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RESEARCH ARTICLE

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A Study of Vitamin D Status in Type 2 Diabetes Mellitus and Association of Vitamin D Levels with Glycemic Control

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ABSTRACT

Diabetes mellitus is a group of metabolic disorders, characterized by chronic hyperglycemia due to relative or absolute deficiency of insulin. There can be a defect in insulin secretion, or its action (insulin resistance) or both. Diabetes mellitus (DM) is a worldwide health problem with rising prevalence, which seems due to change in life style, increasing obesity rates and aging of the population. Osteopenia and bone fragility is one of the problems faced by many diabetics without the nature of cause of either residual insulin secretion or high insulin requirement. Studies also show that patients with type 2 diabetes (T2DM) have an increased risk of hip and vertebral fracture, thus hinting the link between the two distinct pathologies. **Aim of this study** is to find out the association between Vitamin D levels with glycemic Control and to measure the vitamin Levels in Diabetes Patients.

Conclusion: There is Negative association between Vitamin D and glycemic control.

Materials & Methods: This Cross sectional study was conducted in the Hail region, KSA, during the period of 1st January 2018 to 21st March 2019. Total 210 study patients aged 18–60 years; their Vitamin D levels were estimated and their socio-demographics data were collected regarding nationality, femininity, oldness, marital status, educational level, and occupation and analyze by SPSS 17.

Result: Only 0.5 percent of the total sample was Vitamin D sufficient, while 37.1 percent was insufficient. Majority i.e. 62.4 % had deficiency of vitamin D. It can be observed that majority of patients i.e. 53.8 % had diabetes for 5-10 years. Only 9 patients out of a total of 210 had diabetes for more than 15 years.

Conclusion: levels are Vitamin D levels in Diabetic patients are found to be low in Diabetic patients. Negative association is present between HbA1c levels and derangements in serum Vitamin D.

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Diabetes Mellitus, Vitamin D: BMI, HbA1c

Introduction

Type 1 DM can occur at any age. It occurs most commonly in juveniles but can also occur in adults, especially in those in their late 30s and early 40s [1]. Type 1 diabetes mellitus (DM) is a catabolic disorder in which circulating insulin is very low or absent, plasma glucagon is elevated, and the pancreatic beta cells fail to respond to all insulin-secretary stimuli. The pancreas shows lymphocytic infiltration and destruction of insulin-secreting cells of the islets of Langerhans, causing insulin deficiency. Patients need exogenous insulin to reverse this catabolic condition, prevent ketosis, decrease hyperglucagonemia, and normalize lipid and protein metabolism [2].

One theory regarding the etiology of type 1 DM is that it results from damage to pancreatic beta cells from infectious or environmental

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agents. In a genetically susceptible individual, the immune system is thereby triggered to develop an autoimmune response against altered pancreatic beta cell antigens or molecules in beta cells that resemble a viral protein. Approximately 85% of type 1 DM patients have circulating islet cell antibodies, and the majority also has detectable anti-insulin antibodies before receiving insulin therapy. Most islet cell antibodies are directed against glutamic acid decarboxylase (GAD) within pancreatic beta cells [3].

Currently, autoimmunity is considered the major factor in the pathophysiology of type 1 DM. Prevalence is increased in patients with other autoimmune diseases, such as Graves disease, Hashimoto thyroiditis, and Addison disease. Approximately 95% of patients with type 1 DM have either human leukocyte antigen (HLA)-DR3 or HLA-DR4. HLA-DQs are considered specific markers of type 1 DM susceptibility [4].

Recent evidence suggests a role for vitamin D in the pathogenesis and prevention of diabetes mellitus. Vitamin D deficiency is also an important independent predictor of development of coronary artery calcification in individuals with type 1 DM [5].

The etiology of type 1 DM has a strong genetic component. Nevertheless, identical twins have a concordance rate for type 1 DM of less than 50%. In studies of identical twin pairs in which 1 twin has type 1 diabetes, antibodies to the islet cell and to insulin are positive for several years in the non-diabetic twin before overt diabetes develops [6].

