DISSERTATION ON

"A STUDY ON THE PREVALENCE OF VITAMIN D DEFICIENCY AND THE EFFECT OF VITAMIN D ON THE GLYCEMIC CONTROL AND LIPID PROFILE IN TYPE II DIABETES MELLITUS PATIENTS IN A RURAL TERTIARY CARE HOSPITAL"

Dissertation submitted to

THE TAMILNADU DR.M.G.R MEDICAL UNIVERSITY

In partial fulfilment of the regulations

For the award of the degree of

M.D PHARMACOLOGY - BRANCH-VI

CHENNAI MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE

IRUNGALUR, TRICHY- 621 105.

THE TAMILNADU DR.M.G.R MEDICAL UNIVERSITY

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APRIL-2016

CERTIFICATE

This is to certify that this dissertation entitled **"A STUDY ON THE PREVALENCE OF VITAMIN D DEFICIENCY AND THE EFFECT OF VITAMIN D ON THE GLYCEMIC CONTROL AND LIPID PROFILE IN TYPE II DIABETES MELLITUS PATIENTS IN A RURAL TERTIARY CARE HOSPITAL"** is a bonafide research work of **Dr.JEGAN. A** in partial fulfilment of the requirements for M.D Branch-VI (Pharmacology) Examination of the Tamil Nadu Dr. M.G.R Medical University to be held in APRIL -2016. The period of study was from 2013-2016.

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DECLARATION

I, **Dr.A.JEGAN** solemnly declare that the dissertation title **"A STUDY ON THE PREVALENCE OF VITAMIN D DEFICIENCY AND THE EFFECT OF VITAMIN D ON THE GLYCEMIC CONTROL AND LIPID PROFILE IN TYPE II DIABETES MELLITUS PATIENTS IN A RURAL TERTIARY CARE HOSPITAL"** was done by me at Chennai Medical College Hospital and Research Centre, Irungalur ,Trichy, under the supervision and guidance of my professor and head of the department **Dr.S.Manickavasagam .M.D.,**

This dissertation is submitted to The Tamil Nadu Dr. M.G.R Medical University, towards the partial fulfilment of requirement for the award of M.D. Degree (Branch –VI) in Pharmacology.

Place: Irungalur
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The work done by Dr.A.Jegan on titled **"A STUDY ON THE PREVALENCE OF VITAMIN D DEFICIENCY AND THE EFFECT OF VITAMIN D ON THE GLYCEMIC CONTROL AND LIPID PROFILE IN TYPE II DIABETES MELLITUS PATIENTS IN A RURAL TERTIARY CARE HOSPITAL"** is under my supervision and I assure that this candidate has abide by the rules of the Ethical Committee.

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The research proposal submitted by **Dr. A. Jegan,** Postgraduate Student, Department of Pharmacology, Chennai Medical College Hospital and Research Centre, Tiruchirapalli, was discussed and analyzed by the Institutional Ethics Committee of the CMCH&RC. The committee approved the research project subject to existing rules and regulations.

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"A study on the prevalence of vitamin D deficiency and the effect of vitamin D on the glycemic control and lipid profile in type II diabetes mellitus patients in a rural tertiary care hospital"

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ACKNOWLEDGEMENT

I am thankful to **Dr.P.G.Sankaranarayanan M.D.,** The Dean, Chennai Medical College Hospital and Research Centre, Irungalur, Trichy for permitting me to carry out the study.

I sincerely express my heartfelt gratitude to my guide **Dr.S.Manickavasagam M.D.,** Professor and Head of the department, Department of Pharmacology for his constant encouragement, innovative suggestions and valuable guidance in every step of this study.

I express my sincere gratitude to my co-guide **Dr.M.Paramasivam M.D.,** Professor of department of medicine for his constructive suggestions and constant encouragement throughout the period of the study.

I sincerely thank my Associate Professor **Dr.P.Revathi, M.D.,** Department of Pharmacology for her valuable guidance and support during my study.

I express my heartiest thanks to **Dr.C.K.Elandevan, M.D., Dr.T.Nivethitha,M.D., Dr.K.Kanagasanthosh., M.D.,** Assistant Professors Department of Pharmacology for their support during my study.

I extend my gratitude to **Dr.Ramprabhakar,M.D** Assistant Professor, Department of Community Medicine for his guidance and support to complete my study.

I am very thankful to the Vice Principle, Director and Medical Superintendent of our institution for permitting me to carry out the study.

My sincere thanks to co-post graduates for their support during the study

I would like to acknowledge the assistance rendered by tutors and the technical staffs who helped me to perform the study.

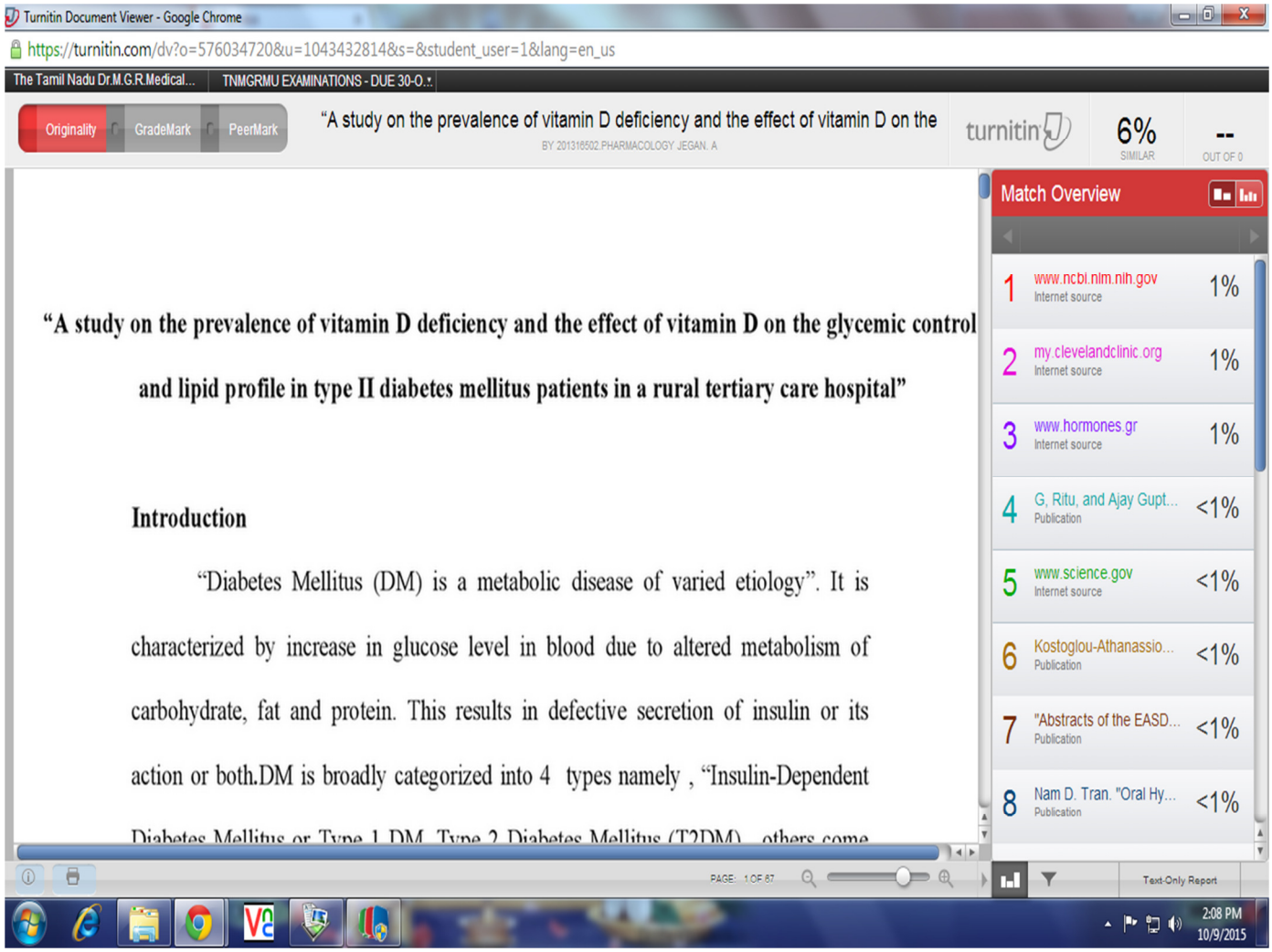
I am grateful to my parents and volunteers who participated in this study. I owe my special thanks to my family members for their moral support in conducting the study.

CONTENTS

ABBREVIATIONS

IDDM -Type 1	-	Insulin-Dependent Diabetes Mellitus
T2DM	-	Type 2 Diabetes Mellitus
GDM	-	Gestational Diabetes Mellitus
MODY	-	Maturity Onset Diabetes Mellitus of Young
HbA_1c	-	Plasma glycosylated haemoglobin
HPLC	-	High Performance Liquid Chromatography
HOMA-IR	-	Homeostatic Model Assessment- Insulin Resistance
25(OH)D	-	Vitamin D
RDA	-	Recommended Dietary Allowance
DBP	-	Vitamin D binding protein
VDR	-	Vitamin D receptor
SREBP	-	Sterol Response Element Binding Protein
LCTMS	-	Liquid Chromatography Tandem Mass Spectrometry
BMD	-	Bone Mineral Density
DEXA	-	Dual Energy X-ray Absorptiometry

TC	-	Total Cholesterol
HDL	-	High Density Lipoprotein
LDL	-	Low Density Lipoprotein
TG	-	Triglycerides
ANOVA	-	Analysis Of Variance
CVD	-	CardioVascular Disease
ICMR	-	Indian Council of Medical Research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"A study on the prevalence of vitamin D deficiency and the effect of vitamin D on the glycemic control and lipid profile in type II diabetes mellitus patients in a rural tertiary care hospital"
Introduction
"Diabetes Mellitus (DM) is a metabolic disease of varied etiology". It is characterized by increase in glucose level in blood due to altered metabolism of carbohydrate, fat and protein. This results in defective secretion of insulin or its action or both.DM is broadly categorized into 4 types namely , "Insulin-Dependent
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ABSTRACT

Objectives: The World Health Organization (WHO) describes diabetes mellitus (DM) as a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defective insulin secretion, insulin action or both.Studies have been going on regarding the extra-skeletal diseases associated with vitamin D [25(OH)D]deficiency including T2DM(type2 diabetes mellitus).This study is aimed to evaluate the prevalence of vitamin D deficiency and the effect of vitamin D on the glycemic control and lipid profile in type II diabetes mellitus patients in a rural tertiary care hospital.

Methods: Glycosylated hemoglobin (HbA_1c),fasting and postprandial blood sugar,25(OH)D, serum insulin levels and lipid profile levels were estimated among 90 T2DM patients who were randomized into three groups (N= 30) belonging to both sex in the age group of 36-60 years.Group oneT2DM on metformin monotherapy with normal Vitamin D levels.Group twoT2DM on metformin with deficient serum Vitamin D levels.Those with deficient serum Vitamin D level on metformin and cholecalciferol were allocated under group three.25(OH)Dwas measured by chemiluminescence method and HbA_1c was measured by high-performance liquid chromatography.

Results: The results were analyzed by proper statistical methods at the end oftwelfth week. Intergroup comparisions were done by one way ANOVA and Benferroni test. For intragroup comparisions,two way. ANOVA and Wilk's lambda tests were used. Significant reduction in fasting blood sugar ($p=0.032$),postprandial blood sugar($p=0.024$),serum triglyceride level($p=0.045$),insulin resistance($p=0.037$) andHbA_1c($p=0.046$) were observed.

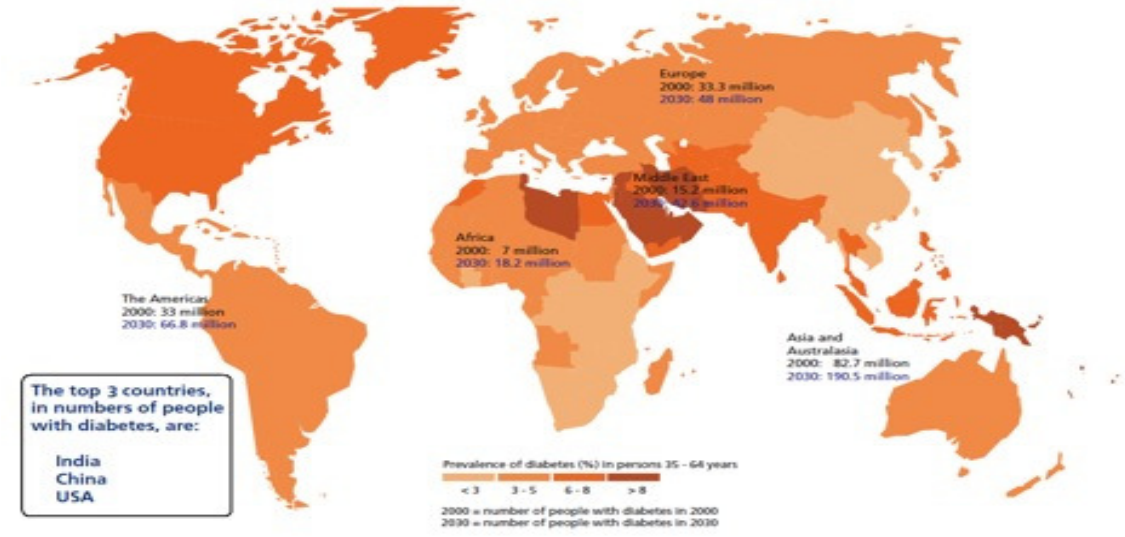
Conclusions: Combinations of metformin and cholecalciferol significantly reduced fasting plasma glucose, postprandial plasma glucose,HbA_1c,insulin resistance and serum triglyceride.Further this combination also showed insignificant reduction in total cholesterol, , low density lipoprotein,(p □ 0.05),with a nonsignificant increase in high density lipoprotein as compared to metformin monotherapy in T2DM.A nonsignificant decrease in fasting serum insulin level and a significant increase in serum 25[OH] D level in T2DM with metformin and cholecalciferol therapy were observed.This study suggests vitamin D supplementation may improve glycemic control and lipid profile in T2DM.

Keywords:Type 2Diabetes Mellitus,Metformin, Vitamin D, HbA_1c,Serum insulin.

INTRODUCTION

The World Health Organization(WHO) describes diabetes mellitus (DM) as a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defective insulin secretion, insulin action or both**.** Diabetes mellitus is classified into four categories namely type 1 - Insulin-Dependent Diabetes Mellitus (IDDM),Type 2 Diabetes Mellitus (T2DM) , Type 3 - others and Type 4 – Gestational Diabetes Mellitus(GDM). Type 2 DM ranges from predominantly insulin-resistant with relative insulin deficiency to a predominantly insulin secretary defect, with or without insulin resistance.

Type I Diabetes mellitus accounts for 5 to 10% and Type 2 accounts for remaining 90 - 95 % of diabetic population.



The incidence of both Type 1 and Type 2 DM is on the rise. DM is estimated by WHO that, in the year 2000, 171 million people had Diabetes and this expected to double by 2030. The International Diabetes Federation estimated that there are 285 million people with diabetes worldwide in 2010 and projects the absolute number will surpass 400 million by 2030. India tops the Diabetes Mellitus population of the world (31.7 million in the year 2000 and estimated to rise by 79.4 million in 2030) followed by China and United States. This global pandemic principally involves T2DM.

