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Quantification of Vitamin D and Fas Protein in Serum from Healthy Patients

Jose Augusto Camargo*

Neuroscience Research Institute, Newlin Medical Clinic, Sorocaba, Professor, School of Medicine, Pontifical Catholic University of Sao Paulo, Sao Paulo, Brazil

***Corresponding author**: Jose Augusto Camargo, Neuroscience Research Institute, Newlin Medical Clinic, Sorocaba, Professor, School of Medicine, Pontifical Catholic University of Sao Paulo, Sao Paulo, Brazil

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Abstract

Background: The effects of vitamin D on the human body have been the subject of numerous studies. Its beneficial effects on the immune system are promising. The aim of this study was to investigate the correlation between serum vitamin D concentration and cell death evaluated by the quantification of Fas apoptotic factor in healthy patients.

Methods: All patients included in the study were prospectively selected among individuals who attended a medical consultation between 2023 and 2024 and who needed to undergo routine laboratory tests on that occasion, including vitamin D levels. The results of serum samples from 77 healthy individuals, collected from the electronic medical records, were analysed. The concentrations of vitamin D and Fas protein measured by electrochemiluminescence and fluorescence methods, respectively, were compared.

Results: Our study reviewed 77 patient records from our archives that met the inclusion criteria. Age ranged from 18 to 95 years and there were 43 females and 34 males. We found a statistically significant and strong relationship between vitamin D and Fas protein levels (p=0.016). There were no significant differences in vitamin D levels according to age or sex.

Conclusion: Our results show an important correlation between vitamin D levels and Fas apoptotic factor in serum from healthy subjects. Factors such as age or sex did not obtain statistical significance. Future studies involving larger samples conducted at various centers that monitor and adjust blood vitamin D levels in each particular case are essential to identify approaches to improving and strengthening the immune system of the world population.

Keywords: Vitamin D; Fas Apoptotic Factor; Programmed Cell Death.

Introduction

The large number of studies conducted by researchers from all over the world highlights the intense efforts in seeking alternatives to help cure pathologies that are difficult to control and that have a poor prognosis, including severe genetic diseases, degenerative diseases, and cancer [1-6]. Many publications try to combine alternatives in order to obtain a better response to the treatments administered, including the concomitant supplementation of vitamin D (25(OH)D).

Vitamin D receptors (VDRs) are widely expressed throughout the body and experimental evidence suggests that they possess antineoplastic activity. Vitamin D is a steroid-like hormone that acts by binding to the VDR and that plays a key role in calcium homeostasis and metabolism. This vitamin also exerts other important effects that are distinct from its classical actions, including regulating immune cell function, hematopoietic cell differentiation and proliferation, and help-seeking protection of innate immunity. Furthermore, blood vitamin D levels have been correlated with good outcomes in patients undergoing allogeneic stem cell transplantation, regulating the immune response and

consequently reducing the risk of graft-versus-host reactions [7-20,21,22].

The binding of vitamin D to its receptor interferes with apoptosis, exerting immunomodulatory and anti-cell proliferation effects that could contribute to the prevention, reduction, and cure of a multitude of pathologies. Benefits for the treatment of diseases such as Alzheimer's, multiple sclerosis, Parkinson's, mood disorders, infectious diseases such as COVID-19, and organ transplants, among countless others, are possible [3-6,23-38]. The defence of the human body related to programmed cell death involves a complex, dynamic and fascinating cellular circuitry mediated by intrinsic and extrinsic pathways of apoptosis that can actively participate in the onset or resolution of these pathologies [1,7,36]. Among cell death markers, Fas protein, which belongs to the tumor necrosis factor (TNF) receptor superfamily, has been widely studied. It is the main pro-apoptotic protein. The interaction of the soluble form of Fas protein with its receptor (FasL) leads to the formation of a cell death-inducing signalling complex that activates different types of caspases, causing mitochondrial damage and, ultimately, proteolytic death [3,24, 25,28,29,38,39].

Since vitamin D can have beneficial effects on immunity, it would be of great clinical importance to analyse and elucidate its interaction with Fas protein in healthy individuals, considering that the vast majority of studies analyse this vitamin in patients with active pathologies. Therefore, the aim of the present study was to quantify and correlate vitamin D and Fas protein in serum obtained from healthy patients without pathologies.

