the source of vitamin D, it ultimately transferred by the bloodstream to the liver where it hydroxylated at C-25 position by microsomal cytochrome P2R1 and mitochondrial cytochrome P27a1; which both known as (25-hydroxylase), the newly formed 25(OH)-1-D3 represents precursor for the active form of vitamin D [(1)]. The 25(OH)-1-D then conveyed by the blood to the kidneys where another hydroxylation process ensued at 1α-position by mitochondrial cytochrome P27b1; which also known as (1α-hydroxylase). This active form named 1, 25(OH)-2-D3 binds to the Vitamin D receptor (VDR) and retinoid X receptor (RXR) to pass to the nucleus and binds to the Vitamin D response element (VDRE) [(2)]. VDRE host genes play a vital role in controlling many cellular activities like regulating calcium and phosphate metabolism,

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bone remodeling, detoxification processes, cell cycle, apoptosis, and immunity response [3]. So the mitochondria have a vital role in the production of active vitamin D., On the other hand, vitamin D has important effects on mitochondrial function. Ricca et al. described the role of Vitamin D in mitochondrial function as they suggest that any defect in the active vitamin D/VDR will reflect as decrease mitochondrial respiration and shift cellular metabolism to the glycolytic pathway [4]. Accordingly, Vitamin D plays a major role in maintaining the physiological activity of the respiratory chain inside the mitochondria to generate energy. So vitamin D deficiency is leading to mitochondrial dysfunction through induction of intracellular pro-inflammatory status [5].

There are many classifications of vitamin D status according to various organizations, most of these definitions recommended that a level of ≥ 20 ng/ml of 25(OH) D to be regarded as sufficient or adequate vitamin D level which meets the needs of at least 97.5% of the population with regards to bone health, whereas a deficient level is < 12 ng/ml, a level between 12–19 ng/ml is insufficient level and a level of > 100 ng/ml denotes vitamin D intoxication (Hypervitaminosis) [6-10].

Serum lactate and pyruvate represent good indicators for mitochondrial function despite that their elevation may be related to other factors such as hypoxia and /or ischemia, low thiamine level, or even poor sampling technique [11]. In contrast, lactate/pyruvate molar ratio (L:P) represents a better scanning index for mitochondrial function as it evaluates the changes in lactate dehydrogenase substrate and product which give us the indirect image about redox state. Any changes in cellular respiration are reflected by changes in both serum lactate and serum pyruvate [11]. Under normal cellular respiration L: P molar ratio value not excessed 20 while value more than 25 will highly suggest respiratory chain defective [12].

Pyruvate, the primary substrate of mitochondrial pyruvate dehydrogenase complex, it also can be a recruit for the citric acid cycle or be converted into another pathway of gluconeogenesis, fatty acids, and non-essential amino acids synthesis [4]. Pyruvate level is controlled by a different mechanism like the efficiency of the mitochondrial pyruvate carrier, the inhibitory effect of pyruvate dehydrogenase kinase pyruvate carboxylase activity that correlated gluconeogenesis. Excess hydrogen from glycolysis that exceeds the mitochondrial transport capability will enforce pyruvate to accept the hydrogen form lactate to maintain glycolysis and elevate serum lactate level [13].

L-Carnitine represents an important component of the inner mitochondrial membrane in addition to it is a vital role in long-chain fatty acid and maintain energy homeostasis as serum level reflect the dietary status of the body. Carnitine plays a central role in long-chain acyl CoA transport to the mitochondrial matrix, where it subjected to β-oxidation enzymes activity located in the matrix. Moreover, it play role in glycolysis, gluconeogenesis, certain amino acids degradation, detoxification of many organic acids and xenobiotics, and interfere with the tricarboxylic acid cycle at pyruvate dehydrogenase, and α-ketoglutarate dehydrogenase [14]. Thus, low carnitine levels will affect all the above pathways that occur in the matrix. This study aimed to assess the association between Vitamin D level and mitochondrial function in children