Extra genetic factors also may contribute. Potential triggers for immunologically mediated destruction of the beta cells include viruses (eg, mumps, rubella, coxsackie virus B4), toxic chemicals, exposure to cow's milk in infancy, and cytotoxins. As beta-cell mass declines with ongoing immunologic destruction, insulin secretion decreases until the available insulin no longer is adequate to maintain normal blood glucose levels. After 80-90% of the beta cells are destroyed, hyperglycemia develops and diabetes may be diagnosed [7].

A meta-analysis suggests a significant association between enterovirus infection and autoimmune/type 1 DM [3]. The role of enterovirus in development of type 1 DM warrants investigation in larger prospective studies (Ann V et al., 2009) [8].

Internationally, rates of type 1 diabetes are increasing. In Europe, the Middle East, and Australia, rates of type 1 diabetes are increasing by 2-5% per year. Scandinavia has the highest prevalence rates for type 1 DM (ie, approximately 20% of the total number of people with DM), while China and Japan have the lowest prevalence rates, with less than 1% of all people with diabetes. Some of these differences may relate to definitional issues and the completeness of reporting [9].

Type 1 DM is associated with a high morbidity and premature mortality. More than 60% of patients with type 1 DM fare reasonably well over the long term. Many of the rest develop blindness, end-stage renal disease, and, in some cases, early death. If a patient with type 1 DM survives the period 10-20 years after onset of disease without fulminant complications, he or she has a high probability of reasonably good health. Other factors affecting long-term outcomes are the patient's education, awareness, motivation, and intelligence level [10].

The morbidity and mortality associated with diabetes are related to the short- and long-term complications. Such complications include hypoglycemia and hyperglycemia, increased risk of infections, micro vascular complications (eg, retinopathy, nephropathy), neuropathic complications, and macro vascular disease. As a result of these complications, people with diabetes have an increased risk of developing ischemic heart disease, cerebral vascular disease, peripheral vascular disease with gangrene of lower limbs, chronic renal disease, reduced visual acuity and blindness, and autonomic and peripheral neuropathy [11]. Diabetes is the major cause of blindness in adults aged 20-74 years, as well as the leading cause of non-traumatic lower-extremity amputation and end-stage renal disease (ESRD).

Controlling blood glucose, hemoglobin A1C (HbA1c), lipids, blood pressure, and weight are important prognostic factors. Patients with diabetes have a lifelong challenge to achieve and maintain blood glucose levels as close to the normal range as possible. With appropriate glycemic control, the risk of both micro vascular and neuropathic complications is decreased markedly. In addition, if hypertension and hyper lipidemia are treated aggressively, the risk of macro vascular complications decreases as well [12]. The benefits of glycemic control and control of co-morbidities are weighed against the risk of hypoglycemia and the short-term costs of providing high-quality preventive care. Studies have shown cost savings due to a reduction in acute diabetes-related complications within 1-3 years of starting effective preventive care.

The most common symptoms of type 1 diabetes mellitus (DM) are polyuria, polydipsia, and polyphagia, along with lassitude, nausea, and blurred vision, all of which are due to the hyperglycemia itself [13].

The disease onset may be sudden, with the presentation of an infection. It is not unusual for type 1 DM to present with diabetic ketoacidosis (DKA); it may occur de novo or develop with the stress of illness or surgery. An explosive onset of symptoms in a young lean patient with ketoacidosis always has been considered diagnostic of type 1 DM (Tuomelihto Jet al., 2001).

By definition, patients with type 1 DM require lifelong treatment with insulin to promote glucose utilization. Rapid-, short-, intermediate-, and long-acting insulin preparations are available. Although pork, beef, and beef-pork insulins were previously used, recombinant human insulin is used almost exclusively in the United States. Commercially prepared mixtures of insulin are also available [14].