Materials and Methods

Population and study sample

All patients included in the study were prospectively selected among those who attended a medical consultation between January 2023 and November 2024 and who needed to undergo routine laboratory tests, including the measurement of vitamin D. Serum samples from 77 healthy individuals, including 43 non-pregnant females and 34 males ranging in age from 18 to 95 years, were analysed. The concentrations of vitamin D and Fas protein were determined by electrochemiluminescence and fluorescence methods, respectively. Other laboratory tests included lipid levels, blood glucose, hematocrit, hemoglobin, leukocyte count, urea, creatinine, and urine, in addition to clinical assessment during the consultation with the researcher. The inclusion criteria were age ≥ 18 years, and a good health status demonstrated by clinical and laboratory analysis and monitored at our service. The exclusion criteria were age < 18 years, pregnancy, and the presence of symptoms and signs suggestive of infectious or inflammatory processes or active pathologies.

Data collection method

The present study employed a retrospective chart review approach. The electronic medical records that support the findings of the article and that are registered at the Newclin Clínica Médica Institute, Sorocaba, São Paulo, Brazil, were used as the primary data source and are confidential. Data were collected and checked against the inclusion and exclusion criteria using a structured form containing information such as sex, age, results of routine laboratory tests, vitamin D, Fas protein, and the patient's clinical condition on the day of the medical consultation.

Determination of vitamin D and Fas protein concentrations

Serum vitamin D levels were measured by electrochemiluminescence and the results were expressed as nanograms per milliliter (ng/ml). Fas concentration was measured by fluorescence using the Fas-L Human ProcartaPlex Simplex 96 Kit (EPX01A- 10260-901; Thermo Fisher Scientific Inc.), according to the manufacturer's instructions, and the results were expressed as picograms per milliliter (pg/ml) (Figure 1).

Statistical analysis

The results were organized in MS Excel spreadsheets (MS-Office 2013) and analyzed using IBM SPSS Statistics, version 26.0 (Statistical Package for the Social Sciences). Categorical variables such as sex, age, Fas protein concentration, and vitamin D concentration were reported as frequency and percentage. Spearman's correlation analysis was applied to determine the degree of relationship between the variables of interest (Fas x vitamin D and age x vitamin D) and the Mann-Whitney test was used to evaluate possible differences between sexes (sex x vitamin D). Significance was set at $p<0.05$.

Results

Seventy-seven patients were studied, including 43 females and 34 males ranging in age from 18 to 95 years. All patients underwent laboratory tests, including the measurement of lipids, blood glucose, hematocrit, hemoglobin, leukocyte count, urea, creatinine, urine, vitamin D, and Fas. These results, together with the clinical assessment during consultation with the researcher, were normal and there were no signs suggesting infectious or inflammatory processes.

Statistical analysis revealed no significant association between age and vitamin D ($p=0.115$) or between sex and vitamin D ($p=0.524$). A strong and statistically significant relationship was found between vitamin D and Fas levels ($p=0.016$) (Table 1).

Variable	Statistics	Vitamin D
	Correlation coefficient (r)	-0.274
FI	Sig. (p)	0.016
	n	77
	Correlation coefficient (r)	-0.274
	Sig. (p)	0.016
Obs Conc		
	n	77

Table 1: Spearman's correlation test was applied to evaluate the degree of relationship between the variables of interest: Vitamin D and Fas. Abbreviations. Fl=Fluorescence, Obs Conc= Value Fas.

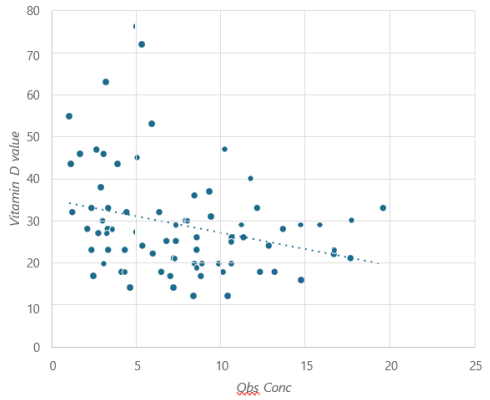

Figure 1: Obs Conc = Fas concentration in pg/ml and vitamin D Value = Vitamin D Concentration in ng/ml.