Patients and methods

Multistage random sampling technique was

intended, the first stage includes a geographical stratification into the left and right bank of Mosul city to involve both banks. The second stage comprises gender stratification, to select male schools and female schools. Lot randomization to select schools on each side. Grading stratification, to choose a cluster of the second class of each grade in the school, in school with one class per grade; we selected the available class. Finally, all the pupils in the chosen classes were 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 November 2018 to April 2019. Three hundred children were selected. One hundred sixty from the west bank and one hundred forty from the east bank of Mosul city. Their age range was 6-12 years with a mean of 8.5± 0.51 with male to female per cent 45:55, respectively.

From each student, a sample of blood for vitamin D estimation was analyzed using VIDAS® 25 OH Vitamin D Total - BIOMERIEUX - France for the determination of 25-hydroxyvitamin D in serum using the Enzyme-Linked Fluorescent Assay (ELF) 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 colourimetric/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 wavelength as specified by the manufacturer [18].

Written informed consents were taken from parents of all children before initiating the study. The ethical committee approved this study. Independent sample *t*-test was used to evaluate differences between means of continuous variables, $p \leq .05$ was considered statistically significant. Receiver operating characteristic (ROC), was used to obtain vitamin D cutoff value predictive of normal mitochondrial function results. Data analysis was executed using the version 17 SPSS program.

Results

Seventy-two (24%) of children had sufficient serum vitamin D level of ≥ 20 ng/ml of 25(OH) D with a mean of 24.87±12ng/ml. Forty-eight (16%) of students had insufficient vitamin D levels ranged between 12–19 ng/ml and a mean of 17.7±2.4 ng/ml. One-handed seventy-eight children (59.3%) of investigated students had level < 12 ng/ml consistent with severe vitamin D deficiency. Only two children (0.7%) had hypervitaminosis D of more than 100 ng/ml, with a mean serum vitamin D level equal to 145± 43ng/ml.

Serum lactate, serum pyruvate, and the lactate/ pyruvate ratio significantly increased (*p*-ranged between.0001-.026*)* in children with insufficient vitamin D levels compared to those with sufficient vitamin D values. L-Carnitine value significantly (*p*=.0001) decreased in children with insufficient vitamin D levels (Table 1).

Table 1: Mitochondrial function tests comparing children with sufficient vitamin D level to children with insufficient vitamin D level

Parameter	Sufficient vitamin D3 Mean ± SD	Insufficient vitamin D3 Mean ± SD	*p*
Lactate	**1085±167.9**	**1625±120.8**	**.0001**
Pyruvate	**66.8±13.8**	**71.4±3.9**	**.026**
L:P ratio	**16.98±4.23**	**22.78±1.35**	**.0001**
L-Carnitine	**34.4±18.15**	**16.8±4.6**	**.0001**

There was a significant elevation in serum lactate and lactate/pyruvate ratio (*p<0.000*) with a significant reduction in serum L-Carnitine (*p<0.000*) in children with deficient vitamin D level in comparison to the group with sufficient vitamin D3 (Table 2).

Table 2: Mitochondrial function tests comparing children with sufficient vitamin D level to children with deficient vitamin D level

Parameter	Sufficient vitamin D3 Mean ± SD	Deficient vitamin D3 Mean ± SD	*p*
Lactate	**1085±167.9**	**1665±96.3**	**.0001**
Pyruvate	**66.8±13.8**	**69±3.8**	**.0515**
L:P ratio	**16.98±4.23**	**24.17±1.2**	**.0001**
L-Carnitine	**34.4±18.15**	**11.4±2**	**.0001**

In the hypervitaminosis D group, there was a significant elevation in serum lactate, lactate/pyruvate ratio (*p<.05*) with a significant reduction in serum pyruvate and L-Carnitine (*p<.05*) in comparison to children with sufficient serum vitamin D group (Table 3). There was no significant difference between students living in either bank of Mosul city concerning the measured vitamin D status and mitochondrial function.