Rapid-acting insulins include regular insulin, lispro, glulisine, and aspart insulin. Regular insulin is a preparation of zinc insulin crystals in solution. Its onset of action is 0.5-1 hour, it peaks at 2.5-5 hours, and duration of action is 6-8 hours. Lispro insulin is a form of regular insulin that is genetically engineered with the reversal of the amino acids lysine and proline at B28,29 in the B chain. Aspart insulin has aspartic acid substituted for proline in position 28 of the B chain. Both of these insulins are absorbed more quickly and have a rapid onset (5-10 min), peak (1 h), and duration (4 h) of action. Therefore, they have the advantage that they may be administered shortly before eating. Semilente insulin is like regular insulin and is a slightly slower rapid-acting insulin. It contains zinc insulin microcrystals in an acetate buffer and is not readily available in the United States [14].

Intermediate-acting insulins include neutral protamine Hagedorn (NPH) insulin, which contains a mixture of regular and protamine zinc insulin, and Lente insulin, which contains 30% Semilente insulin and 70% Ultra Lente insulin in an acetate buffer [12].

Long-acting insulins include Ultra Lente insulin, containing large zinc insulin crystals in an acetate buffer, and insulin glargine, a newer long-acting insulin that has no peak and produces a relatively stable level lasting more than 24 hours. Both insulins can supply basal 24-hour insulin with a single daily injection.

Mixtures of insulin preparations with different onsets and durations of action frequently are administered in a single injection by drawing measured doses of 2 preparations into the same syringe immediately before use. The exception is insulin glargine, which should not be mixed with any other form of insulin. Preparations that contain a mixture of 70% NPH and 30% regular human insulin (ie, Novolin 70/30, Humulin 70/30) are available, as is Humulin 50/50, but the fixed ratios of intermediate-acting to rapid-acting insulin may restrict their use. In addition, a 25/75 mixture of NPH and lispro insulin is available [14].

A new basal insulin, insulin degludec, was found to be safe and effective and compared favorably with insulin glargine in a phase II clinical trial of patients with type 1 DM [15]. Both regular human insulin (RHI) and rapid-acting insulin analogs (RAIA) are effective at lowering postprandial hyperglycemia in various basal bolus insulin regimens used in type 1 DM. While RAIAs may be slightly better at lowering HbA1c, the differences are clinically insignificant.

Although emergency physicians rarely start new therapy for patients with diabetes, being acquainted with the various forms of insulin and the common regimens is useful.

When treating patients with type 1 DM, the goal is to provide insulin in a manner that is as physiologic as possible. Insulin replacement is given as a basal insulin (either long-acting [glargine or detemir] or intermediate-acting [NPH]) and preprandial (premeal) insulin (either rapid-acting [lispro, aspart, or glulisine] or short-acting [regular]). For patients on intensive insulin regimens (multiple daily injections or insulin pumps), the preprandial dose is based on the carbohydrate content of the meal (the carbohydrate ratio) plus a correction dose if their blood glucose level is elevated (eg, 2 additional units of rapid-acting insulin to correct the blood glucose from a level of 200 mg/dL to a target of 100 mg/dL). This method allows patients more flexibility in caloric intake and activity, but it requires more blood glucose monitoring and attention to the control of their diabetes [16].

Insulin is sensitive to heat and exposure to oxygen. Once a bottle of insulin is open, it should be used for no more than 28 days and then discarded, even if insulin remains in the bottle. Use of old insulin can result in a lack of clinical effectiveness. Insulin in a pump reservoir for longer than 3 days may lose its clinical effectiveness (although insulin aspart has now been approved for use for up to 6 days in a pump). Sometimes, insulin distributed from the pharmacy has been exposed to heat or other environmental factors and may be less active. If a patient is experiencing unexplained high blood sugar levels, new insulin vials should be opened and used [14].