Discussion

Our chart review retrieved 77 individuals for data collection between January 2023 and November 2024 and the results showed an important correlation between serological vitamin D levels and Fas apoptotic factor. A higher concentration of 25(OH)D has been associated with several treatment benefits, an improved prognosis, prevention of severe symptoms, and a decrease in hospital admissions, as well as a lower risk of death, which decreased with each increase in its concentration [1,8,11]. Studies have also shown that the prevalence of vitamin D deficiency is high among patients with serious illnesses and the known risk factors for vitamin D deficiency are common in individuals with a history of low sunlight exposure, a diet low in this nutrient, increased body fat percentage, genetic factors, presence of gastrointestinal diseases that impair its absorption, use of chemotherapy, and receiving palliative care, among others [13,17,27,34,38].

A considerable number of studies have demonstrated a role of vitamin D in the immune system, promoting the production of antimicrobial peptides that help defend against infections; in the modulation of T and B cells and in the production of cytokines; in reducing the risk and attenuating the symptoms of acute respiratory infections such as the flu and cold and, more recently, COVID-19; in the prevention of autoimmune diseases, progressive degenerative diseases such as multiple sclerosis, Alzheimer's disease, Parkinson's disease, type 1 diabetes, systemic lupus erythematosus, Hashimoto's disease, and psoriasis [11,12,15,19,26].

On the other hand, hypervitaminosis D, a rare condition resulting from the administration of excessive doses of vitamin D or its prolonged use without medical supervision, can have toxic effects. Since vitamin D is fat soluble and can accumulate in the body, its supplementation can lead to both important health benefits and a series of complications.

Great efforts are made by researchers around the world in discovering new treatments, in contributing to existing treatments, and in preventing the most varied pathologies that plague humanity. The number of cells that die by apoptosis, also called programmed cell death, in the human body over a period of 24 hours can vary greatly but estimates indicate that about 50 to 70 billion cells die by apoptosis each day in a healthy adult. This process is essential for maintaining the body's homeostasis, eliminating damaged, diseased, senescent, and no longer necessary cells that can become harmful and trigger diseases in the body. Unlike necrosis, which is an uncontrolled cell death, apoptosis occurs in a controlled manner and does not cause inflammation. In general, cells are constantly undergoing cycles of renewal and the number of cells killed by apoptosis may be higher in some tissues and lower in others, depending on cellular activity and the necessities of the body.

The combination of existing treatment options for the most varied diseases, especially serious illnesses and diseases in advanced stages or with a poor prognosis, with 25(OH)D must be carefully evaluated, particularly when doses much higher than those recommended by the current medical literature for healthy individuals are administered [6,21,22,40-43] The important effect of vitamin D on the apoptotic process demonstrated here makes us reflect on whether, in many cases, we are not neglecting an important resource to help improve the quality of life of patients by preventing, alleviating suffering, more effectively treating and, ultimately, curing illnesses that affect the world population.

Conclusion

Our study demonstrated an important activity of vitamin D in immunological processes mediated by the apoptotic pathway. A strong and statistically significant relationship was observed when vitamin D levels were compared. New multicenter studies should be conducted to reach a consensus on the necessary supplementation levels, observing the individual needs of each specific case. When prescribing or administering doses considered to be too high for

patients treated for serious illnesses or pathologies with a poor prognosis, such as extreme exertion, the risk of toxicity must be disregarded. What must prevail is the certainty that all possible efforts and existing resources have been used to maintain the most important asset, which is LIFE!!!

Ethics Approval: This study was exempt from ethics approval by our institutional review board because the data set used in the analysis was completely de-identified.

Consent to Participate: Informed consent was obtained personally from all patients before data collection from their medical records. Participants were fully informed about the purpose of the study, their role in the study, and the confidentiality of their information. Data collection for the study was only started after the participants had acknowledged and agreed to these terms.

Human and Animal Rights: Not applicable.

Consent for Publication: Patient's consent to report this case has been obtained on the condition that all details that would enable any reader to identify the person will be omitted.

Standard of Reporting: Care guidelines have been followed in this study.

Availability of Data and Materials: The data supporting the findings of the article is recorded in the medical records of the treating service (Instituto Newclin Clínica Médica especializada de Sorocaba, São Paulo, Brasil), and is confidential.

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Conflict of Interest: The authors declare no conflict of interest, financial or otherwise.

Acknowledgements: Declared none.

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