Recent studies have observed that there exists a role of vitamin D in the pathogenesis of Type2 Diabetes Mellitus.[1,2,3] Several studies had reported that Vitamin D improves glycemic control in T2DM,[4] while few studies observe no such effect.[5] Few recent studies have observed that vitamin D deficiency exists in GDM. The effect of Vitamin D on serum lipids in T2DM is uncertain.[6] Some studies have reported an inverse association of Vitamin D with triglycerides and total cholesterol[7] level while few other studies reported no such effect.

AIM AND OBJECTIVES

The present study was structured for evaluating the prevalence of vitamin D deficiency and the effect of vitamin D in the glycemic control and lipid profile in type II diabetic mellitus patients in a rural tertiary care hospital.

REVIEW OF LITERATURE

Diabetes Mellitus – An Overview

Type 1 DM

Type 1 DM is the result of interactions of genetic, environmental, and immunologic factors that ultimately lead to the destruction of the pancreatic beta cells and insulin deficiency due to autoimmune beta cell destruction. Most individuals have evidence of islet-directed autoimmunity. Individuals with a genetic susceptibility have normal beta cell mass at birth but begin to lose beta cells secondary to autoimmune destruction that occurs over months to years.

The major susceptibility gene for type 1DM is located in the HLA region on chromosome 6. Polymorphism in the HLA complex account for 40–50% of the genetic risk of developing type 1 DM. Most individuals with type 1 DM have the HLA DR3 and/or DR4 haplotype. Environmental triggers include viruses (coxsackie, rubella, entero viruses), bovine milk proteins, and nitrosourea compounds. Beta cell mass then begins to decrease, and insulin secretion progressively declines, although normal glucose tolerance is maintained. Features of diabetes do not become evident until a majority of beta cells are destroyed (70–80%). At this point, residual functional beta cells exist but are insufficient in number to maintain glucose tolerance

Type 2 Diabetes

The incidence of T2DM also increases with age, which may be related to decrease in exercise and muscle mass. T2DM occurs in families so that those with a first - degree relative with diabetes have an almost 50% life - time risk. Insulin resistance and abnormal insulin secretion are central to the development of T2DM.

Type II DM was defined by the WHO criteria as follows:

(a) fasting plasma glucose ≥7.0 mmol/l; or

(b) with an oral glucose tolerance test, two hours after an oral dose of plasma glucose ≥11.1 mmol/l; or

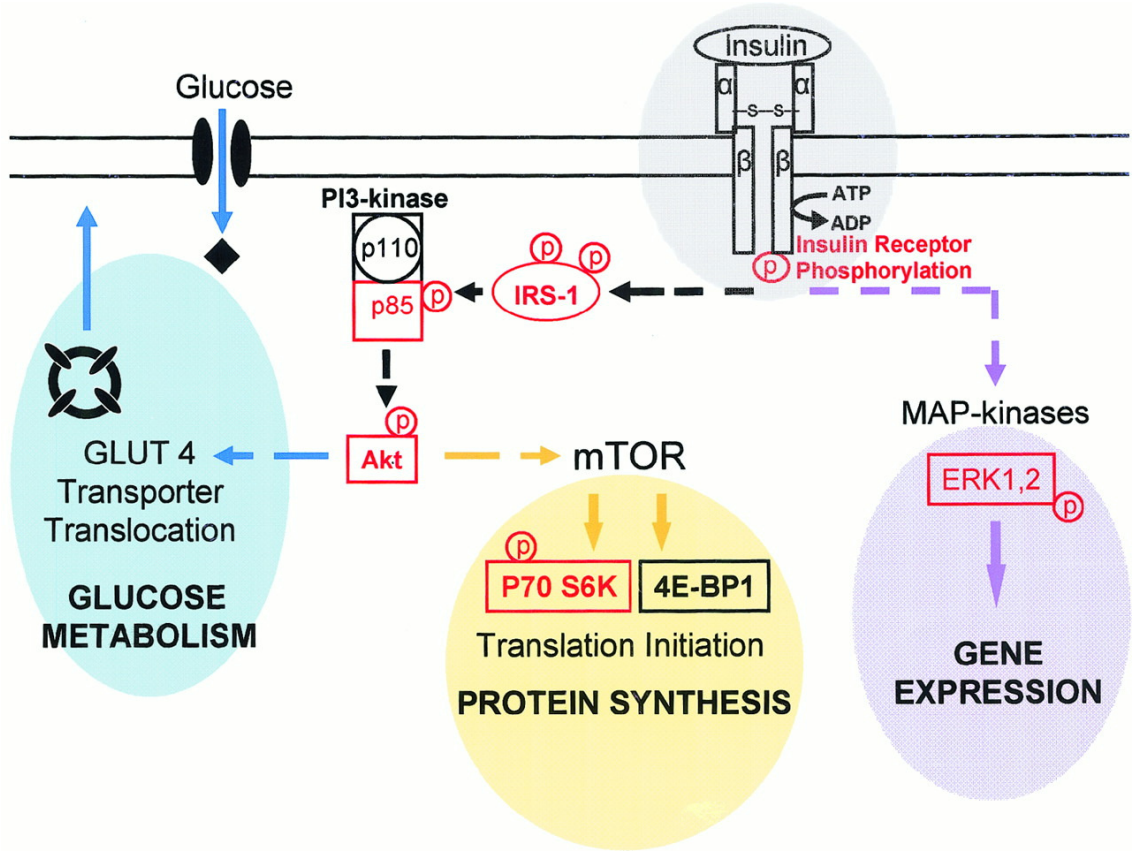
(c) $HbA_1c > 6.5\%$

Type 2 diabetes (T2DM) is marked by insulin resistance (IR). In IR, insulin is adequately or Overproduced by pancreatic β cells, but is ineffectively utilized by the target cells of adipose, hepatic and skeletal muscles tissues. As a response to hyperglycemia, β cells further increase insulin production leading to hyper insulinemia, which is often indicative of a pre-(T2DM) stage. Hyper insulinemia is associated with hypertension, obesity, dyslipidemia, and glucose intolerance, These conditions are collectively known as metabolic syndrome.[26,27]

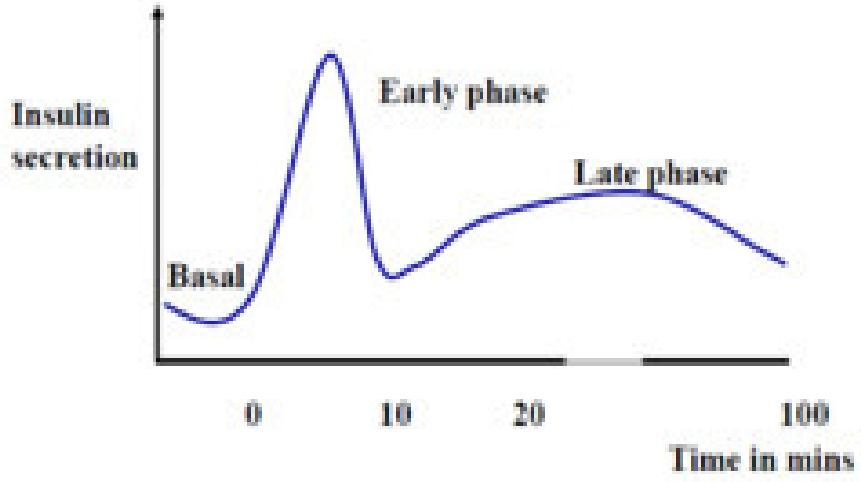
Free Fatty Acids (FFA) are the major players effecting insulin resistance, the underlying mechanism by which this happens is still unclear.

Inhibition of glucose transport by FFA has been found to be linked to insulin-mediated signals. Binding of insulin to the heterotetrameric membrane receptor results in insulin receptor substrate-1(IRS-1) phosphorylation and IRS-1-associated phosphatidylinositol 3 phosphate kinase (PI3kinase) activation.

Insulin Signalling Pathway



This affects downstream effectors such as Akt/PKB which activates the glucose transporter Glut4. Glut4 is then translocated to the membrane and imports glucoseinto the cell.Activation of RAS – MAP kinase pathway regulates cell growth and glycogen synthesis.



The major cellular mechanism for the termination of insulin action is receptor mediated endocytosis and recycling. Protein kinase C – mediated serine phosphorylation of the insulin receptor plays a major role in the pathogenesis of hyperglycemia induced insulin resistance.

MANAGEMENT OF DIABETES MELLITUS

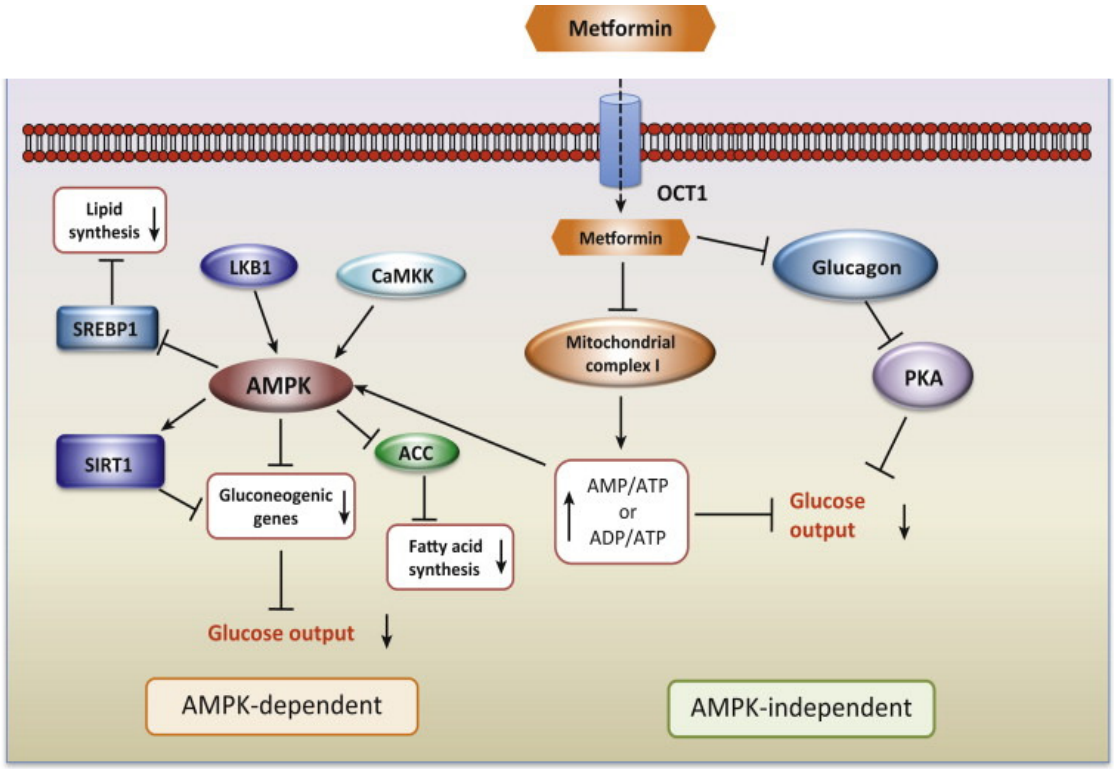
- Diet/lifestyle modification
- Regular Exercise
- Medication

Oral agents including Biguanides (Metformin), Alpha Glucosidase Inhibitors, Dipeptidyl peptidase 4 inhibitors,Insulin Secretagogues, Thiazolidinediones, Bile Acid sequestrants Parenteral agents including Insulin, GLP-1 receptor agonists, Amylin agonists

- Management of associated conditions including Dyslipidemia, Hypertension, Obesity & Coronary heart disease
- Screening and managing complications of diabetes including Retinopathy, Cardiovascular disease, Nephropathy, Neuropathy & others

Metformin in Type 2 Diabetes Mellitus:

Metformin (1,1-dimethyl biguanide), is a first line oral anti- diabetic drug. Metformin primarily reduces hepatic glucose production and also improves peripheral glucose utilization. Metformin activates AMP-dependent protein kinase (AMPK), a serine – threonine protein kinase which acts like a fuel gauge in monitoring energy status. It enters hepatic cells through organic cation transporters (OCT-1) and binds to the mitochondrial matrix where it inhibits complex -1of the electron transport chain.This subsequently leads to a decrease in ATP production.This increased AMP/ATP ratio inhibits Fructose 1,6 biphosphatase ,the regulatory enzyme of gluconeogenesis and results in decrease in hepatic gluconeogenesis.Further the energy pathways utilizing ATP get shut-down resulting in decreased glucose,protein,lipid synthesis and increase in fatty acid oxidation and glucose utilization.Metformin also regulates the expression of key genes involved in gluconeogenesis (SHP,KLF-15,TORC-2,CBP) through activation of AMPK resulting in long term suppression of hepatic glucose production.Metformin causes down regulation of the sterol response element binding protein (SREBP) thereby decreases lipogenesis.[28]



Metformin reduces fasting plasma glucose and insulin levels, improves the lipid profile, and promotes modest weight loss. Metformin is administered orally and undergoes dose dependant absorption.Its oral bio-availability is 50 to 60% with a serum half life of 1.5 to 3 hours. Metformin does not undergo hepatic metabolism and excreted unchanged by kidneys. The initial starting dose of 500 mg once or twice a day can be increased to 1000mg b.i.d. Fewer gastro intestinal side effects like diarrhea, anorexia, nausea, metallic taste occur due to metformin intake. Metformin is effective as monotherapy and can be used in combination with other oral antidiabetic agents or with insulin. The major toxicity of metformin, lactic acidosis is very rare.It also causes Vitamin B12 deficiency. Metformin should not be used in patients with renal insufficiency

[GFR < 60 ml/min], any form of acidosis, congestive cardiac failure, liver disease, or severe hypoxemia.

Vitamin D Deficiency

Vitamin D deficiency is pandemic, yet it is one of the most under-diagnosed and under-treated nutritional deficiency[8] in the world. Vitamin D deficiency is widespread in individuals irrespective of their age, gender, race and geography. Vitamin D is photosynthesized in the skin on exposure to UVB rays. [9] Sun exposure alone ought to suffice for vitamin D sufficiency. However, vitamin D deficiency is widely prevalent despite plentiful sunshine even in tropical countries like India.

Vitamin D deficiency has a bearing not only on skeletal but also on extra skeletal diseases.[9] Owing to its multifarious implications on health, the epidemic of vitamin D deficiency in India is likely to significantly contribute to the enormous burden on the healthcare system of India.[10] Cultural and social taboos often dictate lifestyle patterns such as clothing that may limit sun exposure and vegetarianism which certainly limits vitamin D rich dietary options.

The socioeconomically backward people constitute a large percentage of the population in India. The underprivileged generally suffer from overall poor nutrition. Vitamin D rich dietary sources are limited and unaffordable to most Indians. Vitamin D supplements are available, but most Indians are not aware that they need additional vitamin D.

Additionally, the cost of these supplements is essentially prohibitive to the majority. Fortification of staple foods with vitamin D may prove to be a more viable solution towards attaining vitamin D sufficiency in India.

There are scores of research papers in the literature reporting poor vitamin D status from all over India and some from other countries of the Indian subcontinent too. Many of these studies measured serum 25-hydroxyvitamin D levels in ostensibly healthy subjects. Biochemical evidences of suboptimal bone health are elevated alkaline phosphatase (ALP) and elevated PTH levels (secondary hyperparathyroidism or SHPT). These were often reported in research papers, especially with respect to their correlation with vitamin D status. However, the most accurate and convincing measure of bone mineral density (BMD) is DEXA (Dual Energy X-ray Absorptiometry).

