

Vitamin D Level In Patients With Multiple Sclerosis In Comparison With Healthy Controls

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Abstract

Background: Multiple sclerosis is a chronic, immune – mediated inflammatory and neurodegenerative disease of the central nervous system (CNS). The prevalence of multiple sclerosis is highest where environmental supplies of vitamin D are lowest.

Vitamin D is a steroid aid to maintain the normal level of calcium in the circulation, enhancing immune function, and may protect people from multiple sclerosis.

Objective: To evaluated the effectiveness of vitamin D and calcium level in patients with multiple sclerosis.

Design: Two groups, healthy and patients based in hospital.

Setting: Both healthy and patients of the Iraqi population in Al-Hakeem hospital in AL-Najaf city.

Population: 36 patients (11 male, 25 female), and 36 healthy (15 male, 21 female).

Methods: Study was carried out in the department of biochemistry, Collage of Medicine, Kufa University in period from February to August 2005.

The samples was measured by the high performance liquid chromatography (HPLC) technique. This technique was of choice in measuring the low levels of vitamin D in sera.

Results: The normal values of vitamin D levels were in 19.21 ng/ ml \pm 5.51, and calcium levels were 10.79 mg / dl \pm 1.19 in both sexes (males and females).

The result were taken from also showed that a first group a high significant decrement ($P < 0.01$).

In serum of thirty-six patients affected with multiple sclerosis, the vitamin D level average in this group were 6.46 \pm 0.91 ng / ml, and calcium level were 8.69 \pm 1.85 mg / dl.

الخلاصة

يعتبر تصلب الأنسجة المتعدد هو التهاب مناعي مزمن متوسط الشدة وهو مرض يصيب الجهاز العصبي ويسبب تلف في الأعصاب . حدة هذا المرض تكون الاعلى عندما يكون مستوى فيتامين د قليل. يعتبر فيتامين د من الاستيريدات التي تحافظ على مستوى عنصر الكالسيوم في الدورة الدموية و قد تم قياسه بواسطة تقنية كروماتوغرافيا السائل عالية الأداء. أن هذه التقنية هي الانسب لقياس التراكيز الواطنة من هذا الفيتامين في الدم. في هذه الدراسة تم فحص 36 شخصا من المرضى (11 ذكور, 25 اناث) و 36 شخصا اصحاء (15 ذكور, 21 اناث) لتحديد مستوى فيتامين د في أمصالهم . لقد كان معدل فيتامين د في الاصحاء مساوي الى 19,21 نانوغرام لكل مليلتر في كلا الجنسين.

ان النتائج المستفاة من المرضى أظهرت انخفاضاً واضحاً و ملحوظاً في مستويات فيتامين د في مصل الدم لدى 36 شخصاً . حيث اظهرت النتائج ان معدل فيتامين د في المرضى هو 6.46 نانوغرام لكل مليلتر .

Introduction

Multiple sclerosis (MS) is a slowly progressive, often disabling disease of the central nervous system (CNS),

characterized by disseminated patches of demyelination in the brain and spinal cord. Women are affected more often than men. Age at onset of the clinical symptoms is typically between

20 and 40 y(1). A role for vitamin D in MS has been suggested (2,3,4,5).

Multiple Sclerosis is regarded as a complex multicausal disease. The etiological factors comprise (a) genetic factors, (b) dysfunction of the immune system (autoimmunity), and (c) environmental factors(6). The environmental factors, possibly contributing to susceptibility for MS, are sunlight and nutrition indicates that food intake may be an influencing factor determining the disease susceptibility (2,3,4). An association has been reported in Norway between the relatively low risk of MS along its Atlantic coast and the relatively high dietary intake of fish oil, a rich source of vitamin D₃ (2,6).

Vitamin D: It is a steroid. Steroids are group of substances, prohormone with antirachitic properties(4). Vitamin D also appears to have other roles within the body, such as enhancing immune function (7), From animal and human studies, researchers have hypothesized that vitamin D may protect people from multiple sclerosis (4).

The influences of (1, 25-(OH)₂D) on cells of the nervous system were recently reviewed by Garcion. It appeared that (1, 25-(OH)₂D) had effects on neurons, oligodendrocytes, as well as astrocytes, but the exact pathways of these effects remain to be established. In general, the influences of (1, 25-(OH)₂D) on these cells seem to be neuroprotective and anti-inflammatory (8).

