

RESEARCH ARTICLE

Effect of Vitamin D, Calcium and Phosphate on The Low Back Pain

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ABSTRACT

Objective: We have aimed to analyze and examine the relationship between varied levels of vitamin D, Ca²⁺ and PO₄⁻³ in the serum of patients suffering particularly from chronic low back pain and further to investigate its impact on pain and even on functional capacity.

Methods: serum of 150 patients (70 female and 80 male) with, aged between 35 and 60 years, was participated in the study. Control group include 100 healthy volunteers. Level of vitamin D estimated using ELIZA kits while Ca²⁺ and PO₄⁻³ level were measured by spectrophotometer.

Results: results indicate that all patients have low level of vitamin D (21ng/mL) when compared to the normal subjects (62.8 ng/mL) with p- value 6E-05. while for Ca²⁺ level was equal to (7.95ng/mL) refereed to low than that of normal subjects (11.97 ng/mL) with P- value 0.0534 and (0.33 ng/mL) for PO₄⁻³ concentration compared with (0.09 ng/mL) for normal human (p-value 0.01).

Conclusion: Vitamin D deficiency may be referred to man cause of low back pain, Vitamin D levels should be observed in patients with musculoskeletal pain at risk of Vit D deficiency. Vitamin D levels can used for Diagnostic study.

Keywords: Low back pain, Vitamin D, Ca²⁺ ion concentration, PO₄⁻³ ion concentration.

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INTRODUCTION

One of the highly prevalent musculoskeleton pain is low back pain which may lead to severe condition of loss of functionality and labour.¹ In accordance with the period of pain, we can classify it in 3 types as acute (lasting < 6 weeks), subacute (lasting amid 6 and 12 weeks), and chronic (lasting more than 12 weeks).² About 30% of acute low back pain, in due course of time, become chronic.³ Acute low back pain affect in muscle spasm in locomotor system, while chronic cases may lead to incapacity of performing the routine tasks.⁴ Studies reveal about muscle atrophy in patients deficient of vitamin D. Also, the evidence about significantly higher atrophy rates in type II-a muscle fibers, is provided by biopsies conducted on atrophic muscles.¹

According to the recent studies, vitamin D deficiency encourages to stage of resistant chronic musculoskeletal system pain and even neuromuscular dysfunction. Henceforth, it is clear that vitamin D deficiency may have potential damaging impacts on musculoskeletal system.⁵ The very first indications of vitamin D deficiency may be some weakness of proximal muscles and widely spread pain. Ther could be some complaints especially about lower extremity pain.

Proximal muscles weakness may lead to difficulty during walking and result in the antalgic walking pattern.^{6,7} It can be summarized that chronic low back pain (CLBP) is one of the symptoms originated from vitamin D deficiency. Ultimately, it affectsthe quality of life or in other terms functional insufficiency. Relevant literature show very few researches connecting chronic musculoskeletal pain and vitamin D, with contradictory outcomes. Here, we have aimed to analyse the same.

Reported prevalence of LBP varies from 12–33% to 11–84%. In a year, 40.2% of LBP patients have persistent symptoms. 36.1% of them show some positive development in deteriorating the problem, and 14.2% experience an enhancement of their symptoms.⁸

Radiculopathy, specific LBP and nonspecific LBP are three diagnostic categories for LBP Nonspecific LBP (more than 90% LBP cases) comes with symptoms without clear particular cause e.g. infection, malignancy,

spondyloarthritis, spinal stenosis and fracture.⁹ Age, sex, body mass index, habbit of smoking, psychological factors and strenuous physical activity are known key factors with LBP.¹⁰

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Growing evidence suggests an association of chronic low back pain with reduced levels of vitamin D.¹¹ However, the pain due to osteomalacia is not clear to associate with vitamin D and chronic low back pain. Vitamin D influences our immune system.^{12,13} Inflammatory cytokines' regulation by vitamin D is found to be correlated with chronic pain symptoms. Also, there are opposite data on association of lesser levels of vitamin D and chronic LBP.⁵

Also, the observation of vitamin D administration on the improvement of chronic pain is mentioned in some studies.¹⁴ In contradiction to this, there is disparity in the effect of treatment using vitamin D amid randomized and double-blind clinical trials if we compare the studies of other designs.¹⁵ We undertook this randomized double-blind clinical trial based on an experimental design as there are lesser studies on this idea.