Research articles reporting BMD, as measured by DEXA, in the context of vitamin D status of ostensibly healthy individuals, are few. BMD data, as they correlated with the vitamin D status in ostensibly healthy Indians is compiled. A few articles also reported results of interventions studies such as vitamin D supplementation and vitamin D fortified foods.[11]

Mounting evidence suggests that vitamin D deficiency could be linked to several chronic diseases, including cardiovascular disease and malignancies.[12] Vitamin D is produced endogenously when the skin is exposed to sunlight and can be obtained exogenously from a few natural food sources, from food fortification and from supplements. Simple exposure to sunlight is

thought to provide most of the vitamin D [13] requirement of the human population. However, skin synthesis of vitamin D may not compensate for the low nutritional intake in India, even in countries with high supplies from food fortification and supplements. For assessment of vitamin D nutritional status the concentration of 25-hydroxyvitamin D (25(OH)D) in serum is considered to be an accurate integrative measure reflecting an individual's dietary intake and cutaneous production.[14] A substantial percentage of the elderly and adolescents have a low concentration of 25(OH)D; in the elderly this percentage ranges were less.

Low vitamin D status seems to be aggravated by disease and immobility, and by a low frequency of supplement use. Adequate vitamin D(3) by synthesis in the skin or from dietary and supplemental sources is essential for bone health throughout life. Vitamin D deficiency is defined as a 25(OH)D concentration <20 ng/mL (50 nmol/L); vitamin D sufficiency as a 25(OH)D >30 ng/mL (75 nmol/L),[15] and insufficiency as 21-29 ng/mL. Vitamin D deficiency and insufficiency has been linked to a wide variety of chronic diseases including common cancers, autoimmune, cardiovascular, and infectious diseases. Healthcare professionals need to be aware of the vitamin D deficiency pandemic. Guidelines for sensible sun exposure and supplemental vitamin D of 800-1000 IU/day are needed.

In most cases, vitamin D deficiency occurs when individuals do not get enough exposure to sunlight and do not take foods that are rich in vitamin D.[13] Vitamin D deficiency is a global problem and it is known as an

essential factor involved in different immune functions besides skeletal and muscle development .A recent study reported that most of the nonspecific etiologies of common symptoms can result from vitamin D deficiency; although some researchers emphasized that more studies need to be done to prove that vitamin D deficiency can lead to common symptoms of unknown etiologies such as headache and fatigue.[14] Another study found that the prevalence of non-specific muscle pains among the population might result from vitamin D deficiency.

Vitamin D deficiency has been defined differently from country to country. Some consider people are at risk of vitamin D deficiency at serum 25(OH) D concentrations <30 nmol/L.[15] Others define vitamin D deficiency based on 50 nmol/L[16] as the lowest acceptable value for serum 25(OH) D concentrations. The recommended dietary allowance (RDA) is 600 international units (IU) (15 mcg) per day for children and adults from 9 to 70 years old and the tolerable upper intake level for the same age group is 4000 IU (100 mcg).

To avoid any adverse health effect of taking vitamin D above the tolerable upper intake, total vitamin D intake should remain below the intake level. India is a vast tropical country extending from 8.4º N latitude to 37.6° N latitude. Majority of its population lives in areas receiving ample sunlight throughout the year and hence there was disbelief that Vitamin D (Vit D) deficiency is uncommon in India. However from the data available in the published literature, Vit D deficiency is very common in India in all the age groups and both sexes across the country.

The causes of vitamin D deficiency among the Indian population remain uncertain, as well as the factors that may increase their risk for low vitamin D. Studies indicate that lack of sun exposure among population results from cultural practices such as conservative clothing and use of completely covered dresses, in addition to their lifestyle habit of spending most time indoors. Women have higher rates of vitamin D deficiency than men. The level of serum vitamin D is low and it is not varied during the year from season to season in most people due to some common reasons such as skin pigmentation, limited sun exposure and low intake of vitamin D food sources.

A study done on vitamin D status in a multi-ethnic population found that 83% of the Middle Eastern population and some ethnic groups such as Africans and South Asians[15] have low serum vitamin D level. There are increased rates from 30 nmol/L to 12 nmol/L of vitamin D deficiencies in girls wearing conservative clothes in comparison with females living in suburban and urban areas. The prevalence of a lower level of serum vitamin D (<25 nmol/L) is most common in India and other developing countries, darker skin pigmentation, limited sun exposure, higher latitude and lack of foods fortified with vitamin D.[9]

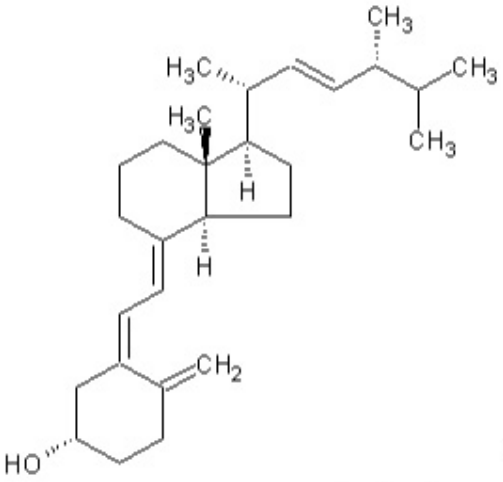
In different countries, the lack of governmental regulations for recommended food fortification with vitamin D is one of the most prevalent barriers[20] to achieve desirable levels of serum vitamin D. Further studies in this area could be the next step to highlight the importance of increasing the number of foods fortified with vitamin D based on population need. As well, raising the public consciousness on sun exposure as a significant source of vitamin D

besides the natural food sources is highly recommended. Recent studies point out that serum vitamin D levels are improved in North America and Europe; however, vitamin D deficiency continues to be prevalent in many regions of the Asian countries including India. As a result, this population is at risk of many vitamin D-related diseases and this is one of reasons for conductance of this study.

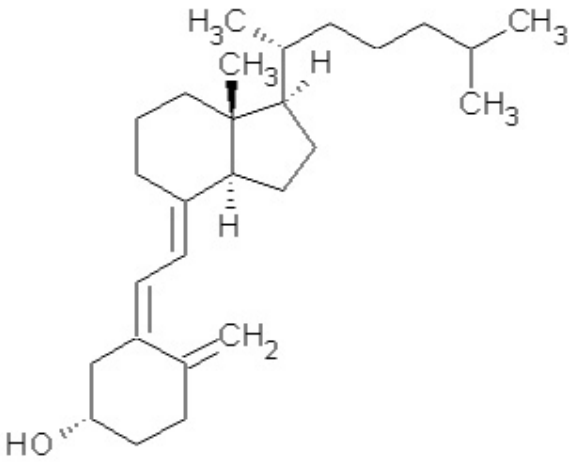
More studies need to be done on vitamin D deficiency among the Indian population with diabetes mellitus. Most of the literature reviews have indicated that the severity of vitamin D deficiency in this population which can lead to serious implications for the growth of future generations and the overall health of the community.

Vitamin D – an overview

Vitamin D is important to facilitate calcium absorption and it has an essential role in maintaining bone and skeletal health. Serum 25-hydroxyvitamin D [25(OH) D] is the measurement variable for vitamin D status. The human body can obtain vitamin D through exposure to sunlight, from diet and dietary supplements. Vitamin D from the sunshine and diet is metabolized within 3 days by the liver into 25(OH) D, which is used to determine a patient's vitamin D status.



Vitamin D2 - ergocalciferol



Vitamin D3 - cholecalciferol

Vitamin D is converted from 7-dehydrocholesterol to 25-hydroxyvitamin D3 when it is transported to the liver by a binding protein; 25-hydroxyvitamin D is further converted to 1, 25(OH) 2D in the kidney as the final step in the activation of vitamin D. When there is not enough sun exposure, serum 25-hydroxyvitamin D decreases gradually, with a half-life of at least 2 months. The maximum number of days for serum levels of the [25(OH) D] metabolite after getting the highest sun exposure in the summer is about 30 – 60 days.

Vitamin D from Sun

Skin exposure to solar UV-B radiation is a significant source of vitamin D. For example, someone exposed to the sun wearing only a bathing suit will get vitamin D2 equivalent to the ingestion of approximately 20,000 IU. Because of the shorter atmospheric distance between the earth's surface and the sun (which is directly above the Equator) and the ozone layer being naturally thinner in

areas close to the Equator (which make it easy to absorb UV radiation), the geographic regions around the Equator which include Middle Eastern countries have the greatest UV rays. Moreover, seasons contribute essentially to vitamin D deficiency in all populations.

A higher rate of vitamin D deficiency occurs during winter and spring, even if people in the Middle Eastern countries and other areas are taking vitamin D supplements because of the positioning of the sun's rays as it hits the earth, which can affect "both quantity and quality of solar radiation reaching the earth's surface". However, some studies done at low latitude areas e.g. 24 N° show a prevalence of low vitamin D. In addition, obesity (because of the possible sequestration of vitamin D in body fat) and darker skin in Saudi Arabia are common factors that increase the time required for sun exposure to get the ideal vitamin D level.

The enhanced fat solubility and decreased bioavailability of vitamin D produces low serum vitamin D levels with obesity. Exposing the skin to sunlight to absorb vitamin D is a major factor in increasing the body's circulating serum vitamin D. In addition, there are different factors that affect the amount of vitamin D synthesized by the skin through sunlight exposure such as individual, geographical and seasonal variations . Risk of common skin cancers is decreased when serum vitamin D is around 75 nmol/L. Risk of skin cancer is one of the barriers to sun exposure. However, too much sun may result in sunburn and damage the skin, which may lead to skin cancer. If a person is going to be exposed to the sun for a long time, it is better to use sunscreen protection.

Vitamin D in Food

Not too many foods are considered good natural sources of vitamin D. For example, fatty fish, eggs, organ meats and UV-irradiated mushrooms are some of the main sources of vitamin D.

In addition, in Saudi Arabia fortified foods are limited to very few dairy products and cereals.[22] The Middle Eastern countries are poor in food fortification in general and in vitamin D specifically. Only wheat flour fortification is mandatory in terms of legislation in most of the Middle Eastern countries.

In Canada, there are more foods that fortified with vitamin D compared to the Middle Eastern countries. Examples of foods fortified with vitamin D in Canada are orange juice, grain products, soy beverage, eggs, some meat and alternatives, and some fats and oils.

Vitamin D from Supplements

Dietary supplements are one source of vitamin D. There are different kinds of supplementation such as over-the-counter and prescription supplements. In some cases, use of supplements is needed to fulfill the body's vitamin D requirement. A study reported that after one year of vitamin D supplementation, there is evidence of increased leanmass, bone area, and bone mass.Positive outcomes with vitamin D supplementation were significant for patients with chronic diseases along with vitamin D deficiency. More studies are needed in

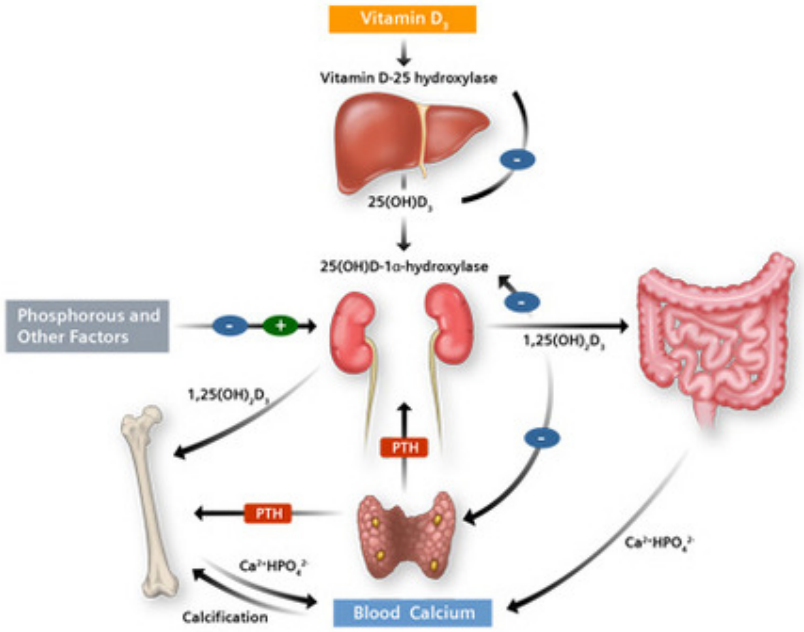
this area to determine the role of vitamin D supplementation- on overall health outcomes. With severe vitamin D deficiency; vitamin D supplementation may help to improve vitamin D serum level as shown among study participants where their serum vitamin D level was elevated to sufficient range. In another study, exceeding the upper intake level of vitamin D may have resulted in hypercalcemia, hypercalciuria, and hyperphosphatemia.

The recommended dietary allowance (RDA) for vitamin D is 600 international units (IU) (15 mcg) per day for children and adults 9 to 70 years old and for the same age group the tolerable upper intake level (UL) is 4000 IU (100 mcg).

Vitamin D metabolism

Vitamin D can be synthesized in sufficient amounts by most vertebrates on adequate exposure of the skin to sunlight (UVB rays). It is critical that most vertebrates obtain a sufficient amount of vitamin D either from their diet or from adequate exposure of the skin to sunlight. The term vitamin D refers to compounds vitamin D3 (cholecalciferol) or vitamin D2 (ergocalciferol). Vitamin D3 is produced in the skin on exposure to sunlight. Vitamin D3 is derived from 7-dehydrocholesterol by ultraviolet irradiation of the skin. Vitamin D3 is also found in animal food sources e.g., fatty fish (e.g., salmon, mackerel and tuna) cod liver oil, milk, etc. Vitamin D2 is found in vegetal sources like sun-exposed yeast and mushrooms. Notably, most dietary sources are not sufficiently rich in their vitamin D content. Vitamin D (both forms D3 or D2) is a prohormone

which requires two hydroxylations to finally attain its biologically active form - 1,25(OH)2D. The first hydroxylation occurs in the liver, at position



C25 to form 25-hydroxyvitamin D, also known as 25(OH)D or calcidiol. Previously considered to be inert, 25(OH)D is the major circulating and more active form of vitamin D.It is an indicator of serum vitamin D status. The second hydroxylation occurs at position C1α to form 1,25(OH)2D, also known as calcitriol 1,25(OH)2D is produced primarily but not exclusively in the kidneys. Both 25(OH)D and 1,25(OH)2D are released in blood, where they binds to vitamin D binding protein (DBP) and reaches its target tissues to exert its endocrine functions through the vitamin D receptor (VDR). 1,25(OH)2D is also produced in several extrarenal tissues for its paracrine and autocrine functions. Most cells in the body have VDR. Both metabolites of vitamin D are

capable of regulating a wide variety of genes that have important functions in regulating cell growth and differentiation.