Multiple Sclerosis prevalence and sunlight:

This disease results in multiple and varied neurologic symptoms and signs, usually with exacerbations and remissions at the onset: relapsing-remitting multiple sclerosis, followed in later years by a more chronic progressive course: secondary progressive multiple sclerosis. A

primary progressive form of multiple sclerosis is also recognized. It affects some 350,000 people in the U.S. and 2 million worldwide, and occurs most commonly in young adults. Women, who are affected more than men, have a lifetime risk of about 1 in 200 in the U.S. Vitamin D is a hormone manufactured naturally in the body. Age at onset of the clinical symptoms is typically between 20 – 60 y⁽¹⁾. Vitamin D is responsible for helping the body absorb and effectively utilize calcium. Unfortunately, over 70% of women ages 51-70y and nearly 90% of women over 70 are not getting an adequate intake of vitamin D from food and supplements. Low level of vitamin D is associated with reduced calcium absorption, bone loss and increased risk of fracture in those women⁽⁹⁾. The suggestion that the risk of developing MS is largely determined before the age of 15 y has been questioned by Australian epidemiological data. New evidence has been reported that increased sun exposure during ages 6-15 y is associated with a decreased risk of multiple sclerosis⁽¹⁰⁾. The prevalence in the migrant population from the UK and Ireland in the different regions in Australia showed a significant correlation with latitude and was considerably less than in their countries of origin⁽¹¹⁾.

The Aims of the work

- 1- To estimate vitamin D levels in normal control group in some Iraqi population in Najaf city.
- 2- To estimate vitamin D levels in some patients affected by some neurodegenerative diseases such as multiple sclerosis.
- 3- To determine the sensitivity of measurement of vitamin D in sera by modified current method of HPLC technique

Material and Methods

The present study was carried out in the department of biochemistry, Collage of Medicine, Kufa University in period from February to August 2005. Thirty - six blood samples from (15 males and 21 females) were collected from healthy volunteers and 36 blood samples (11 males and 25 females) were collected from patients in Al-Hakeem hospital in Al - Najaf Government. The HPLC method was the method of choice in this field .The reverse phase HPLC method has a very high sensitivity and needs only a very small amount of sample without any complicated pretreatment for the used serum which may be a major source of errors. The serum stored at deep freeze -20°C until the time of analysis, to prevent any unfavorable decomposition, any hemolytic samples were excluded from the measured groups, all samples were collected during the morning at about 8:00-10.00 o'clock and we make sure that all patients and volunteers, did not take any medicament that may influence vitamin D levels which may alter the final results. Due to the affinity of 25- hydroxyvitamin D to plastic all steps were performed in glass tubes, preferable pointed-bottom glass tubes⁽¹²⁾.

The HPLC separation works with an isocratic method which mean that the chromatography can be carried out with single pump, at 35°C and the reversible phase column was applied.

Chromatograms were detected by the UV- detector. The retention time of 25-hydroxy vitamin D was 30 minutes for each run. Finally the results were quantified by the delivered serum calibrator and calculated by the external standard method which was done by the integration of the peak area. The acetonitrile solution was used as eluant solution. The Conditions for Vitamin D measurement were as follow:

A-Mobile Phase: 100 % Acetonitrile.

B-Flow Rate: 1 ml / min.

C- Wave Length: 256nm- 262nm.

D- Column shim pack: MRC- ODS [Octadecylslyle].

E- Temperature : Room temperature or 35 °c.

Results

The mean of vitamin D levels in the control group was 19.21ng / ml, the other related statistical parameters as serum calcium level was 10.79 mg/dl shown in table 1.

Table 2 shows that there is a highly significant decrement ($P < 0.01$) in mean serum 25- hydroxy vitamin D levels in patients with multiple sclerosis in men and women if compared with age matched control group of healthy volunteers, there is 35.2% decrement in serum vitamin D levels in those patients, the level of vitamin D was 6.46 ng /ml, while the calcium level was 8.69 mg /dl, other statistical values are included in table 2.

Table 1. Vitamin D Level in Sera of Healthy Males and Females.