Type 2 diabetes mellitus comprises an array of dysfunctions resulting from the combination of resistance to insulin action and inadequate insulin secretion. It is disorders are characterized by hyperglycemia and associated with microvascular (ie, retinal, renal, possibly neuropathic), macrovascular (ie, coronary, peripheral vascular), and neuropathic (ie, autonomic, peripheral) complications. Unlike patients with type 1 diabetes mellitus, patients with type 2 are not absolutely dependent upon insulin for life. This distinction was the basis for the older terms for types 1 and 2, insulin dependent and non–insulin dependent diabetes. However, many patients with type 2 diabetes are ultimately treated with insulin. Because they retain the ability to secrete some endogenous insulin, they are considered to require insulin but not to depend on insulin. Nevertheless, given the potential for confusion due to classification based on treatment rather than etiology, these terms have been abandoned [16]. Another older term for type 2 diabetes mellitus was adult-onset diabetes. Currently, because of the epidemic of obesity and inactivity in children, type 2 diabetes mellitus is occurring at younger and younger ages. Although type 2 diabetes mellitus typically affects individuals older than 40 years, it has been diagnosed in children as young as 2 years of age who have a family history of diabetes.

Diabetes mellitus is a chronic disease that requires long-term medical attention both to limit the development of its devastating complications and to manage them when they do occur. It is a disproportionately expensive disease; in the United States in 2002, the per-capita cost of health care was $13,243 for people with diabetes, while it was $2560 for those without diabetes. The emergency department utilization rate by people with diabetes is twice that of the unaffected population [10].

Pathophysiology

Type 2 diabetes is characterized by the combination of peripheral insulin resistance and inadequate insulin secretion by pancreatic beta cells. Insulin resistance, which has been attributed to elevated levels of free fatty acids in plasma, leads to decreased glucose transport into muscle cells, elevated hepatic glucose production, and increased breakdown of fat.

For type 2 diabetes mellitus to occur, both defects must exist. For example, all overweight individuals have insulin resistance, but diabetes develops only in those who cannot increase insulin secretion sufficiently to compensate for their insulin resistance. Their insulin concentrations may be high, yet inappropriately low for the level of glycemia.

Beta cell dysfunction is a major factor across the spectrum of pre-diabetes to diabetes. A study of obese adolescents by Bacha et al confirms what is increasingly being stressed in adults as well: Beta cell function happens early in the pathological process and does not necessarily follow stage of insulin resistance [10]. Singular focus on insulin resistance as the "be all and end all" is gradually shifting, and hopefully better treatment options that focus on the beta cell pathology will emerge to treat the disorder early.

Role of Vitamin D in Diabetes Mellitus

There are also evidences suggesting that altered calcium and vitamin D homeostasis plays a role in the development of diabetes. Low levels of activated vitamin D are associated with glucose intolerance, diabetes, insulin resistance and metabolic syndrome; all increasing the potential for the development of type 2 diabetes [13].

Vitamins can be taken up from the foods like fish and oils but the major source is sunlight. Vitamin D exerts its effects by binding to its nuclear receptor (VDR). VDR are present on pancreatic β-cells and vitamin D is essential for normal insulin secretion. Insulin secretion is reduced in animal models with vitamin D deficiency [12].

Vitamin D refers to vitamin D2 (Ergocalciferol) or vitamin D3 (cholecalciferol). Ergocalciferol is produced from irradiated fungi or yeast. Cholecalciferol is produced in skin or found naturally in fatty fish such as salmon or mackerel. Both forms of vitamin D can be used to fortify food, however only cholecalciferol can be made endogenously in skin. When the skin is exposed to ultraviolet B (UVB) radiation between the wavelengths of 290 and 315 nm, 7-dehydrocholesterol, a compound present in the skin, is converted to previtamin D3. Then it isomerizes to form vitamin D3 [14].