Table 3: Mitochondrial function tests comparing children with hypervitaminosis D to children with sufficient vitamin D level

Parameter	Hypervitaminosis D3 Mean ± SD	Sufficient vitamin D3 Mean ± SD	p-value
Lactate	**839±19.8**	**1085±167.9**	**.04**
Pyruvate	**88±5.6**	**66.8±13.8**	**.034**
L:P ratio	**9.56±0.84**	**16.98±4.23**	**.0001**
L-Carnitine	**64±2.8**	**34.4±18.15**	**.025**

Receiver operating characteristic (ROC) analysis showed the area under the curve of

0.990 ± SE 0.001, a cut off value 20.950 ng/ml of Vitamin D was significantly (p=.000) associated with sensitivity of 100 % and 1-specificity of .060 value with presence of L:P molar ratio of ≤ equal 20 (Figure 1).

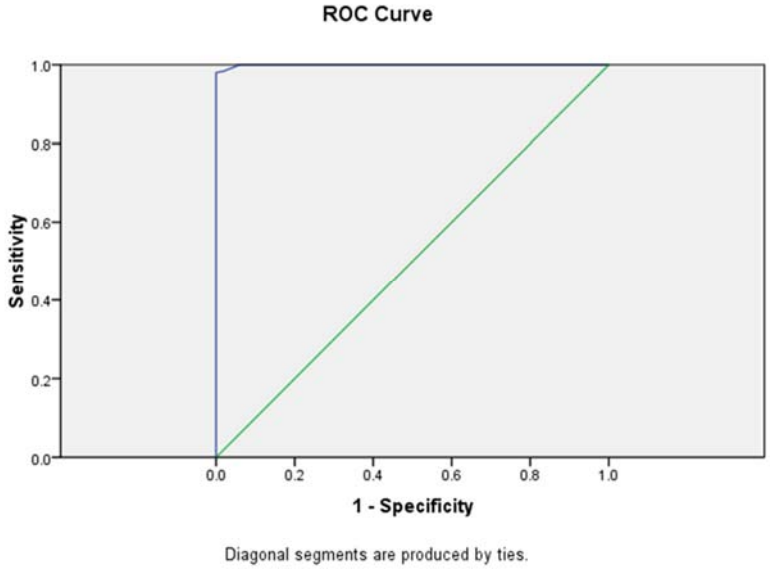

Figure 1. ROC Analysis (sensitivity vs 1-specificity)

Discussion

There was a significant difference in mitochondrial function between studied children with sufficient vitamin D level and the compared groups, which support that sub physiological concentration of calcitriol, at least in part, enhance mitochondrial dysfunction, normal serum concentrations of 25 (OH)D is essential for optimal cellular function and protect from the excessive oxidative stress-related DNA damage [19].

The results of this work revealed that value less than 12ng /mL will cause significant elevation of cellular oxidation status above the 20 which reflect an increase in oxidative stress whereas value of high serum vitamin D above 100 ng /mL significantly lower cellular oxidation below the normal range which reflects inefficient respiration by deactivation of pyruvate dehydrogenase complex (PDHC). Data are also accumulating that suggests that mitochondrial dysfunction is likely fueled by sustained intracellular inflammation, as in the case with vitamin D deficiency [20-22]. Vitamin D supports cellular oxidation and reduction (redox) control by maintaining normal mitochondrial functions [23, 24]. Vitamin D3 modulates pyruvate levels by modulating pyruvate kinase and pyruvate carboxylase activities [24].

Sustained, adequate serum 25(OH)D concentrations should allow target tissues to keep many of the harmful processes to be under control[25]. The minimal adequate level is 30 ng/mL[19]. In the current study, cut off value of vitamin D of 20.95 ng/ml was discovered to be associated with normal mitochondrial function or healthy mitochondria, which is in fact in harmony with the vitamin D status classification approved by most of the organizations that recommend a level of ≥ 20

ng/ml of 25(OH) D to be regarded as sufficient or adequate and which meet the needs of at least 97.5% of the population with regards to bone health [6,26-28].