Vitamin D and Skeletal Health

Vitamin D is a hormone of skeletal integrity[13]. Rickets, osteomalacia and osteoporosis[23, 24] are widely prevalent all over the world. The most well recognized function of 25(OH)D involves regulation of calcium and phosphorus balance for bone mineralization and re modeling. Without adequate levels of25(OH) D in the bloodstream, dietary calcium cannot be absorbed. Low calcium levels lead to an increase in serum PTH concentration, which leads to increased tubular reclamation of calcium in kidneys and resorption from the skeleton at the cost of lowering bone density. In the long term this leads to weakened and brittle bones that break easily. Approximately 40%–60% of total skeletal mass at maturity is accumulated during childhood and adolescence. Rickets results from inadequate mineralization of growing bone. Thus it is a childhood disease and it is manifested as bone deformities, bone pain and weakness. Biochemical abnormalities consistently include hypophosphatemia, elevated alkaline phophatase levels and serum 25(OH)D levels are usually below 5 ng/mL. Chronic vitamin D deficiency in adults results in osteomalacia, osteoporosis, muscle weakness and increased risk of falls.. Epidemiological support for skeketal benefits of vitamin D is well known

Vitamin D: Extraskeletal Effects

Biochemical studies have implicated vitamin D deficiency in many chronic diseases including, but not limited to, infectious diseases, autoimmune diseases, cardiovascular[9] diseases, diabetes, gestational diabetes mellitus[8] and malignancies. Numerous epidemiological publications support the extraskeletal benefits of vitamin D and they cannot be ignored even though majority of these are association studies or small randomized controlled trials. Nevertheless, stronger evidence is required with the aid of more robust and reliable statistical methods such as randomized controlled trials (RCTs). These RCTs should be well-designed, well-executed and conducted worldwide to generate dependable and incontrovertible data, in order to assess the benefits of vitamin D supplementation not only as a preventive measure but also as adjuvant therapies.

Role Of Vitamin D in Diabetes Mellitus

The presence of vitamin D receptors (VDR) and binding of vitamin D with vitamin D binding proteins (DBP) in β cells of pancreas facilitates the secretion of insulin from pancreatic β cells.Vitamin D promotes pancreatic β-cell function in numerous ways[29].

1.Direct actions: Activation of vitamin D occurs in pancreatic β-cells by intracellular 1-α-Hydroxylase enzyme.Vitamin D enhances insulin secretion by forming $1,25(OH)_2D_3$-RXR-VDR complex which binds to vitamin D responsive

elements (VDRE) found in the insulin gene promoter region, enhancing the transcriptional activation of the insulin gene and increase insulin synthesis

2.Indirect actions: Insulin secretion is a calcium-dependent process and is influenced by calcium influx through the cell membrane. Vitamin D regulates calbindin, a cytosolic calcium-binding protein found in β-cells. It acts as a modulator of depolarization-stimulated insulin release via regulation of intracellular calcium. Decreased intracellular calcium level in insulin target tissues may contribute to peripheral insulin resistance via impaired signal transduction pathways leading to decreased glucose transporter activity. Vitamin D promotes increased calcium influx into the cells, leading to increased transport of glucose into the muscle

3.Vitamin D enhances insulin sensitivity by stimulating the expression of insulin receptor (INS-R) and/or by activating peroxisome proliferator-activated receptor-δ (PPAR- δ) implicated in the regulation of fatty acid metabolism in skeletal muscles and adipose tissue.

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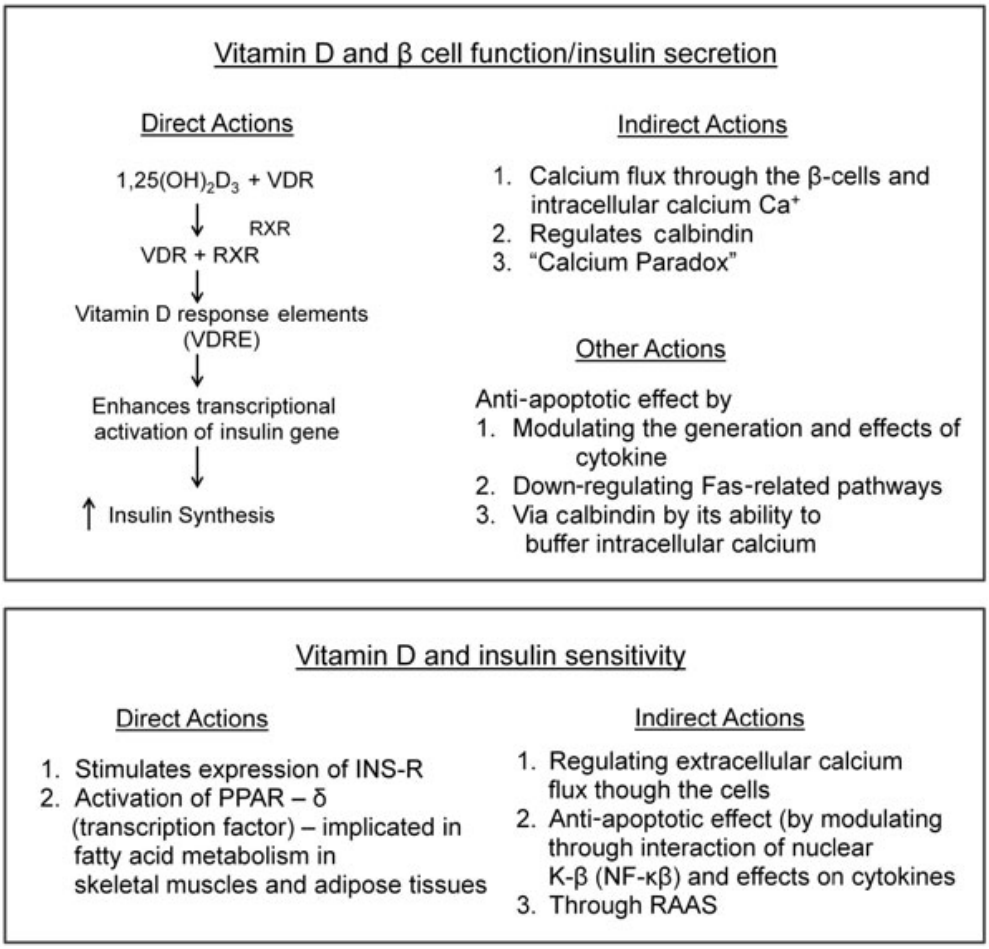

Further vitamin D receptor gene polymorphisms (FokI BsmI,TaqI) is associated with increased risk of T2DM.[30]

Cardiovascular Health

Cardiovascular diseases (CVDs), including congestive heart failure and coronary artery diseases[19] are a major cause of morbidity and mortality worldwide. There is accumulating epidemiological evidence from observational studies suggesting that CVDs are associated with vitamin D deficiency[20]. Increased risk of hypertension was associated with people living at higher

latitudes.25(OH)D level < 21 ng/mL was associated with increased risk of hypertension, diabetes, obesity and high triglyceride levels—all associated with increased cardiovascular mortality. Various studies have reported reduced 25(OH)D concentrations in patients with previous and prevalent cardiovascular or cerebrovascular diseases.

Immunity

As early as in the 19th century, cod liver oil (a rich source of vitamin D) was used for treating tuberculosis (TB). Skin exposure to sunlight was an effective therapy for treating Mycobacterium infections of the skin. In 1903, Finsen received the Nobel Prize for demonstrating that Lupus vulgaris, the epidermal form of TB, could be cured using light from an electric arc lamp. In early 1900s, growing awareness of benefits of sun exposure pertaining treatment of infectious diseases led to the development of sanatoriums in sun-rich areas. These sanatoriums enabled regimented sun exposure, diet and exercise. These sanatoriums primarily hosted TB patients. Recent studies have linked vitamin D deficiency with increased risk of developing TB, otitis media, upper respiratory tract infections and influenza.

Malignancies

Adults living at higher latitudes are more likely to develop various carcinomas including colorectal carcinoma, prostate carcinoma, ovarian carcinoma,[24] breast carcinoma, lung carcinoma and oesophageal carcinoma[30].

Retrospective and prospective epidemiologic studies showed that when 25(OH)D levels were <20 ng/mL there was a 30%–50% increased risk of developing and dying of colorectal, prostate, breast, pancreatic, and esophageal carcinomas.

Vitamin D Deficiency – Global Distribution

Estimates of about one billion people are reportedly suffering from vitamin D deficiency and it is a widespread problem.Vitamin D deficiency has been associated with different chronic diseases, such as rickets in children and osteoporosis in adults, because it has an important role in bone metabolism and many cellular and immunological processes.The Canadian Health Measures Survey reported that vitamin D > 50 nmol/L was considered as a sufficient level of serum vitamin D to meet the recommended dietary allowance (RDA), while < 50 nmol/L was considered insufficient level of serum vitamin D. Serum vitamin D <30 nmol/L represents the deficiency level. About 32% of Canadians were below the vitamin D cut-off of 50 nmol/L and 10% were considered vitamin D deficient (< 30 nmol/L). The national average of serum [25(OH) D] levels was 64 nmol/L . The best marker of vitamin D stores is serum 25-hydroxyvitamin D [25(OH) D]; thus, vitamin D deficiency is diagnosed by measuring serum 25-hydroxyvitamin D. In addition, vitamin D deficiency can be identified through clinical assessment of rickets in children or osteomalacia in adults. However, vitamin D deficiency can occur for some time before the bone manifestations of the clinical deficiency state present to medical attention.The

healthier level of [25(OH) D] is higher than the normal range recommended for the population and how the ideal [25(OH) D] levels are defined varies from country to country. However, Health Canada stated that people who had serum [25(OH) D] < 30 nmol/L are considered at risk of vitamin D deficiency.

A general perception is that the Middle East is a hot area with adequate sunshine. Although there are no fixed boundaries of the region, most geographical sites mentioned include countries like Egypt, Bahrain, Iran, Iraq, Israel, Jordan, Kuwait, Lebanon, Oman, Palestine, Qatar, Saudi Arabia, Syria, Turkey, the United Arab Emirates, and Yemen. Despite the adequacy of sunshine, hot weather and arid climate which allow maximum vitamin D production in humans (through skin exposed to UVB light for about 5-30 minutes depending on the time of day, season, latitude, and skin sensitivity),there have been many studies that show the population of this area being deficient in Vitamin D. Vitamin D has several important physiological functions in the human body of all age groups, from infancy to old age. Worldwide, it is estimated that almost 1 billion people suffer from Vitamin D deficiency. We know that sunlight is a major source, yet the populations of countries receiving most of it are estimated to suffer the most, with worst figures and prevalence rates. According to a European study, people living in sunny countries have more deficiency than other countries because of inadequate exposure to sunlight. A study done on Saudi Arabia's population found that they have a high prevalence of vitamin D deficiency at (28nmo/L- 33 nmol/L) in both summer and winter seasons and it was even higher in the winter season.

Researchers have surmised that vitamin D deficiency among Saudi women can result from a lack of public awareness. However, post-menopausal women had high serum vitamin D levels in the study among Saudi female outpatients while another study on healthy Saudi women has the opposite. The study on Saudi female outpatients as study groups had higher serum vitamin D levels possibly because many clinicians tend to routinely recommend calcium and vitamin D supplements to post-menopausal women, without measuring their vitamin D status, as a protection against osteoporosis.

Testing Vitamin D Status

Plasma 25(OH)D or calcidiolis the most reliable marker of vitamin D status. Immunoassays such as radioimmunoassay (RIA), enzyme linked immunosorbant assay (ELISA), chemiluminescence immunoassay and protein binding assays are used in routine testing of 25(OH)D in clinical laboratories. LCTMS (liquid chromatography tandem mass spectrometry) is the widely accepted reference method for 25(OH)D measurement. However, LCTMS is tedious, expensive and time consuming and therefore seldom used commercially. Since vitamin D under nutrition is largely silent and subclincal, the indication for testing remains controversial. At present 25(OH)D test is the "most ordered test" in the USA. A similar trend has just begun in the upper socioeconomic stratum in India too. This clearly shows increasing awareness pertaining widespread prevalence of vitamin D deficiency among Indian clinicians. A 25(OH)D test using antibody based technologies in India costs an

individual approximately INR 1500, which is unaffordable for most Indians. Surely, vitamin D status needs to be improved in most individuals in India and not just the privileged or a select few. However, testing every individual's vitamin D levels, in a population with such a high prevalence of vitamin D deficiency is not economically and practically feasible. Furthermore, whether subclinical vitamin D deficiency in otherwise healthy individuals should be treated or not, and to what target level of serum 25(OH)D remains controversial. It will be more cost-effective to implement aggressive nationwide vitamin D supplementation and food fortification programs for the benefit of all ostensibly healthy individuals.

Health Problems Associated with Vitamin D Deficiency

People who are suffering from severe serum vitamin D deficiency experience some symptoms of numbness, paresthesia (abnormal or impaired skin sensation), muscle cramps, laryngospasm, tetany and seizures, while those with minor deficiency may complain from having muscle weakness or pain. In addition, a deficiency may cause rickets, osteomalacia, osteoporosis, multiple fractures, and growth retardation.

Another study found that low bone mineral density in adults is strongly associated with low serum 25-hydroxyvitamin D level and low serum vitamin D leads to reduced ability to absorb calcium. In pregnant women, low vitamin D can be noticeable in the fetal skeleton during the 19th week of pregnancy. In some cases low serum 25-hydroxyvitamin D levels is associated with colon

cancer mortality. A study done on the American population showed that vitamin D insufficiency/deficiency is associated with cardiovascular disease deaths. Vitamin D is also important for some of the metabolic syndromes such as hypertension, obesity insulin resistance and glucose intolerance. Other studies show that vitamin D deficiency is associated with several chronic diseases such as cancer, infection asthma, and dermatopathies (skin problems of unknown etiologies), insulin resistance, diabetes and related microvascular complications and retinopathy. Vitamin D insufficiency can cause calcium malabsorption and secondary hyperparathyroidism. Multiparity and menopause also seem to be statistically associated with hypovitaminosis D.Obesity has indirect effects on physical activity by decreasing movement and as a result, sun exposure may be restricted which will decrease vitamin D levels. Recent studies found that vitamin D deficiency is also associated with autoimmune disorders, CVD and cancers.

Vitamin D Status of Ostensibly Healthy Indians

Countrywide studies have reported vitamin D deficiency in as high as 70%–100% of ostensibly healthy individuals. High prevalence of vitamin D deficiency was reported from northern to southern and western to eastern India, in ostensibly healthy children, adolescents, young adults and those ≥50 years old. All over India, vitamin D deficiency was highly prevalent in pregnant women and lactating mothers. Vitamin D status of these mothers correlated well with their neonates and their exclusively breastfed infants. Subjects from rural

and urban areas presented a similar picture. Relatively, fish are a rich source of vitamin D. The residents of Bengal (eastern India) eat more fish compared to the rest of the Indians. Surprisingly, their vitamin D status appears to be just as poor as in the rest of the country. Similarly, even healthy young soldiers with sufficient intake of calcium, adequate sun exposure and regular exercise regimen were found to be vitamin D deficient as were young sportswomen. Among resident doctors from Mumbai (western India) and also doctors from eastern India, most were vitamin D deficient. Vitamin D deficiency was also observed in most of 2119 healthcare professionals studied from all over India.Evidently, countrywide prevalence of vitamin D deficiency is undeniable.

Correlation of Vitamin D Status with Bone Health of Ostensibly Healthy Adolescents and Adults in India

Among otherwise healthy adolescents and adults studied, along with low serum 25(OH)D levels, a significant number of subjects also revealed other biochemical and clinical manifestations of vitamin D deficiency. Biochemical evidences of suboptimal bone health are: elevated alkaline phosphatase, a surrogate marker for increased bone turnover, and elevated PTH levels (secondary hyperparathyroidism or SHPT). ALP and PTH were often reported in research papers, especially with respect to their correlation with vitamin D status. However, the most accurate and convincing evidence of bone density is DEXA. Only research articles reporting BMD, as measured by DEXA, in the

context of vitamin D status of ostensibly healthy individuals are observed in various studies.