Experimental Part

1. The study included 150 patients with low back bone pain (80 males and 70 females) aged from 35 to 60 years with 100 normal subjects as control 60 males and 40 females nearly in the same age rang.
2. Blood collection: 5 mL blood sample was collected from Patients who visited the privet clinic and the control was collected from relatives and friends in the college.
3. Other patients with different diseases were excluded from this study.
4. The blood was centrifuged at 6000 rpm using hitch centrifuge. Then, the serum was collected to be used for determination of vitamin D, Ca²⁺ ion and phosphor.
5. Vitamin D was estimated by ELIZA technique as illustrated below.
6. Calcium ion and phosphorus was measured using spectrophotometric method using ready for use kit as shown below.

Assay for Vitamin D

Principle of the Assay

The ELISA works upon methodology of competitive binding enzyme immunoassay technique. An antibody (particular to vitamin D) is pre-coated over microtiter plate provided in the assay it has been observed and found that while reacting, vitamin D competes with a designated amount of biotin-labelled vitamin D for sites on a pre-coated Monoclonal antibody mentioned above. non-required conjugate and unbound sample or standard are washed with the help of washing solution provided with the kit Next, avidin conjugated to horseradish peroxidase (HRP) is clubbed to every microplate well and incubated for 15 min minutes. Then a (TMB) as substrate solution is added to each well. The enzyme-substrate reaction is terminated with the supplement of a sulphuric acid solution 0.01 molar. Data is collected by using spectrophotometer at 450 nm \pm 2 nm. Concentration curves help to identify the concentration of vitamin D in the samples.

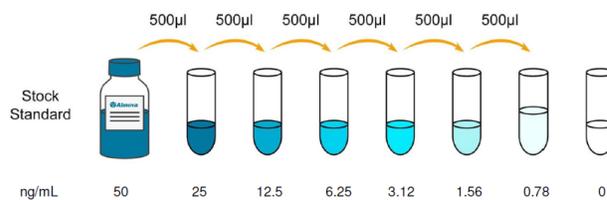


Figure 1: Serial dilution for the stock solution

List of Components

Component	Quantity
Assay Plate	96 wells
Standard	2 vials
Sample Diluent	20 mL
Assay Diluent A	10 mL
Assay Diluent B	10 mL
Detection Reagent A	60 µL
Detection Reagent	120 µL
Wash Buffer (25 x concentrate)	30 mL
Substrate	10 mL
Stop Solution	10 mL
Plate sealer for 96 wells	5

Reagent Preparation

- Wash Buffer solution is providing with the kit. About 30 mL of wash buffer was diluted with deionized water to prepare 750 mL of Washing Buffer.
- Standard solution
The Standard was reconstituted considering 1.0 mL of sample diluent; it produces a solution of 50 ng/mL. Prior to prepare serial dilutions as shown in the Figure 1, which are not allowed to be made in the wells directly, the standard were stood for 15 minutes (minimum) The undiluted standard is considered as the high standard (100 ng/mL). The sample diluent is represented as the zero standards (0 ng/mL) at 450 nm.
- Detection Reagent A and B
The working solution was diluted using the assay diluent A and B up to (1:100), respectively.

Assay protocol of Vitamin D

All reagents were kept at room temperature and were mixed thoroughly with method of gentleswirling before going for pipetting; foaming must be avoided. Working standards along with samples and all reagents were prepared as in prior sections.

1. A volume (50 µL) standard, blank, or sample was placed in specific well.
2. Detection reagent A was added (50 µL) as working solution to each well and covered with the plate sealer. The plate was gently tapping to ensure through mixing, then was incubate for 1 hour at 37°C.
3. Every well was aspirated and washed, the process was repeated 3 times for 3 washes. Then each well washed by filling with wash Buffer (approximately 400 µL) with the help of a squirt

bottle, multichannel pipette, manifold dispenser or auto-washer removing liquid at every step for better results and kept idle for 1~2 minutes. with the help of aspirating or decanting, we remove any wash buffer after the last wash. Further it gets inverted the plate and blot it against clean paper towels.

4. Detection reagent B working solution (100 µL) was added to each well; covered with a new plate sealer then incubated for 45 minutes at 37°C.
5. The aspiration/wash process was repeated for five times as conducted in step 3.
6. Substrate solution (90 µL) was added to each well, covered with a new plate sealer and incubated within 15-30 minutes at 37°C. (it is sensitive to light).
7. Stop solution (50 µL) was added to each well; if colour change does not appear uniform, gently tap the plate to ensure thorough mixing.
8. The optical density was determined for each well at once, using a microplate reader set to 450 nm.