Also, depending on the analyzed ROC findings, which displayed that 100 % of all studied children who have a level of Vitamin D ≥ 20.950 ng/ml would be correctly identified as having normal mitochondrial function by the finding of L/P ratio of ≤ equal 20 and only 6.0 % of those with vitamin D less than 20.95 ng/ml would be wrongly identified as having a normal mitochondrial function. Accordingly, a cut off value of Vitamin D ≥ 20.950 ng/ml has 100% sensitivity in its association with normal mitochondrial function.

In conclusion, both hypervitaminosis D and hypovitaminosis D produce a significant defect in mitochondrial function in children.

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حالة فيتامين (د) ووظيفة الميتوكوندريا عند الأطفال

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الملخص

الهدف من الدراسة: وجود اضطراب وظيفي في الميتوكوندريا ممكن تحديده عن طريق نقص فيتامين (د)، والهدف من الدراسة تقييم العلاقة بين وظيفة الميتوكوندريا، ومستوى فيتامين (د) عند الأطفال.

منهجية البحث: هذه الدراسة هي دراسة استطلاعية أجريت على أطفال المدارس الابتدائية في مدينة الموصل، وشملت الدراسة 300 طفل تتراوح أعمارهم بين 6 و 12 سنة. تم قياس 25 D (OH) في المصل كما، وتم تقييم وظيفة الميتوكوندريا عن طريق قياس مستوى اللاكتيت، والبيروفيت ، و إل- كارنتين في المصل.

النتائج: كانت هناك زيادة ذات اهميه إحصائية في مستويات اللاكتيت، البيروفيت ونسبة اللاكتيت / البيروفيت) تراوحت قيمة p بين 0.0001-0.026 (في الأطفال الذين لديهم مستويات غير كافيه من فيتامين د (12-19 نانوغرام / مل) مقارنة مع أولئك الذين لديهم قيم كافية من فيتامين د (20- 100 نانوغرام / مل). وكان هناك ارتفاع ذو دلالة إحصائيه في مستويات اللاكتيت والبيروفيت و نسبة اللاكتيت / البيروفيت ($p <.000$) مع انخفاض الدلالة الإحصائية في مستوى إلكارنتين ($p <.000$) عند الأطفال الذين يعانون من نقص مستوى فيتامين د (أقل من 12 نانوغرام / مل) بالمقارنة مع المجموعة التي لديها مستوى كافٍ من فيتامين (د) في مجموعة فرط الفيتامين د 100 < نانوغرام / مل) ، وكان هناك ارتفاع ذو دلالة إحصائية في مستوى اللاكتيت، ونسبه اللاكتيت / البيروفيت في المصل ($p <.05$) مع انخفاض ذو أهمية إحصائية في مستوى البيروفيت و إلكارنتين ($p>.05$) مقارنة مع مجموعة لأطفال الذين لديهم كمية كافية من فيتامين د في المصل، وأظهر تحليل خصائص تشغيل جهاز الاستقبال (ROC) أنه مع وجود منطقة تحت المنحنى 0.990 SE ± .001 ، فإن قيمة القطع البالغة 20.950 نانوغرام / مل من 25 D (OH) كان لها ارتباط ذو دلالة إحصائية ($p = .000$) بحساسية 100٪ و 1 - خصوصية 0.060 مع وجود نسبة مولارية اللاكتيت: البيروفيت ≤ 20.

الاستنتاج: يجب المحافظه على قيمة القطع البالغة 20.950 نانوغرام / مل من 25 D (OH) في المصل لضمان الوظيفة الطبيعية للميتوكوندريا، ترتبط قيمة 100 < 25 D (OH) نانوغرام / مل في المصل بوظيفة الميتوكوندريا غير الطبيعية.

الكلمات الدالة: فيتامين (د)، وظيفة الميتوكوندريا، الأطفال، اللاكتيت، البيروفيت، إل- كارنتين.