Group Type		Vitamin D			Calcium	
Sex	Age yrs	No.	X ng/ml	SD ng/ml	X mg/dl	SD mg/dl
Male	20-65	15	24.56	±4.52	10.87	±2.22
Female	20-65	21	14.37	±1.86	10.72	±2.22
Total	20-65	36	19.21	±5.51	10.79	±2.19

Table 2. Serum Vitamin D Levels in Multiple Sclerosis Patients and Controls.

Group Type	Multiple Sclerosis Patient (20-65) yrs					Control Group (20-65) yrs					C S
	Vitamin D			Calcium		Vitamin D			Calcium		
	No.	X ng/ml	SD ng/ml	X g/dl	SD mg/dl	No.	X g/ml	SD ng/dl	X mg/dl	SD mg/dl	
Male	11	4.99	0.98	8.77	±1.80	15	24.56	±5.52	0.87	±2.22	HS
Female	25	6.62	0.85	8.46	±1.98	21	14.37	±1.86	0.72	±2.22	HS
Total	36	6.46	0.91	8.69	±1.85	36	19.21	±6.51	0.79	±2.19	HS

Discussion

1-Determination of Vitamin D Levels in Sera of Healthy:

The mean of vitamin D levels in the control group was 19.21 ng / ml, the other related statistical parameters as serum calcium level was 10.79 mg/dl. The value in our study in fact can be relatively considered as sub-normal value. Holick. in 2000 ⁽¹³⁾ reported that the normal range of serum 25-hydroxy vitamin D level in human is 25-56 ng/ml in both sex. In addition to that, he mentioned that the optimal 25-hydroxy vitamin D value is 45-50 ng/ml.

The decrement in vitamin D level in healthy Iraqi population may be attributed to the malnutrition and unavailability of animal sources nutrition or insufficiency of such types of diet in Iraqi people food due to the previously imposed embargo and low income of these people, where the diet is mainly composed of carbohydrate and fiber components. The vegetarian diet of a major sector of Iraqi people adversely affects 25-hydroxyvitamin D status⁽¹⁴⁾.

In our country and specially our city due to social behaviors and due to religious reasons, the women must cover all the body with black cloths, and wear a veil and some times black gloves when they go outdoors. These factors act as a good reason for preventing the cutaneous production of vitamin D in those women, even when they live in a sunny climate.

Many people leave for work early in the morning, return home after dark, and drive to and from work, so that, during winter, they have limited sunlight exposure for five out of every seven days ⁽¹⁵⁾. Window glass allows only 5% of the UV-B light range that produces vitamin D to get into home, latitude, time of year (virtually none available in winter), clouds can block UV-B light. Pollution, smog and ozone can block UV-B light, and the darker the pigmentation or more tanned the skin, the less UV-B penetrates. In veiled Moslem women indicates that, in the absence of sunlight exposure, they have a range below 8 ng/ml of vitamin D concentration ⁽¹⁶⁾.

However, our result was in agreement with reference value for serum 25- hydroxy vitamin D concentration that obtained from Australia and New Zealand which revealed that the 25- hydroxy vitamin D level ranges between 10-20 ng/ml ⁽¹⁷⁾. In U.S.A., it was reported that 70% of the United States population has 25-hydroxy vitamin D level below 35 ng/ml ⁽⁸⁾.

A recent study from Harvard in USA in June 2004 found that 24% of healthy adolescents had 25-hydroxy vitamin D levels less than 35ng/ ml. The research was done on a population of 7 million military personnel in U.S. Army and Navy between 1992 and 2004. Because vitamin D levels are strongly influenced by skin color, separate analyses were conduct among whites, blacks, and Hispanics'. The

average age of onset of multiple sclerosis cases was 28.5 years old⁽¹⁸⁾.

2- Serum Vitamin D and Multiple Sclerosis:

A study of 25- hydroxy vitamin D concentration in the resident adult population in the Catchments' area of one of Birmingham hospitals in the UK and other European countries has found 22% of healthy adult Asian couples (both sexes) had 25- hydroxy vitamin D less than 10 ng / ml with normal concentration in white controls⁽¹⁴⁾.