Vitamin D intervention studies that measured resultant BMD outcomes are reviewed discussed. Most studies did not show any correlation of BMD with vitamin D status. Notably, among 90 adults, who were 20–30 year-old soldiers from Indian paramilitary forces, with adequate nutrition, sun exposure and physical exercise, BMD was lower when compared to Caucasians. Among men, osteopenia was noted in 50% at the lumbar spine, 35% at the hip, and 50% at the forearm.

Additionally, 10% of men had osteoporosis of the lumbar spine. Among women, osteopenia was noted in 32% at the lumbar spine, 14% at the hip and 21% at the forearm. The authors speculated that the effect of childhood malnutrition may have contributed to lower peak bone mass accumulation in these subjects. BMD studies emphatically underline the need for adequate nutrition, sun exposure and physical exercise from the very beginning of one's life, to attain peak bone mass, and later to maintain it. Indubitably, vitamin D status in India is grim and needs to be reckoned with.

Vitamin D Sufficiency via Sun Exposure Is Not a Tenable Solution for Most Indians

Vitamin D deficiency is a major health concern in India, notwithstanding the brightly shining sun. The adequacy of exposure to sunlight of an individual's bare skin required to photosynthesize vitamin D is grossly ill understood. Darker

skin has high melanin content which acts as a natural sunscreen. Therefore, darker skin produces a significantly lesser amount of vitamin D when compared with the individuals with fairer skin, such as Caucasians.Thus, for Indian skin tone, minimum direct sun exposure required daily is more than 45 min to bare face, arms and legs to sun's UV rays (wavelength 290–310 nm). With the exception of those who perforce need to work outdoors in the sun, most Indians do not get adequate sun exposure to produce sufficient amounts of vitamin D endogenously. Indian social and or religious norms related to public modesty dictate that most parts of an individual's body, irrespective of gender, be covered. The not so D-lightful price of urbanization in big cities a majority of people live in very high population density areas. They perforce live in overcrowded tenements, which are closely packed and 3–4 stories high. Consequently, direct sunlight does not reach inside most parts of the dwellings, thereby disallowing any sun exposure to an individual in the privacy of one's home. Additionally, lack of space offers limited options for outdoor activities. Atmospheric pollution of metropolitan India also factors in with respect to vitamin D status.

The extreme discomfort of the scorching heat associated with most sunny days of Indian summer instantly extinguish any desire for sun exposure and a person's primary focus is on finding ways to avoid the sun, at all costs. Usage of sun screen lotion aggravates the existing status. Therefore, in the Indian scenario, vitamin D sufficiency cannot be attained by depending on adequate sun exposure.

Nutritional Factors Attributing to High Prevalence of Vitamin D Deficiency in India

Vitamin D sufficiency by dietary intake is the only tenable solution for Indians. However, this solution itself has a barrage of problems. Most dietary sources of vitamin D have very low vitamin D content. Most of the food items rich in vitamin D are of animal origin. Commonly, a dietary source of vitamin D for vegetarians is milk, provided milk has been fortified with vitamin D. Milk is rarely fortified with vitamin D in India. The vitamin D content of unfortified milk is very low (2 IU/100 mL). Additionally, milk and milk products are unaffordable to the socioeconomically underprivileged. Another concern in India is the rampant dilution and/or adulteration of milk and milk products.

Low dietary intake of calcium in conjunction with vitamin D insufficiency is associated with secondary hyperparathyroidism (SHPT). SHPT is further exacerbated by induced destruction of 25(OH)D and 1,25(OH)2D by 24 hydroxylase [64]. 24 hydroxylase is the key enzyme of vitamin D catabolism and is regulated by 1,25(OH)2D, PTH and FGF23 (Fibroblast Growth Factor 23) levels. FGF23 is a phosphate regulator. High serum phosphate levels increase production of FGF23 in bone osteocytes *via* the action of 1,25(OH)2D. Subsequently, FGF23 reduces renal phosphate resorption, indirectly suppresses intestinal phosphate absorption and also suppresses PTH and 1,25(OH)2D synthesis. Overproduction of FGF23 can result in increased morbidity associated with vitamin D deficiency.This regulatory mechanism may explain the low

25(OH)D levels in rural subjects on a high phytate and/or low calcium diet, despite plentiful sun exposure. Most studies reported calcium intake much lower than the RDA (Recommended Daily Allowance) defined by the Indian Council of Medical Research (ICMR). Only two studies reported adequate calcium intake. In both these publications the study subjects were paramilitary soldiers. ICMR's RDA for calcium intake in India is lower than that of the western world.

Calcium balance is a function of intake and excretion.Even though the Indian diet is low in calcium content, it also has a lower protein content and therefore low endogenous acid production, which may reduce urinary calcium loss. Therefore, the amount of dietary calcium required to maintain calcium balance may be lower than for those in the Occident. The protein-induced alterations in calcium homeostasis (and possibly in bone mass) have been attributed to increments in endogenous acid production and net acid excretion due to the oxidation of the constituent sulfur containing amino acids. On the other hand, the high salt content of Indian diet is likely to increase urinary calcium excretion. A direct relation between high sodium intake and lower bone mass has been reported.

Intake of caffeine from tea and coffee is very high in India. Most Indians consume milk as part of their tea or coffee. The proportion of milk is very low in these drinks. Thus calcium intake through these beverages is low. Vitamin D is stable during cooking. It is stable up to 200°C. However, thermal stability of vitamin D is an inverse function of both temperature and time. In India, milk is boiled for several minutes before consumption. Before the same lot of milk is

consumed in entirety, it is subjected to two-three rounds of boiling. In India, most of the times, beverages like tea and coffee are boiled for several minutes to get the right flavor. This boiling may reduce the content of any vitamin D that there may have been left after boiling of the milk itself. Therefore, these beverages may not contribute significantly to either calcium or vitamin D intake in Indians. Vitamin D is a fairly robust vitamin. The preceding statements about its thermal degradation have been made as precautionary stance to not overstate the thermal robustness of this micronutrient. Additionally, studies have reported association of high caffeine intake with increased risk of low bone mineral density, osteoporosis, and osteoporotic fractures in middle-aged women. This situation is exacerbated in women with low calcium intake, especially in lean subjects.

High prevalence of lactose intolerance in India is a major deterrent pertaining milk consumption, further lowering intake of calcium and vitamin D in these individuals. Ethnic and geographic variations of lactose intolerance were observed, with a higher prevalence in southern and eastern India compared to northern India.

Indian diet has high phytate content. Phytate is the principal storage form of phosphorus in many plant tissues, especially the bran portion of grains and other seeds. Phytate is indigestible to humans. Phytates chelate micronutrients such as calcium and iron, and thus reduce intestinal absorption of these nutrients. Benefits of sun exposure in rural subjects owing to an agrarian life were seen by significantly higher 25(OH)D levels. However, possibly owing to high phytate

content in diet, these levels were still insufficient in most individuals. Possibly, high phytate content in the diet of soldiers in northern India may have contributed to their vitamin D insufficiency, despite adequate sun exposure, nutrition and physical exercise.

Dietary habits in India have changed significantly. Many people remove a substantial proportion of bran from whole wheat flour before kneading to improve texture and fluffiness of chapatis(unleavened flat bread). Consumption of white bread is also very high. Most people prefer processed, split and polished pulses to whole seeds due to the ease of shorter time required for cooking and the consequent lowered expense of cooking fuel. Consumption of instant (or not) noodles and burgers also is on the rise across all socio-economic strata, with the exception of the impecunious.

High phytate in Indian diet especially among th socio-economically lower classes stems from the elementary and immediate need of sufficiency of the calorific need. Cereals and legumes are more affordable and easily available than vegetables, milk and other dairy products. Besides, they are sources of protein for the vegetarians. Many cereals are also sources of calcium, however due to chelation by phytates its bioavailability is limited.

In the scenario of inadequate calcium intake, vitamin D insufficiency and high phytate content in diet, environmental pollutants such as fluoride add insult to injury. Toxins like fluoride affect bone metabolism severely in the conjunction with inadequate calcium intake, especially in children.

Indians in general adhere to traditional cooking styles and practices, irrespective of their migration to any part of the world. In tropical climate perishable food items putrefy quickly. Notably, in India there is no perceptible government regulation on the hygiene and microbial quality control of fresh produce that reaches from the producer to the end-consumer. Consumption of uncooked fresh produce, especially vegetables, milk, *etc.*, is generally considered ill-advised. As in the rest of the world, in India too, slow cooking is widely practiced. This culinary practice, however, is ill-advised bearing in mind the thermal instability of many vitamins. Its thermal stability is inversely related to temperature and time. Cooking gas flame reaches temperature above 1900°C and coal stove heat reaches 300–700°C. Water boils at 100°C.

Baking is done mostly above 175°C but the temperature in the food does not reach such high temperatures, therefore stability of vitamin D during baking is well within acceptable range. Pertaining shallow and deep-frying of food, most cooking fats and oils have smoke points above 180°C. Shallow and deep frying of foods is very popular in India. When foods are fried, vitamin D in the food comes out into the cooking medium and is thermally degraded.

Pressure cooking temperatures vary depending on the pressure withstood by the cooker used and may range from 100°C to 120°C. Short-time (as short as possible) pressure cooking is definitely advisable to retain at least some of the thermally more stable essential nutrients in cooked food, including vitamin D.

Protein malnutrition and poor overall nutrition resulting from poverty: Perforce, in the sordid context of poverty, focus on a balanced diet is always on the back burner. It is very convenient to attribute dietary patterns or cooking traditions, than face a reality as grim as poverty. Factually, balanced diet is only an occasional treat to the impecunious. An old adage, "When a poor man eats chicken, one of them is sick", says it all. Publications indicating wide prevalence of vitamin D deficiency in healthy Indians have studied subjects mostly from lower and upper middle classes. Individuals below poverty line were not represented well in these studies. Hence, poor nutrition observed in these studies may also stem from lack of awareness of the features, benefits and necessity of balanced nutrition.

Vitamin D Fortification in USA and Canada

Despite predominantly non-vegetarian dietary pattern, approximately 60% of the intake of vitamin D from food comes from fortified foods in USA and Canada. In USA, vitamin D fortification of foods is voluntary, but it is strictly regulated pertaining categories of foods, functional use and level of use, thus limiting over-fortification. Vitamin D fortified milk has been available in USA since the 1930s. Vitamin D is added to most milk sold in the United States, although it is not added to all milk products like cheese and ice cream. Some manufacturers also add it to cereal, soymilk, rice milk, and orange juice, usually along with calcium. In USA either form, D2 or D3, may be used for fortification, but commonly D3 is used. In Canada, the law mandates fortification of milk,

milk alternatives and margarine. Similar to USA, for other permitted foods, vitamin D fortification D is voluntary, but fortification level is limited.

Statistics on Vitamin D Deficiency in Middle East Countries

In countries that have similar cultural practices and lifestyle habits outside of the Middle Eastern countries such as Tunisia, the prevalence of vitamin D deficiency was 47.6% and the deficiency increased with age. Those in the age group between 50-59 years have a deficiency of about 59.5% while those in the age group between 20-29 years have less deficiency of about 35.8%. In addition, the severe deficiency level of serum vitamin D was 12.5 nmol/L and it is commonly prevalent among a certain group of women.

About 70.5% of veiled women had the deficiency, which is higher than that of unveiled women at 48.9%.However, vitamin D deficiency is infrequent in the United States and Japan. In Japan, 7.9% of the study participants have low levels of vitamin D; while 14.5% women in the United States study have hypovitaminosis D lower than 37.5 nmol/L. In the United States, more foods are fortified with vitamin D compared to Europe. In Canada, 10% of Canadians were considered as being vitamin D deficient (< 30 nmol/L).

Many of the young and aging people in the Middle East suffer from hypovitaminosis D. A study done in Lebanon showed that 41% have severe deficiency, levels lower than serum 12.5 nmol/L. About 84% of young females had serum vitamin D levels lower than 30 nmol/L .The Gulf region (Bahrain, Kuwait, Oman, Qatar, Saudi Arabia, and United Arab Emirates, Iran, Iraq) has a

prevalence of insufficiency and deficiency of serum vitamin D at levels of 25(OH) D < 30ng/mL (< 74 nmol/L).

The National Academy of Sciences Institute of Medicine advocates for a daily intake of 400 to 600 IU/day for adults 18 to 70 years old and 400 to 800 IU/day for those older than 70 years. The American Association of Clinical Endocrinologists recommends a higher intake of 1000 to 2000 IU/day to reach a target vitamin D serum concentration of 30 to 50ng/mL. Another study showed that 70% of Iran's and 80% of Saudi Arabia's populations have low serum vitamin D levels. Another study done about vitamin D deficiency in the Middle East showed that Emirati (UAE) women have more vitamin D deficiency than western women living in UAE. About 83 % of Saudi women who have low levels of vitamin D have been reported to also have back pain.

Sunning Practices

Vitamin D deficiency is a widespread disease in many countries around the world in different age groups such as North America, Europe and the Middle East. Despite the high latitude and long sunlight hours in the Middle Eastern countries, lack of sunlight exposure is the major cause of vitamin D deficiency among the populations. Young male athletes have normal vitamin D levels especially those who spend most of their sport practice outdoors although athletes are still at risk of vitamin D deficiency.

One of the studies among Saudis report a lack of sun exposure with only 6 male participants out of 200 participants exposing themselves to sunlight for vitamin D from the sun.Lack of sun exposure was also reported as one of the most important predictors of hypovitaminosis D in developing countries .Another study on vitamin D deficiency among Saudi married couples reported that males were significantly exposing themselves to sunlight more than their female partners. Lack of sun exposure is also one of the common results of sedentary lifestyle in the Gulf region. In addition, sun exposure is greatly affected by clothing style that covers most of the body, which leads to reduced vitamin D production in the skin Thus, limited sun exposure in the Middle East appears to be mostly due to cultural practices, clothing styles and limited outdoor activity.

Food Habits

Dietary factors have an important role in vitamin D deficiency among the Middle Eastern populations. Their intake of foods rich in vitamin D is limited and this has been shown as one of the main risk factors of vitamin D deficiency. There is also a lack of awareness in Delhi (India) on the value of taking vitamin D supplements. Lack of food and dairy products fortified with vitamin D is a problem in many countries of the Middle East. For example, in Saudi Arabia, the Saudi Ministry of Health is trying to change this by increasing milk fortification.

However, the Saudis have low consumption of milk and dairy products due to lactose intolerance, and in addition, phytates in bread can limit the absorption of calcium in the gut.

The food habits in some Middle Eastern regions have changed from healthy foods such as dates, milk, fresh vegetables and fruit, whole wheat and fish to fast and unhealthy foods such as fried chicken and beef burgers which are low in vitamin D. In addition, some Gulf countries rely on importing a lot of food to meet their population needs while they export a lot of seafood. People in the Gulf countries tend to gather a lot and cook and eat foods at home together, which lead to high intakes of calories per day.

Men versus Women

Men's work environment is mostly different than women's in terms of having a lot of time working outdoors, thus exposing themselves to sunlight. Most of women have indoor environment workplaces. As a result, women (70%) have more deficiency of < 25 nmol/L of serum vitamin D than men (40%).There are not enough studies that show the difference between men and women in terms of vitamin D deficiency. Some studies considered vitamin D deficiency based on gender, but with no actual comparisons between both genders in terms of vitamin deficiency and its risk factors.In terms of risk factors affecting vitamin D level and controlling for some confounding variables between genders, most men were wearing lighter clothing than women; however, there were no differences in physical activity, medication intake, and skin colour.

Dietary patterns were quite similar between men and women in the study of Saudi married couples; however, the intake for both fresh milk and soft drinks were higher among men.