Standard Curve

Standard curve was done by the data may be linearized by plotting the log of the concentrations ranged from 1 ng/mL to 100ng \ ml versus the log of the absorption.

Determination of PO₄⁻³ and Ca²⁺ Concentration

The serum Ca²⁺ and PO₄³⁻ concentration have been estimated according to the manufactured kit protocol perches from Promega company.

RESULTS AND DISCUSSION

In general, there is no similar studies in the literature to compare our results with it so our discussion depends on our hypothesis. “Bone is a living active tissue that’s continually being removed and replaced. This process is known as bone turnover”. Bone made up of matrix (collagen fibres) and cortex (primarily calcium and phosphorus).¹⁶

Higher quantity of minerals over collagen matrix ensure the strength of any new bone. Improper mineralization also brings Osteomalacia resulting in soft bones and can be painful also.¹⁷

This research is concerned with the measurement of concentrations of vitamin D, Ca²⁺ and PO₄⁻³ ions in the serum of backbone pain patients. Results indicate that there is a significant effect of the data variance with diseases. As shown in Table 1, there is a significant decrease in vitamin D, calcium ions and PO₄⁻³ ions in female patients in comparison to healthy people.¹

It’s clear from this data vitamin D is very effective in this disease with a very high p-value, the other biochemical variables also appeared a significant p-value indicated its effective development of backbone disease in female. Figure 2

shows the concentration of vitamin D in both group female patients and normal volunteers. From the same figure, it’s clear that the concentration of the vitamin D is very low in patients with respect to normal values.

Any lacking in vitamin D concentration is likely to develop backbone disease. vitamin D can be synthesised throw on or more metabolic pathways in addition to that come from food. Calcitriol (1, 25 [OH]₂ Vitamin D₃, which is present naturally in the skin, is converted to vitamin D with the aid of sun light on the skin.¹⁸ Some reasons such as illness and fragility are the most reason for many people to be at risk of osteomalacia due to the lack in produced enough quantity of vitamin D through exposure to sunlight for. On another hand, they may be having dark skin or may be not obtain much sunlight in some parts of the world. Cooler climates are possibly less efficient source of vitamin D.⁵

There is another reason caused a deficiency in vitamin D such as, few foods and chemicals preservatives with the food or diet prevent the absorption of vitamin D and normal absorption of calcium from the stomach or intestine.^{1,10}

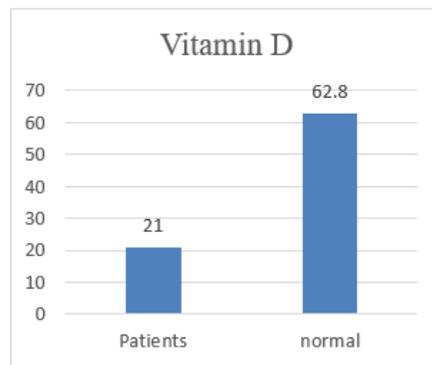


Figure 2: Concentration of vitamin D in patients (female) and normal volunteers.

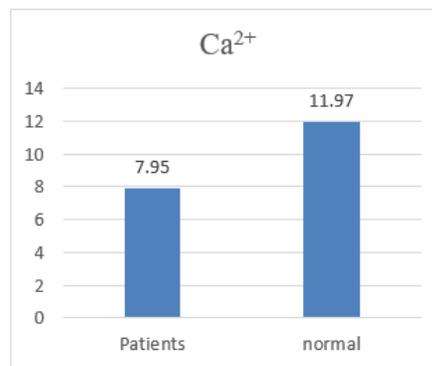


Figure 3: Shows the concentration of calcium in patients (female) and normal volunteers.