Methodology

The subjects were selected after taking written consent on a detailed prescribed Proforma. In addition, every subject was explained verbally the possible benefits and risks associated with participation in the study. They were explained the possible impact on the results of not following the instructions by the investigator.

[1] BMI was calculated through formula that is (BMI = weight in Kg/ Height in meter2) by taking weight in Kg with the help of weighing machine and height in meter with the help of height scale. Height and weight were measured using a standard weight machine with height scale that was available at the laboratory

[2] Waist measurement was done with the help of measurement tape.

[3] Systolic and diastolic blood pressure measurement was done by aneroid sphygmomanometer.

[4] Random blood sugar was measured by automatic biochemical analyzer (Hitachi 902) on photometric technique, described below.

[5] Glycated hemoglobin levels were measured by turbid test. (Turbidimetric inhibitor immunoassay) described below.

[6] Serum Vitamin D was measured by Electrochemiluminesence method, described below.

[7] Serum Calcium Levels were measured by (Hitachi 902 automatically calibrates itself by using calibrator)

Procedure for Determining Random Blood Glucose Levels (RBS)

The RBS was tested in the Hitachi 902 analyzer in DDRRL by Hexokinase / glucose-6-phosphate dehydrogenase method. The procedure is simple once the reagents are installed into the analyzer. DDRRL uses ready to use reagents manufactured by Cobas for this particular test.

Procedure

[1] Use samples collected in tubes containing Sodium fluoride.

[2] Install the reagents into the analyzer.

[3] Program the analyzer and calibrate it using the Cobas calibrator.

[4] Centrifuge the samples at 3500 rpm for 10 min to separate serum and erythrocyte debris.

[5] Carefully install the tubes into the analyzer and run them.

The reading is displayed by the machine within 10

Reference Ranges

Reference values taken for all serum markers are according to DDRRL criteria.

- ❖ Obese and underweight i.e. (BMI more than 30 and less than 18.5kg/meter square.)
- ❖ Fasting blood glucose 65 – 110 mg/dl
- ❖ Random blood glucose 140- 160mg/dl
- ❖ HbAIC ≤6.5% (CONTROL)
- ❖ Serum calcium 8.5-10.5 mg/dl
- ❖ Serum Vitamin D
- Less than 20ng/dl (Group A) Vitamin D deficiency
- 21– 32ng/dl (Group B) Vitamin D Insufficiency
- Greater than 32ng/dl (Group C) Sufficient Vit

Data Management

All questionnare

Statistical Treatment

All the variables in the data will be analyzed via SPSS (Statistical Package for Social Sciences) version 16.0 on computer. Threshold for statistical significance will be set at $p < 0.05$.

Mean and standard deviation will be computed for numerical variables like age, serum vitamin D levels and HbAIC levels

For descriptive analysis serum HbAIC levels will be divided into two groups namely controlled and uncontrolled, Duration of disease into four groups and serum Vitamin D levels will be divided into three groups namely A, B, C.

Less than 20ng/dl (Group A) Vitamin D deficiency

21– 32ng/dl (Group B) Vitamin D Insufficiency

Greater than 32ng/dl (Group C) Sufficient Vitamin D

Then will be presented in the form of frequencies and percentage. Frequency and percentage will also be computed to describe their categorical variables like gender and life style.

To find out the correlation between serum HbAIC levels in two groups and the serum Vitamin D levels in the three groups the chi square test will be applied.

Results

Age Distribution

Mean age of the sample is 45.77 years. Sample was divided for analysis in two age groups i.e. group 1 (<45 years) and group 2 (> 45 years).Greater proportion fell into the >45 age group i.e. 58.1 %. Detailed age distribution of sample is presented in Fig 4.1.