Vitamin D deficiency was detected in a group of female MS patients who were subjects of a study on osteoporosis. Of these patients ($n=52$), 70% had a subclinical vitamin D deficiency, defined as serum 25OHD < 20 ng/ ml or 50 nmoll. This study consecutively recruited female MS patients who were admitted to a tertiary care hospital because of deterioration in their clinical status, and there was no appropriate control group. Results suggested that low circulating 25OHD levels contributed to low bone mineral density (BMD)⁽²⁰⁾.

Another group of MS patients ($n=54$), of whom 64% had a subclinical vitamin D deficiency, defined as serum 25OHD < 20 ng/ml or 50 nmoll, had more rapid bone loss and more frequent fractures than healthy age- and gender-matched controls. Levels of 25OHD were on average 8 – 37.5 ng/ ml or 2037.5 nmoll lower in MS patients than they were in the three control groups: men, pre- and postmenopausal women. In the analyses, lack of sunlight exposure, a vitamin D-deficient diet, immobility, and corticosteroid treatment contributed to the low 25OHD serum concentrations⁽²¹⁾.

Mahon *et al* (2003)⁽²²⁾ reported that 48% of MS patients ($n=39$) had a

subclinical vitamin D deficiency at baseline, defined as serum 25OHD < 20 ng /ml or 50 nmoll. Only a few reports have investigated the association between MS and 25OHD serum concentration. Vitamin D deficiency was detected in a group of female multiple sclerosis patients who were subjects of a study on osteoporosis. Results suggested that low circulating 25OHD levels contributed to low bone mineral density⁽²⁰⁾. Levels of 25OHD were on average 8-15 ng/ml lower in MS patients than they were in the three control groups: men, pre- and postmenopausal women. In the analyses, lack of sunlight exposure, a vitamin D-deficient diet, and corticosteroid treatment contributed to the low 25OHD serum concentrations⁽²¹⁾.

While in our study we reported that the level of serum 25 OHD equal to 6.46 ng/ml. Moreover, seasonal variation of (1, 25(OH) 2 D) serum concentration had been established in multiple sclerosis Patients, in addition to differences between male and female concentration, postmenopausal women, lack of sunlight exposure, and a vitamin D-deficient diet. It is regarded as a complex multicausal disease.

The results that are shown in table - 2-explain the decline of vitamin D to its lowest level in multiple sclerotic patients. However, the decrements in the calcium levels in those patients refer to the low levels of this ion in most elderly Iraqi population at this age. The results in our study if compared with that obtained from other studies in the world it showed that there is clear differences between them due to special sociality.

This issue also was discussed by Taha *et al.*, in 1984, they reported that “people of cultures such as

Bedouins living in the Nagged Desert, who are required to have most of the skin surface covered by clothing, are prone to develop vitamin D deficiency⁽²³⁾.

Increased family incidence and association with certain allotypes suggests genetic susceptibility. The genetic epidemiology indicate that multiple sclerosis is not a single-gene disorder⁽²⁴⁾. Autoimmune responses to myelin components may play an important role in the initiation of multiple sclerosis. The autoimmune character of multiple sclerosis is supported by the presence of numerous T lymphocytes in multiple sclerosis lesions and various deviating immune parameters for multiple sclerosis patients⁽²⁴⁾. Among the postulated environmental etiological factors for multiple sclerosis is infection by a latent virus, possibly by a human herpes virus or retrovirus, in which viral activation and expression trigger a secondary response. However, no virus has yet been identified that causes multiple sclerosis⁽²⁵⁾. Other environmental factors, possibly contributing to susceptibility for multiple sclerosis, are sunlight and nutrition. Phytic acid may reduce the absorption of calcium in the gut. Obesity has been associated with vitamin D deficiency⁽²⁶⁾.

Conclusions

The most important conclusions from the current work are:-

- 1- Vitamin D can be measured successfully by the HPLC technique with high accuracy and precision with the current modified method.
- 2- The normal values of vitamin D levels in the controls group in Al-Najaf city were in general lower than that reported by other investigators in western countries or USA.

3- The level of vitamin D is extremely affected by the seasonal variation in Iraqi and that may be related to the amount of light reaches the earth during summer and winter terms in our region.

4- Vitamin D deficiency is predominant in low socioeconomic people.

5- Serum vitamin D level in patients was relatively very low and such patients must be given a supplementation of vitamin D and calcium to compensate the loss in this vitamin.

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