Table 1: Average value and standard deviation for female patients

	Vitamin D (ng/mL)		Ca ²⁺ (ng/mL)		PO ₄ ⁻³ (ng/mL)	
	Patients	normal	Patients	normal	Patients	normal
average	21	62.8	7.95	11.97	0.33	0.09
STD	±5.47	±4.25	±2.08	±2.101	±0.2	±0.06
P-value	6E-05		0.0534		0.0128	

Table 2: Average values and standard deviation for male patients

M	Vitamin D		Ca ²⁺		PO ₄ ³⁻	
	Patients	normal	Patients	normal	Patients	normal
average	20.6	62.8	8.8	11.97	0.31	0.09
std	±4.9	±4.3	±0.99	±2.1	±0.46	±0.06
p- value	6.4E-05		0.1083		0.284512	

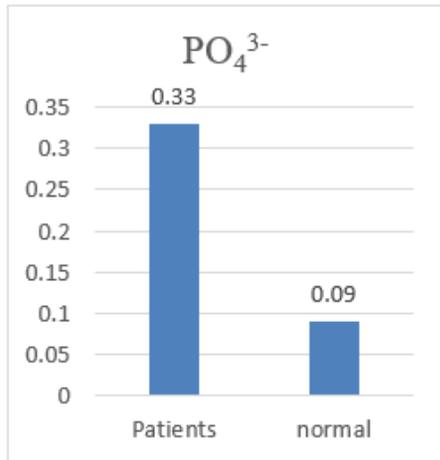


Figure 4: Concentration of PO₄³⁻ in female patients and normal

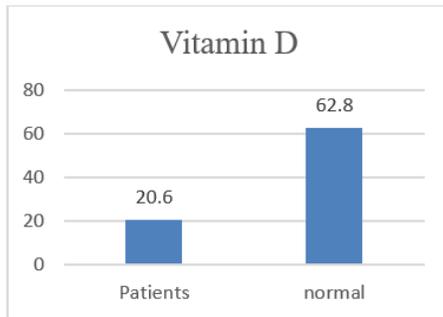


Figure 5: Concentration of vitamin D in patients and control in male

The normal requirement of vitamin D for normal human body is around 10 µg/400 units per a day to protect from osteomalacia. The human body naturally can produce up to 100 µg/4,000 units per a day through many metabolic pathways and can be stored in the body for a few weeks. There are many causes for vitamin D deficiency such as gut problems, untreated coeliac disease or previous stomach surgery, liver disease, kidney failure and Epilepsy tablets.

From these results (Figure 3), it seems that there is a decrease in Ca²⁺ concentration in female patients in compared with that normal values. Some literature refers to backbone pain and calcium, alkaline phosphatase were aggressively correlated with vitamin D. Also, in these patients, glucose presented a downward or negative correlation with vitamin D. The LBP problem is a challenge to health care providers and due to ignorance in developing countries.⁶ They didn't report early symptoms to gain early treatment in addition to occupational compulsions in rural areas compare to sedentary lifestyle in urban youth.¹⁹

The researchers have recommended frequent and initial screening for vitamin D along with albumin, glucose, protein,

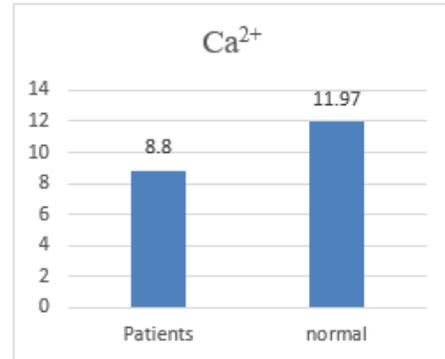


Figure 6: Concentration of Ca²⁺ in patients and control in male

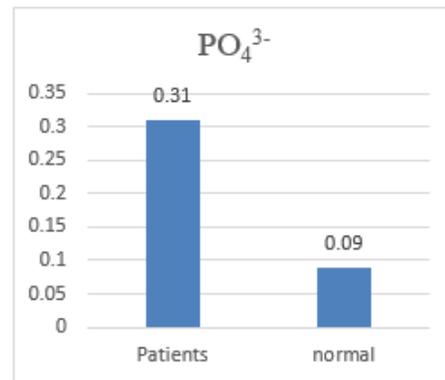


Figure 7: Concentration of PO₄³⁻ in patients and control in male

calcium, phosphorus, CRP in order to ensure general health check-up for non-specific body pain, especially LBP.¹

On another hand, there is an increase in PO₄³⁻ ions concentration in serum of female patients compared with that of normal subjects as in Figure 4.

While for male it seemed that vitamin D has a highly significant effect with respect to that of Ca²⁺ and PO₄³⁻ ions as in Table 2, and as shown in Figures 5, 6, and 7.

From the above table and figures represents a significant difference between vitamin D concentration in both normal and patients (Figure 5). In the same time there is a significant decreased in Ca²⁺ concentration and increase in PO₄³⁻ concentration this may be the cause of vitamin D deficiency.

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