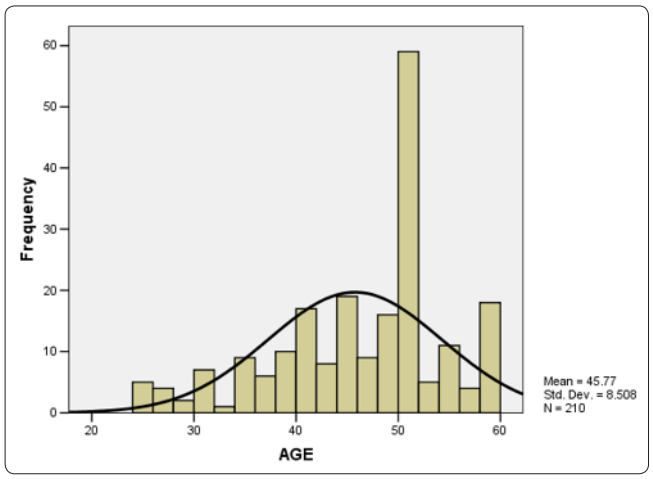
Age Range: 24-60 years

4.1. Age Distribution (n=210)

Age (Years)	Frequency (n=210)	Percentages (%)
<45 years	88	41.9
>45 years	122	58.1

Mean (+SD) age=45.77(+8.508) years

4.1 Histogram of Age Distribution (n=210)



Gender Distribution

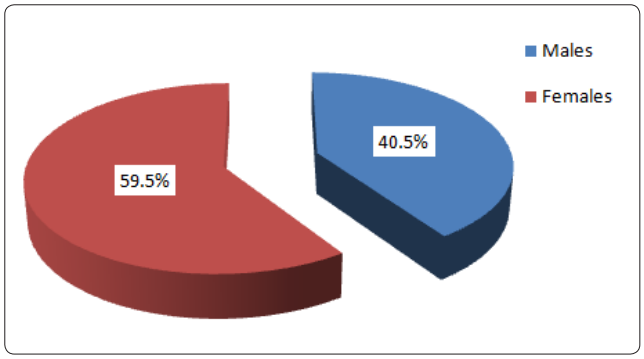
Female gender constituted 59.5% of total sample in this study compared to 40.5% males. Gender distribution is presented as table 4.2 and Fig 4.2 in a pie-chart.

Ratio: M:F=0.68:1

Gender Distribution (n=210)

Age (Years)	Frequency (n=210)	Percentages (%)
<45 years	85	40.5
>45 years	125	59.5

Gender Distribution (n=210)



Duration of Diabetes Mellitus

Range of duration of having Diabetes Mellitus of the sample was 16 years. Mean duration was 7.79 (+3.67) years. For description, sample was divided into 4 groups on the basis of duration of diabetes mellitus as presented in table 4.3. It can be observed that majority of patients i.e. 53.8 % had diabetes for 5-10 years.

Only 9 patients out of a total of 210 had diabetes for more than 15 years. Fig 4.3 presents the duration of diabetes across the sample in a bar chart.

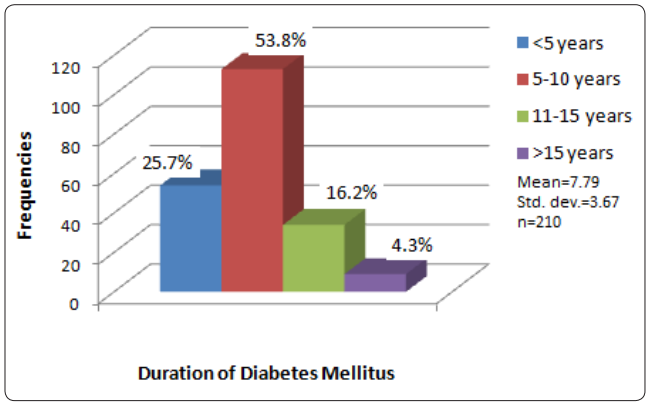
Range=2-18 years

Table 4.3. Duration of Diabetes (n=210)

Age (Years)	Frequency (n=210)	Percentages (%)
<5 years	54	25.7
5-10 years	113	53.8
11-15 years	34	16.2
>15 years	09	4.3