Genetic and Epigenetic Factors Affecting Vitamin D Status

Genetic factors leading to vitamin D deficiency in Indians, due to their effect on expression of genes that modulate vitamin D metabolism may not be ruled out. Genetic factors such as polymorphisms in 7 dehydroxylase reductase, DBP, 1 alpha hydroxylase, VDR, 25 hydroxylase, 24 hydroxylase have been studied. However, a clear association between these polymorphisms and vitamin D status is yet to be established. Epigenetic factors too may be important that pertain to heritable changes in the gene expression, while the DNA sequence is unchanged. These are several possibilities including post-translational modifications of histones and methylation, acetylation and phosphorylation, and also aberrant expression of microRNAs. Interaction between genetic and environmental factors, modulated by epigenetic factors has been reported. 1α hydroxylase and 24 hydroxylase have been shown to be epigenetically controlled. Information regarding association between genetic and/or epigenetic factors and vitamin D status is inconclusive and warrants further study.

Population Based Approaches to Improve Vitamin D Status in India: Supplementation, Food Fortification and Educational Programs

There is protracted debate ongoing on issues pertaining optimal levels of intake of vitamin D, preferred form of vitamin D for human use and extraskeletal benefits of vitamin D. However, one thing is clear that Indians need more vitamin D. Vitamin D can be obtained from three sources: sun exposure (limitations of which has been discussed earlier), vitamin D supplements and vitamin D fortified foods. There is urgent need to prioritize development of national level programs to make available, quality-regulated and affordable vitamin D supplements and vitamin D fortified foods to the Indian populace. Very importantly, the government needs to implement measures to educate the Indian populace about the current status of vitamin D in India and also the modes to attain vitamin D sufficiency.

Vitamin D Supplements Available in India

Supplements commonly available are - D3 (cholecalciferol), 1,25(OH)2D3 and 1 alpha hydroxyl vitamin D3 (alfacalcidol). Some formulations have calcium too. Multivitamin formulations are also available and contain about 400 IU of D3.Vitamin D3 supplement of 60,000 IU is the highest selling one and is available in powder form in sachets or as oil-based capsules. Recommended dose on the label is once per week. The sachets indicate that half a sachet per week may also be taken.The other vitamin D supplements mentioned here are present in lower doses (0.25 μg or 500 IU) and daily intake (1–4 times/day) may

be recommended by the clinicians. Calcium supplementation is generally recommended with vitamin D intake.

The cost of a single dose of 60,000 IU of vitamin D3 is about INR 30. Intake of 60,000 IU of vitamin D3 per week may be advisable for a short duration, for patients with severe vitamin D deficiency, but a regular weekly dose may be lead to toxicity problems. A lower dose of vitamin D not exceeding the limit of 4000 IU per day would be advisable for otherwise healthy individuals to avoid toxicity. This will also reduce the cost of the supplement and become more affordable to the common people of India.

Vitamin D Supplementation Studies in Ostensibly Healthy Indians

Vitamin D supplementation studies in ostensibly healthy were compiled. Supplementation resulted in significant improvement in vitamin D status, but a large proportion of the population had still did not attain sufficiency. The following study is noteworthy. In India, physicians often prescribe D3 60,000 IU per week for 8 weeks for vitamin D deficiency. Twenty two healthy Indians with subnormal serum 25(OH)D levels were supplemented with oral D3 60,000 IU/week and calcium 1 gm/day for 8 weeks. At 8 weeks the mean 25(OH)D levels increased from 10.16 (3.96) ng/mL to 22.4 (6.8) ng/mL. Twenty two of the 23 subjects had 25(OH)D levels > 20 ng/mL. At the end of 12 months however, all the subjects were vitamin D deficient, once again. To sustain optimal 25(OH)D levels vitamin D supplementation would need to be ongoing

after the initial loading. Some supplementation studies, in ostensibly healthy adolescents and adult subjects, resulted in improved bone health.

More frequent and lower doses, not exceeding 4000 IU/day of vitamin D supplementation may be better for maintenance of serum 25(OH)D level. Most of the studies from India mention vitamin D status in terms of serum 25(OH)D levels as below or above 20 ng/mL (or 50 nmol/L). However, one must not lose sight of the fact that the aim of all interventions in terms of vitamin D status should be ≥ 30 ng/mL or above to derive both skeletal and extraskeletal benefits of this D-lightful nutrient, but with a precautionary stance to not exceed 100 ng/mL.

Need for Vitamin D Fortified Food Products in India

Vitamin D sufficiency via sun exposure is untenable for most Indians. Vitamin D rich dietary sources are mostly unaffordable and limited. Vitamin D supplements are unaffordable and not feasible as a population based approach. Fortification of widely consumed staple foods with vitamin D is the only viable solution towards attaining vitamin D deficiency in India. Unlike supplementation strategies, fortification of food with vitamin D poses a negligible risk of toxicity.

Feasibility of Fortification of Foods with Vitamin D in India

Food fortification is a much more economically viable approach compared to vitamin D supplementation. While the cost of fortified food items will be more than unfortified foods, it will be lower than supplementation.

1. Adaptability to fortified food by the consumers is much better than to supplementation. Food fortification requires relatively less change in food habits and preferences, leading to better efficacy of fortification programs, lowered cost to the consumer and a larger profit to the food manufacturers. Clearly a win-win partnership for all.
2. Food fortification may be a better choice compared to supplementation strategies, especially when targeting those who need it the most—women (including non-pregnant, pregnant and lactating), infants, children (especially girls, who are sidelined, more often than not, in India) and senior citizens.
3. Use of staple foods such as chapatiflour, rice, *etc.*, for fortification may have certain advantages over other fortification matrices. Indian government offers cereals, chapatiflour, rice, lentils, *etc.*, at subsidized rates to the socioeconomically underprivileged citizens. Additionally, cost of these products is generally tightly regulated by the government. Thus, no large fluctuations are observed in the cost of these food items in the open market also. This will ensure continued consumption of these foods fortified with vitamin D, by those who avail of them and result in better and sustained economical feasibility of the fortification programs in the long run.
4. Two vitamin D fortification studies in ostensibly healthy subjects were reported in the literature. Underprivileged toddlers, fed with fortified laddoos, resulted in significant increase in serum calcium and vitamin D

levels and also in total body less head (TBLH) bone mineral content (BMC).More cost effective food items could be fortified with vitamin D.

In another study, 776 subjects (boys and girls) were given fortified milk, which resulted in significant improvement in their vitamin D status.These results support the strategy of fortification of foods in India for redressing malnutrition problems in India.

Food Items Which Could Be Fortified with Vitamin D in India

1. Milk: The whole array of different grades of milk available could be fortified—whole milk, toned, double toned and skim milk.
2. Milk curd and yogurt.
3. Infant formulas.
4. Butter, ghee(clarified butter) and oils, to use as spreads or to spike already cooked food.
5. Soy milk, soy curd (tofu), orange juice and mango juice may be fortified to cater to the needs of the lactose intolerant individuals and those who are allergic to milk proteins. Processed cheese also has very low lactose content and is rich in calcium and may be fortified for the benefit of the lactose intolerant. Due to high prevalence of dyslipidemia, metabolic syndrome and cardiovascular diseases in India, these fortified items will also offer healthier choices to the general population.
6. Widely consumed and affordable staple food items such as chapatiflour, maida(all purpose wheat flour, used to make bread and other bakery

products), rice and rice flour may be suitable vehicles for fortification strategies in the Indian scenario.

Foods Fortified with Vitamin D Available in the Indian Market

Vitamin D fortified milk from Amul (an Indian dairy cooperative, located in Anand, Gujarat, India) is the only fortified milk product found in the general market. It is 4.5% fat, homogenized milk fortified with calcium 150 mg, vitamin A 75 μg and vitamin D 0.5 μg (20 IU), *etc*., per 100 mL. The expiry date of this milk is 120 days if the carton is unopened. Some breakfast cereals fortified with vitamin D along with other micronutrients are also available. However, the exorbitant prices of these products are essentially prohibitive for consumption by the common people of India.

Impact of vitamin D deficiency on Indian Healthcare System

In India, bone diseases such as rickets, osteomalacia and osteoporosis have been (Nordin, 1966; Malhota and Mithal, 2008; Sp and Teotra, 2008) and still are widely prevalent. As per 2001 Census Report about 163 million people were above the age of 50 years. About 15%–20% of persons above the age of 50 years would be developing osteoporosis . As presented in this review, BMD studies of ostensibly healthy Indians show that a significant proportion of younger Indians too are suffering from this silent disease. Some staggering figures and estimates pertaining rickets, osteomalacia and osteoporosis are mentioned in some reviews (Malhota and Mithal, 2008; Sp and Teotra, 2008).

Vitamin D status in India is grim not only in the lower and but also in the upper socioeconomic classes. Considering the skeletal benefits of vitamin D, sufficiency in women of reproductive age would at the very least result in - improved women's health, improved outcome of childbirth and birth of infants who may have a better chance of a healthy existence from the beginning of their lives. Vitamin D sufficiency in growing children would mean allowing for their full growth potential - physically, mentally and psychologically. In children, rickets translates into life-long deformity, osteomalacia - a painful and subpar existence. Sufficiency of vitamin D in elderly patients would insulate them from osteoporosis, a silent disease with constant threat of fractures, especially hip fractures resulting in the end of an independent existence. This fact indicates the heavy load on the healthcare system and the economy of the nation due to bone diseases alone.

This review of literature clearly depicted that extra skeletal effects of vitamin D and its beneficial role in, cardio vascular diseases, malignancies, respiratory tract infections and skin disorders emphasize the need of vitamin D supplementation in deficient individuals. Vitamin D improves insulin sensitivity and promotes insulin secretion in diabetes mellitus.

MATERIALS AND METHODS

This chapter includes the study design, participant recruitment, inclusion and exclusion criteria, sample size, Grouping the participants, anthropometric analysis, blood sample collection, biochemical parameters including determination of HbA1C, fasting and post prandial blood sugar, serum insulin, serum 25(OH)D, serum calcium, renal function test including blood urea and serum creatinine, SGOT and SGPT, lipid profile including total cholesterol, triglycerides, HDL, LDL,. All the data collected are analyzed statistically by ANOVA method.

Ethics Approval

The study was conducted after obtaining institutional ethical clearance certificate from Institutional Ethical Committee (IEC), Research Cell of Chennai Medical College Hospital and Research Centre (Affiliated to The Tamilnadu Dr. M.G.R. Medical University), Irungalur, Tiruchirapalli. The approval letter was enclosed as Appendix A.

Study Design

A prospective, open labelled, randomized, single centre study performed in a tertiary care teaching hospital conducted at outpatient clinics. Total duration of the study period was 12 months (from June 2014 to May 2015). All patients were on oral first line anti-diabetic drug (Metformin) at the time of recruitment. All participants had a stable diet pattern.

Informed Consent

Written informed consent was obtained from each subject who were willing to participate in the study, after being explained personally about the purpose, potential risks regarding the study.

Inclusion and Exclusion Criteria

Inclusion Criteria

Our inclusion criteria were any Indian men and women aged between 36-60 years who have been previously diagnosed as type 2 diabetes mellitus and under metformin treatment.

Exclusion Criteria

Individual on other drugs (steroid, diuretics, statins, anti-epileptics, calcium and vitamin D supplements), type 1 diabetes, type 2 diabetes mellitus with ketosis or coma or reduced level of consciousness within the past 6 months, patient on insulin therapy (or) on anti diabetic drugs other than metformin, alcoholics, patients with hepatic dysfunction, renal dysfunction, renal calculi, hyperparathyroidism, pregnancy and lactating mothers.

Sample Size

A total of 90 type II diabetes mellitus subjects in the age group of 36 to 60 years on metformin therapy attending the diabetic clinic at Chennai Medical College Hospital and Research Centre, Irungalur, Tiruchirapalli were

included in this study. The selected subjects were grouped into three groups of 30 each and the detailed study description was depicted in table 1.

Table 1: Description of study groups (n=90)

Group	No. of subjects	Subject descriptions
I	30	Type II diabetes mellitus on metformin therapy + normal serum vitamin 25(OH)D level
II	30	Type II diabetes mellitus on metformin therapy + decreased serum 25(OH)D level
III	30	Type II diabetes mellitus on metformin therapy + decreased serum 25(OH)D level + Cholecalciferol 60000 IU weekly

Group I: Includes 30 patients tested at zero time, 4 weeks, 8 weeks and 12 weeks. The patients were considered as control groups where only metformin therapy followed and this group have normal vitamin D level. The age of patients for group 1 ranged from 36 – 60 years.

Group II: Includes 30 patients tested at zero time, 4 weeks, 8 weeks and 12 weeks. The patients were considered as test group 2 where metformin therapy followed and this group have decreased vitamin D level. The age of patients for group 2 ranged from 36 – 60 years.

Group III: Includes 30 patients tested at zero time, 4 weeks, 8 weeks and 12 weeks. The patients were considered as test group 3 where metformin therapy followed and this group have decreased vitamin D level and advised to take cholecalciferol 60000 IU weekly. The age of patients for group 3 ranged from 36 – 60 years.

All the subjects were provided with a general questionnaire which includes thorough past and present medical history and detailed medication information. None of the subjects included in this study are allowed to change their medication regimens during the entire study period in order to avoid the experimentation bias.

Demographic and Clinical Data

Baseline demographic data including age, sex were determined, medications prescribed for diabetes treatment were documented using a structured questionnaire during the subject's visit and validated from medical records. Clinical characteristics including body weight, height, body mass index, systolic and diastolic blood pressure of all subjects were measured.

Anthropometric Measurements

- All the measurements were recorded by a single observer
- A digital scale was used for measuring the weight, which was adjusted to the nearest 0.1 kg. A wall – mounted stadiometer was used for measuring

the height and was adjusted to the nearest 0.1cm . BMI was calculated as BMI = Weight (kg) / height $(m)^2$.

Blood pressure determination

Blood pressure is measured is measured for the study subjects at 0,4,8 and 12 weeks of study period.

Table 2: Blood pressure levels

Criteria	Blood pressure levels	
	Systolic (mmHg)	**Diastolic (mmHg)**
Normal	Less than 120	Less than 80
At risk (pre hypertension)	120-139	80-89
High	140 and above	90 and above

Blood sample collection

Before taking a blood sample, it is extremely important to be fully familiar with the collection system being used. It does not just make an unprofessional impression to fiddle about with the blood collection equipment, it will make the patient feel increasingly anxious, which will have a negative influence on the condition of the veins. The blood samples were collected from the patients before and after their diet to determine the fasting and post prandial glucose level determination respectively.

BIO CHEMICAL EVALUATION

BLOOD SAMPLE COLLECTION

About 4ml of overnight fasting blood was collected during each visit by vene puncture under aseptic precautions in a sterile dry clean container and anticoagulant was added. The samples were centrifuged and used for the Fasting Glucose,Insulin,HbA1c,Lipid profile,Vitamin D3,SGOT, SGPT, Urea and Creatinine estimations.. Complete hemogram was done by Auto analyzer method.