Mean (+SD) duration=7.79 (+3.67) years

Duration of Diabetes Mellitus (n=210)



1. Correlation of Vitamin D Levels with Duration of Diabetes:

Correlation of Vitamin D Levels With Duration of Diabetes (n=210)

	Duration of Diabetes Mellitus	Vitamin D Levels	p-value*
Duration of Diabetes Mellitus	1	-.250*	0.00
Vitamin D Levels	-.250*	1	0.00

*using Pearson Correlation Coefficient

2. Correlation of Vitamin D Levels with Glycemic Control:

Correlation of Vitamin D Levels With Glycemic Control (n=210)

	HbA1c	Vitamin D Levels	p-value*
HbA1c	1	-.303*	0.00
Vitamin D Levels	-.303*	1	0.00

*using Pearson Correlation Coefficient; HbA1c=Glycosylated hemoglobin

If you will be using categorical values (ordinal classification) of these variables in data then spearman correlation (non-parametric correlation) is applicable

Discussion

Vitamin D refers to vitamin D2 (Ergocalciferol) or vitamin D3 (cholecalciferol). Ergocalciferol is produced from irradiated fungi or yeast. Cholecalciferol is produced in skin or found naturally in fatty fish such as salmon or mackerel [17]. Both forms of vitamin D can be used to fortify food, however only cholecalciferol can be made endogenously in skin. When the skin is exposed to ultraviolet B (UVB) radiation between the wavelengths of 290 and 315 nm, 7-dehydrocholesterol, a compound present in the skin, is converted to previtamin D3. Then it isomerizes to form vitamin D3 [18].

Once vitamin D enters the circulation bound to vitamin D–binding protein. This complex is transported to the liver, where vitamin D undergoes hydroxylation in the 25 position to form 25-hydroxyvitamin D (25[OH]D), which then circulates to the kidney and is hydroxylated at the 1 position by the 1-α-hydroxylase to form the hormonal form of vitamin D, 1,25-dihydroxyvitamin D (1,25[OH]2D). 1, 25(OH) 2D circulates bound to vitamin D–binding protein, enters the target cell and binds to the vitamin D receptor (VDR) in the cytoplasm, which then enters the nucleus and then increase the transcription of vitamin D–dependent genes for the regulation of bone metabolism, calcium absorption [19].

Circulating 1, 25(OH) 2D is catabolized by the 24-hydroxylase to form 1, 24, 25(OH) 2D, an inactive vitamin D compound. 1, 25(OH) 2D increases its own catabolism by increasing expression of the 24-hydroxylase. Recently, researchers discovered that medications such as anticonvulsants and rifampin can increase the catabolism of 1, 25(OH) 2D by activating the pregnane X receptor, resulting in increased expression of the 24-hydroxylase [20].

To increase the efficiency of calcium absorption optimal vitamin D levels are necessary.Without adequate vitamin D, the body absorbs no more than 10% to 15% of dietary calcium. In the vitamin D–sufficient state, the intestinal calcium absorption increases to 30% to 40% [21].

Over the past few years the definition of vitamin D insufficiency has changed from less than 20 ng/mL to less than 32 ng/mL. According to the clinical trials established that optimal calcium absorption occurs with a 25(OH) D level greater than 32 ng/mL and most protection from fracture comes from a 25(OH)D level greater than 30 ng/mL An optimal 25 (OH)D should be at least 32 ng/mL to prevent secondary hyperparathyroidism and fracture [22].

In another study a large percentage of patients with osteoporosis were included. A study of 1536 postmenopausal osteoporotic women from osteoporosis clinics, evenly distributed across southern and northern latitudes of the United States, found that over half of these women (52%) were vitamin D insufficient (25[OH]D < 30 ng/mL) [23].

Conclusion

Negative association is present between HbA1c levels and derangements in serum Vitamin D.

Negative association is also present between duration of Diabetes and derangements in serum Vitamin D.

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