Blood samples were collected from the subjects to measure the biochemical parameters in all the three test groups on 0^{th} day and at the end of 4^{th}, 8^{th} and 12^{th} weeks of the study period and the following biochemical parameters were determined,

1. Plasma glycosylated haemoglobin (HbA_{1C})
2. Fasting Blood Glucose
3. Post Prandial Blood Glucose
4. Serum Insulin
5. Serum 25(OH)D
6. HOMA – IR insulin resistance
7. Lipid profile
    i. Total cholesterol
    ii. Triglycerides
    iii. High density lipoprotein (HDL)
    iv. Low density lipoprotein (LDL)

About 2ml of anticoagulated blood collected at 2 hrs for Postprandial Glucose estimation

Plasma glycosylated haemoglobin (HbA_{1C})

The levels of HbA_{1C} were measured by high performance liquid chromatography (HPLC). Glycosylated hemoglobin (HbA_1c) is formed by the attachment of glucose to hemoglobin (the oxygen carrying protein found in red blood cells). The percentage of glycosylated hemoglobin in the blood reflects the average blood sugar levels over the preceding 2-3 months. If the blood sugar levels have been high in recent weeks, then HbA1c will also be greater (Table 3).

METHOD :- HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

HPLC uses pressure for fast separations, controlled temperature, in-line detectors and gradient elution techniques.

Table 3: Targets of HbA_{1c} with its ranges

$HbA1_c$ targets	**Percentages**
Non diabetics	4-5.9
Diabetics	>6.5

Fasting and Post prandial blood glucose

METHOD:- GLUCOSE OXIDASE-PEROXIDASE METHOD

PRINCIPLE:-

Glucose in the sample is oxidised to gluconic acid and hydrogen peroxide by glucose oxidase. The enzyme peroxidase catalyses oxidative coupling of 4-aminophenozone with phenol to give pink coloured quinino -imine complex. The intensity of the colour is proportional to the concentration of glucose present in the sample

Fasting blood glucose (FBS) Measures blood sugar levels either after an 8-12 hrs of overnight fast

Postprandial blood glucose (PP) Measures blood sugar 2 hours after intake of a meal or a specific amount of glucose (Oral Glucose Tolerance Test)

Table 4: Interpretation of blood glucose levels

Blood Glucose (mg/dL)	Fasting blood Glucose	Postprandial blood Glucose
Normal glucose tolerance	70-110	70-140
Impaired glucose	110-126	140-220
Provisional diabetes mellitus	>126	>200

Serum insulin

- **METHOD:- RADIOIMMUNOASSAY**
- **PRINCIPLE:-**

  The essential componenets of analytical system are Antigen (Insulin) to be determined , a fixed amount of labelled Antigen(Insulin) and a fixed limited amount of Antibody (antiInsulin antibody). This method offers considerable sensitivity and specificity. With antibodies of high binding affinity and radio labelled antigens of high specific activity, RIA measures substances at concentrations as low as 1 pmol/l. High specificity is due to highly specific nature of antigen-antibody interaction. The normal reference value after overnight fast is 2.6-24.9 μIU/mL.

Insulin levels generally decline in patients with type 1 diabetes mellitus. In the early stage of type 2 diabetes, insulin levels are either normal or elevated. In the late stage of type 2 diabetes, insulin levels decline. During prolonged fasting, when the patient's glucose level is reduced to <40 mg/dL, elevated insulin level plus elevated levels of proinsulin and C-peptide suggest insulinoma.

Serum 25(OH)D

The 25-hydroxy vitamin D test is the most accurate way to measure vitamin D level It is measured by Chemiluminescence immunoassay. The normal range of vitamin D is measured as nanogram per milliliter (ng/mL). Vitamin D deficiency is defined as a 25(OH)D concentration < 20 ng/mL ;

vitamin D insufficiency as 21-29 ng/mL and sufficiency as 25(OH)D > 30 ng/mL .

PRNCIPLE:-

The antiserum was raised against a bovine antiserum conjugate of vitamin D analog lacking the side chain. A radio iodinated vitamin D analog was used as tracer. Samples and calibrators were deproteinised with acetonitrile and analyzed directly.

4.HbA1c

METHOD :- HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

HPLC uses pressure for fast separations, controlled temperature, in-line detectors and gradient elution techniques.

HOMA-IR (Insulin resistance)

This method is used mainly for determining the resistance towards insulin. Insulin Resistance is determined by multiplying fasting blood sugar level in mmol/L with fasting serum insulin level in μIu/ml and further divided by 22.5. The normal reference value is < 2.6.

$$\text{HOMA-IR} = \frac{\text{Fasting blood sugar (mmol/L) X Fasting serum insulin(μIu/ml)}}{22.5}$$

Lipid Profile:

Total Cholesterol (TC)

This test may be measured any time of the day without fasting. However, if the test is drawn as part of a total lipid profile, it requires a 12-hour fast (no food or drink, except water). The normal range of the total cholesterol is 75-169 mg/dL for those age 20 and younger and100-199 mg/dL for those over age 21.

High Density Lipoprotein (HDL)

High levels linked to a reduced risk of heart and blood vessel disease. The reference value of HDL is greater than 40 mg/dL. This test may be measured any time of the day without fasting. However, if the test is drawn as part of a total lipid profile, it requires a 12-hour fast (no food or drink, except water)

Low Density Lipoprotein (LDL)

High levels are linked to an increased risk of heart and blood vessel diseases, including coronary artery disease, myocardial infarction and death. Reducing LDL levels is a major treatment target for cholesterol-lowering medications.The ranges of LDL in blood are

- Less than 70 mg/dL for those with heart or blood vessel disease and for other patients at very high risk of heart disease (those with metabolic syndrome)
- Less than 100 mg/dL for high risk patients (multiple risk factors for coronary vascular diseases)

- Less than 130 mg/dL for individuals who are at low risk for coronary artery disease
- Blood should be collected after a 12-hour fast (no food or drink, except water).

Triglycerides (TG)

Elevated in patients with coronary artery diseases, diabetics, obese individuals, hepatic and thyroid disorders. Increased serum triglyceride level has a genetic predisposition also. The normal value is less than 150 mg/dl. Blood should be collected after a 12-hour fast (no food or drink, except water

LIPID PROFILE ESTIMATION:

The estimation of serum Triglycerides and total Cholesterol were done using Enzymatic method. The estimation of serum HDL cholesterol was done by Immuno-Inhibition method.

LDL cholesterol was determined using the Friedwald's formula

LDL-C = Total Cholesterol — (HDL-C + VLDL-C)

VLDL - C = Triglycerides / 5

The other biochemical parameters measured include:

- Serum Calcium estimation was done at the start of the study and at the end of 12th week. Serum Calcium was measured using Ion selective Electrode method
- Blood Urea and serum creatinine were measured at the start of the study. Estimation of blood urea is by Urease GLDH method and Estimation of serum Creatinine was done by Modified JAFFE'S method.
- Estimation of Liver enzymes (SGOT,SGPT) was done by IFCC method at the start of the study.

Urine Analysis:

It was done at the start of the study. About 5 ml of urine was collected in a sterile container. Urine albumin,glucose and deposits were estimated .

STATISTICAL TESTS USED

1. TESTS FOR NORMALITY

- **Shapiro –Wilk Test** and **Kolmogorov Smirnov Tests** were used to test whether the data follow normal distributions or not.
- Since the test was statistically not significant (p=0.679 i.e p>0.05), the data follow a normal distribution, and **Parametric tests** were used.

2. DIFFERENCES BETWEEN INDEPENDENT GROUPS

- Test of significance used was **One-way ANOVA test.**
- The group means were compared with the overall mean of the sample
- **F Statistic** was used to determine the significance.
- Additionally **post-hoc test (Bonferroni test)** was used to compare the intergroup means.

3. DIFFERENCES BETWEEN DEPENDENT GROUPS

- Test of significance used was **Two-way ANOVA (Repeated measures ANOVA) test.**
- The test was used to compare the same variable under 4 different instances [0,4,8 and 12 weeks]
- **Wilks' Lambda** was used to determine the significance

RESULTS

TABLE-1: Distribution of sample population according to age (N=90)

Age	Frequency	Percent (%)	Cumulative (%)
30-39	14	15.5	15.5
40-49	32	35.5	51.0
50-59	37	41.1	92.1
≥60	7	7.9	100
Total	90	100.0	

Mean ± SD= 49.5± 7.44

Median (IQR) =48.5(36-60)

Comments:

Majority of the sample population (41.1%) were in the age group of 50-59years

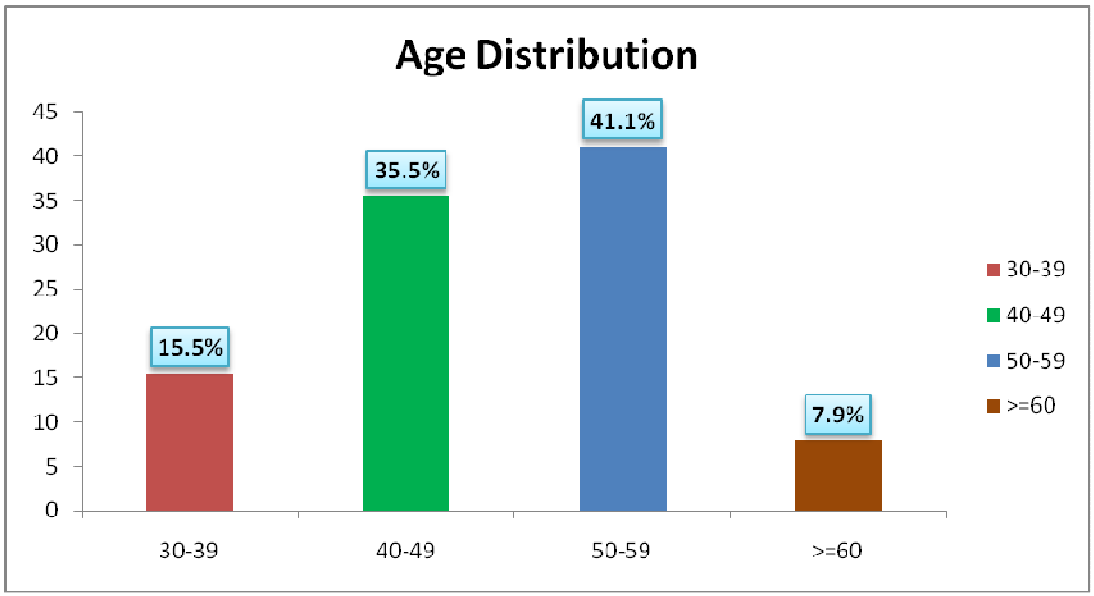

TABLE-2: Distribution of sample population according to gender (N=90)

Gender	Frequency	Percent (%)
MALE	54	60
FEMALE	36	40
TOTAL	90	100

Comments:

Majority of the sample population (60%) were males.

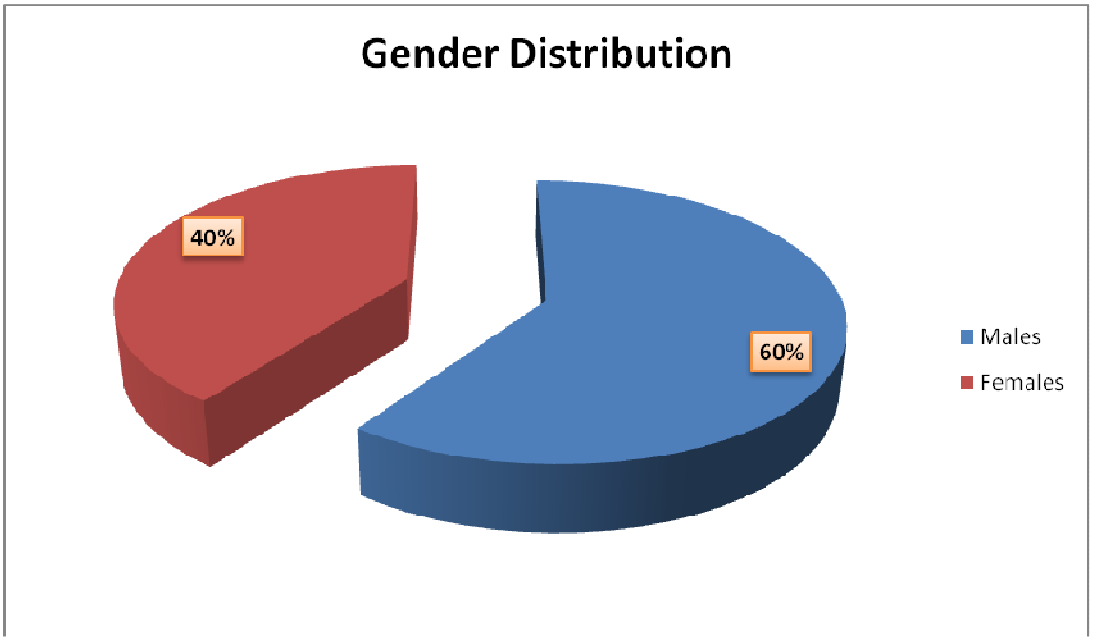

TABLE-3: Descriptive statistics of Weight, height and BMI (N=90)

Measures	Weight (kg)	Height (cms)	BMI
Mean	79.2	166.8	28.5
Median	79	168.1	28.7
SD	9.04	8.02	2.88
Range	57-96	148-180	20.06-34.02

TABLE-4: Descriptive statistics of Mean Fasting Blood Sugar levels of 3 groups at 0,4,8& 12 weeks (N=90)

Measures	0	4	8	12
Group I	119.9	117.4	148.5	112.5
Group II	137.3	136.0	134.1	131.9
Group III	139.2	134.9	130.7	124.9

The mean difference in FBS in 3 groups was statistically significant (p=0.032) [$p<0.05$] according to one way ANOVA(F=11.69).

Post hoc test revealed significant difference in mean FBS between groups II & III

TABLE-5: Descriptive statistics of Mean Postprandial Blood Sugar levels of 3 groups at 0,4,8& 12 weeks (N=90)

Measures	0	4	8	12
Group I	159.9	157.5	154.6	147.7
Group II	177.6	177	174.6	171.8
Group III	177.9	175.0	170.7	163.6

The mean difference in PPBS in 3 groups was statistically significant (p=0.024) [p<0.05] according to one way ANOVA (F=33.72).

Post hoc test revealed significant difference in mean PPBS between groups II & III

TABLE-6: Descriptive statistics of Mean Total Cholestrol levels of 3 groups at 0,4,8 & 12 weeks (N=90)

Measures	0	4	8	12
Group I	192.4	191.2	189.9	189
Group II	194.9	194.1	193.3	192.6
Group III	196.03	191.64	188.58	185.30

The mean difference in Total Cholesterol in 3 groups was not statistically significant (p=0.14) according to one way ANOVA (F=10.08).

TABLE-7: Descriptive statistics of Mean TGL levels of 3 groups at 0,4,8 &12 weeks (N=90)

Measures	0	4	8	12
Group I	234.7	233.3	231.8	230.5
Group II	238.7	238.09	237.6	236.7
Group III	238.6	235.1	227.25	226.01

The mean difference in TGL in 3 groups was statistically significant (p=0.045) [p<0.05] according to one way ANOVA (F=44.3).

Post hoc test revealed significant difference in mean TGL between groups II & III

TABLE-7: Descriptive statistics of Mean LDL levels of 3 groups at 0,4,8 &12 weeks (N=90)

Measures	0	4	8	12
Group I	104.6	103.5	102.5	101.5
Group II	109.9	111.9	109.4	108.5
Group III	114.2	111.03	109.4	109.8

The mean difference in LDL in 3 groups was not statistically significant (p=0.98) according to one way ANOVA (F=158.77).

TABLE-8: Descriptive statistics of Mean HDL levels of 3 groups at 0,4,8& 12 weeks (N=90)

Measures	0	4	8	12
Group I	40	40.7	41.5	42
Group II	38.5	39.1	39.7	40.3
Group III	38.5	40.1	39.9	42.2

The mean difference in HDL in 3 groups was not statistically significant

(p=0.84) according to one way ANOVA (F=97.58).

TABLE-9: Descriptive statistics of Mean Insulin Resistance levels of 3 groups at 0,4,8& 12 weeks (N=90)

Measures	0	4	8	12
Group I	2.19	2.1	1.95	1.83
Group II	3.49	3.4	3.22	3.1
Group III	3.6	3.3	2.9	2.6

The mean difference in Insulin Resistance in 3 groups was statistically

significant (p=0.037) [p<0.05] according to one way ANOVA (F=26.52).

Post hoc test revealed significant difference in mean IR between groups II & III

TABLE-10: Descriptive statistics of Mean HbA1C levels of 3 groups at 0,4,8& 12 weeks (N=90)

Measures	0	4	8	12
Group I	6.44	6.35	6.21	6.15
Group II	7.36	7.27	7.17	6.81
Group III	7.46	7.24	7.03	6.77

The mean difference HbA1C groups was statistically significant (p=0.046) [p<0.05] according to one way ANOVA (F=102.4).

Post hoc test revealed significant difference in mean HbA1C between groups II & III

TABLE-11: Descriptive statistics of Mean Vitamin D levels of 3 groups at 0,4,8& 12 weeks (N=90)

Measures	0	4	8	12
Group I	30.9	31.3	31.9	32.4
Group II	14.33	14.88	16.05	17.13
Group III	12.8	17	21.9	28.1

The mean difference in Vitamin D level in 3 groups was statistically significant (p=0.037) [p<0.05] according to one way ANOVA (F=12.4).

Post hoc test revealed significant difference in mean Vitamin D level between groups II & III

ANALYSIS OF VARIABLES FOR GROUP III [0,4,8,12 weeks]

REPEATED MEASURES

TABLE-12: Descriptive statistics of Mean FBS levels of Group III at 0,4,8& 12 weeks (N=90)

Variables	Time	Mean(CI)	SD	SE	N
FBS	0	139.2(136.8-141.6)	6.49	1.18	30
	4 weeks	134.9(132.5-137.3)	6.42	1.17	30
	8 weeks	130.7(128.2-133.1)	6.52	1.19	30
	12 weeks	124.9(121.8-127.9)	8.07	1.47	30

Wilk's Lambda value = 0.025 F= 351.0 df =3 p= 0.017

Reduction in FBS over time after intervention was statistically significant [p<0.05].

TABLE-13: Descriptive statistics of Mean PPBS levels of Group III at 0,4,8 & 12 weeks (N=90)

Variables	Time	Mean(CI)	SD	SE	N
PPBS	0	177.9(174.8-181)	8.2	1.5	30
	4 weeks	175.0(172.5-177)	6.8	1.2	30
	8 weeks	170.7(168.2-173.1)	6.6	1.2	30
	12 weeks	163.6(159.6-167.9)	10.7	1.96	30

Wilk's Lambda value = 0.80 F= 103.99df=3 p=0.009

Reduction in PPBS over time after intervention was statistically significant[p<0.05].

TABLE-14: Descriptive statistics of Mean Total Cholestrol levels of Group III at 0,4,8 & 12 weeks (N=90)

Variables	Time	Mean(CI)	SD	SE	N
TC	0	196.0(193.3-198.5)	7.1	1.3	30
	4 weeks	191.6(188.9-194.3)	7.1	1.3	30
	8 weeks	188.5(185.7-191.4)	7.7	1.4	30
	12 weeks	185.3(182.7-187.8)	6.7	1.2	30

Wilk's Lambda value = 0.051 F= 169.15df=3 p=0.041

Reduction in TC over time after intervention was statistically significant [p<0.05].

TABLE-15: Descriptive statistics of Mean Triglycerides levels of Group III at 0,4,8& 12 weeks (N=90)

Variables	Time	Mean(CI)	SD	SE	N
TGL	0	238.6(234.9-242.2)	9.6	1.7	30
	4 weeks	235.1(231.4-238.8)	9.9	1.8	30
	8 weeks	227.6(222.6-231.8)	12.3	2.2	30
	12 weeks	226.0(221.8-230.2)	11.2	2.0	30

Wilk's Lambda value = 0.336 F= 17.78df=3 p=0.033

Reduction in TGL over time after intervention was statistically significant[p<0.05].

TABLE-16: Descriptive statistics of Mean LDL levels of Group III at 0,4,8& 12 weeks (N=90)

Variables	Time	Mean(CI)	SD	SE	N
LDL	0	114.2(112-116)	5.3	0.98	30
	4 weeks	111.0(109-112)	4.7	0.86	30
	8 weeks	109.4(107-111)	5.5	1.01	30
	12 weeks	109.8(105-114)	11.4	2.09	30

Wilk's Lambda value = 0.386 F= 14.3df=3 p=0.09

Reduction in LDL over time after intervention was not statistically significant[p<0.05].

TABLE-17: Descriptive statistics of Mean HDL levels of Group III at 0,4,8& 12 weeks (N=90)

Variables	Time	Mean(CI)	SD	SE	N
HDL	0	38.5(37.7-39)	2.1	0.39	30
	4 weeks	40.0(39.5-40.5)	1.4	6.26	30
	8 weeks	39.9(37.3-42.4)	6.8	1.2	30
	12 weeks	42.2(41.5-43)	2.0	0.36	30

Wilk's Lambda value = 0.208 F= 34.27df=3 p=0.07

Rise in HDL over time after intervention was not statistically significant [p<0.05]

TABLE-18: Descriptive statistics of Mean IR levels of Group III at 0,4,8&12 weeks (N=90)

Variables	Time	Mean(CI)	SD	SE	N
IR	0	3.6(3.4-3.8)	0.5	0.09	30
	4 weeks	3.3(3.1-3.4)	0.5	0.09	30
	8 weeks	2.9(2.8-3.1)	0.4	0.08	30
	12 weeks	2.6(2.4-2.8)	0.4	0.09	30

Wilk's Lambda value = 0.021 F= 416.3df=3 p=0.025

Reduction in IR over time after intervention was statistically significant[$p<0.05$].

TABLE-19: Descriptive statistics of Mean HbA1C levels of Group III at 0, 4, 8 &12 weeks (N=90)

Variables	Time	Mean(CI)	SD	SE	N
HbA1C	0	7.4(7.34-7.57)	0.31	0.05	30
	4 weeks	7.2(7.12-7.36)	0.32	0.05	30
	8 weeks	7.0(6.9-7.15)	0.32	0.06	30
	12 weeks	6.7(6.6-6.8)	0.32	0.05	30

Wilk's Lambda value = 0.002 F= 487.2df=3 p=0.029

Reduction in HbA1C over time after intervention was statistically

significant[$p<0.05$].

TABLE-20: Descriptive statistics of Mean Vitamin D levels of Group III at 0,4,8 & 12 weeks (N=90)

Variables	Time	Mean(CI)	SD	SE	N
Vit.D	0	12.8(11.3-14.4)	4.1	0.75	
	4 weeks	17.0(14.7-19.3)	6.0	1.1	
	8 weeks	21.9(19.2-24.5)	7.1	1.2	
	12 weeks	28.1(25.2-30.9)	7.6	1.3	

Wilk's Lambda value = 0.066 F= 127.67df=3 p=0.016

Rise in Vitamin D levels over time after intervention was statistically significant ($p<0.016$)

DISCUSSION

Diabetes Mellitus is a metabolic disorder of multiple etiology with impairment of carbohydrate, protein and fat metabolism. This global pandemic principally involves T2DM, characterized by chronic hyperglycemia resulting from defective insulin secretion or insulin action or both. Type 2 Diabetes Mellitus accounts for 90 – 95 % and Type I accounts for 5 to 10% of diabetic population.. The number of subjects with type 2 diabetes mellitus is on the rise, due to the effects of age, sedentary nature of work and obesity.

Management of T2DM includes lifestyle modifications (diet control, regular exercises), oral anti-diabetic drugs , Parenteral agents including Insulin, GLP1 receptor agonists and Amylin agonists. Metformin (1,1-dimethyl biguanide), is a first line oral anti- diabetic drug. It primarily reduces hepatic glucose production and also improves peripheral glucose utilization.

Vitamin D deficiency is widespread in individuals irrespective of their age, gender, race and geography. Vitamin D is photosynthesized in the skin on exposure to UVB rays. Vitamin D deficiency is widely prevalent despite plentiful sunshine even in tropical countries like India. Vitamin D is a hormone related to skeletal integrity. Recently, the extra skeletal effects of vitamin D have evinced considerable interest. Vitamin D deficiency appears to be related to the development of diabetes mellitus type 2 and metabolic syndrome. Plasma 25(OH)D or calcidiol (a summation of D3 and D2 forms) is the most reliable marker of vitamin D status. Immunoassays such as radioimmunoassay (RIA),

enzyme linked immunosorbant assay (ELISA), chemilumiescence immunoassay and protein binding assays are used in routine testing of 25(OH)D in clinical laboratories.

The increased prevalence of diabetes mellitus is attributed to Low serum vitamin D status.[31] Vitamin D supplementation to T2DM patients increases serurm 25(OH)D level.[32] Studies in humans have shown that vitamin D supplementation in infancy reduces the risk of type 1 diabetes mellitus during early adulthood. As vitamin D modulates insulin receptor gene expression and insulin secretion, vitamin D deficiency is an environmental etiological factor for type 2 diabetes mellitus

Hence this study was conducted to study the prevalence of vitamin D deficiency and the effect of vitamin D on the glycemic conrol and lipid profile in T2DM patients. The study subjects represented type 2 diabetic patients under Metformin treatment. This study was undertaken in 90 T2DM patients on Metformin therapy (500mg OD), who attended the outpatient department of Chennai Medical College Hospital and Research Centre, Tiruchirapalli. The study population were divided into 3 groups of 30 each. A weekly oral dose of 60000 I U of cholecalciferol was administered to the 30 T2DM subjects with vitamin D deficiency belonging to group 3. The biochemical parameters of all the three groups were evaluated at the end of 3rd month (12th week) and the results were statistical analysed by ANOVA method. Additionally, post-hoc test (Bonferroni test) was used to compare the intergroup means.

Vitamin D supplementation significantly reduces Fasting Blood Glucose, Post Prandial Blood Glucose and HbA1c levels ($p< 0.05$) in group III T2DM patients. Further an inverse relation between serum 25(OH) D and HbA1c level[33] was observed Vitamin D exerts this beneficial effect by direct and indirect mechanisms. Vitamin D promotes pancreatic β-cell function and increases insulin secretion in numerous ways.[29] The presence of vitamin D receptors (VDR) and binding of 25(OH) D with vitamin D binding proteins (DBP) in β cells of pancreas[33] leads to the transcription of genes regulated by 25(OH) D and facilitates the secretion of insulin from pancreatic β cells.Activation of vitamin D occurs in pancreatic β-cells by intracellular 1-α-Hydroxylase enzyme.[34] Vitamin D by its direct action enhances insulin secretion by forming $1,25(OH)_2D_3$-RXR-VDR complex which binds to vitamin D responsive elements (VDRE) found in the insulin gene promoter region, enhancing the transcriptional activation of the insulin gene and increase insulin synthesis.[35]

Insulin secretion is a calcium-dependent process and is influenced by calcium influx through the cell membrane. Vitamin D indirectly promotes calcium influx into the β-cells of pancreas by regulating calbindin, a cytosolic calcium-binding protein found in β-cells resulting in increased insulin synthesis.[36]

Vitamin D supplementation along with metformin in group III T2DM patients also significantly reduces Insulin Resistance as compared to group II T2DM patients with metformin monotherapy.

The mechanism by which vitamin D reduces insulin resistance is a complex one. Vitamin D enhances insulin sensitivity by stimulating the transcription of insulin receptor (INS-R) gene[35] and thereby reduces insulin resistance.[37] Further vitamin D exerts anti apoptotic effect by attenuating the expression of pro inflammatory cytokines such as IL-1, IL-6, TNF-α, NF-kβ involved in insulin resistance. Vitamin D also suppresses the renin gene reducing hyperglycemic induced increase in renin levels in pancreatic β cells and blockade of renin-angiotensin activity. This has been proposed as a novel target for management of diabetes and metabolic syndrome.[38] Vitamin D supplementation also increases fasting serum insulin secretion.[29,34,35]

Regarding the analysis of lipid profile, Post- hoc test reveals a significant reduction in triglyceride (TGL) level in group III T2DM patients as compared to group II T2DM subjects. Vitamin D also reduces total cholesterol level and increases high density lipoprotein (HDL) level but insignificantly. Vitamin D exerts its action on lipid metabolism by activating transcriptional factor, peroxisome proliferator-activated receptor-δ (PPAR-δ). It is implicated in the regulation of fatty acid metabolism in skeletal muscles and adipose tissue.[40] The inhibitory effect of vitamin D on lipids facilitates reduction in insulin resistance.

The highlight of the present study is the identification of a higher prevalence of vitamin D deficiency and insufficiency among T2DM study population. Vitamin D supplementation significantly increases serum 25(OH)D level in group III T2DM subjects.

SUMMARY AND CONCLUSION

Diabetes has emerged as a major health problem worldwide, with serious health - related and socioeconomic impacts on individuals and populations. This pandemic growth of diabetes is due to transitioning demographic, socioeconomic, nutritional and lifestyle patterns. There is also an increased proliferation of diabetes mellitus in overweight and obese adults and children. Majority of this escalation is attributed to growth of type 2 diabetes mellitus (T2DM).

The extra skeletal effects of vitamin D have evinced considerable interest. Vitamin D deficiency has been proposed as a risk factor for type 2 diabetes. Vitamin D deficiency has been associated with increased insulin resistance in T2DM patients. Vitamin D decreases fasting blood glucose, post prandial blood glucose and HbA1c level in T2DM patients. Further Vitamin D decreases total cholesterol, triglycerides and increases HDL level in T2DM subjects. Vitamin D also increases insulin sensitivity by promoting insulin synthesis from pancreatic β cells. By these mechanisms, vitamin D can reduce the cardinal manifestations of metabolic syndrome including hyperglycemia, hypertriglyceridemia, obesity and poly cystic ovarian disease.

This study was conducted to study the prevalence of vitamin D deficiency and the effect of vitamin D on the glycemic control and lipid profile in T2DM patients. This study was undertaken in 90 T2DM patients on Metformin therapy (500mg OD), aged 36 to 60 years, who attended the outpatient departments of Chennai Medical College Hospital and Research Centre, Tiruchirapalli. The study population was divided into 3 groups of 30 each. A weekly oral dose of 60000 I U of cholecalciferol was administered to the 30 T2DM subjects with vitamin D deficiency belonging to group 3. Vitamin D status was evaluated using serum 25-hydroxy D level.

The overview of this study highlighted the study design, participant recruitment, inclusion and exclusion criteria, sample size, grouping the participants, anthropometric analysis, blood sample collection, biochemical parameters including determination of fasting and post prandial blood glucose, fasting serum insulin, HbA1C, serum 25(OH)D, lipid profile including total cholesterol, triglycerides, LDL and HDL. All the data collected are analyzed statistically by ANOVA method.

Combinations of metformin and cholecalciferol in group III T2DM patients with vitamin D deficiency significantly reduce fasting plasma glucose, postprandial plasma glucose and HbA1c levels. Further this combination also significantly reduce serum triglyceride level. There is also an insignificant rise in high density lipoprotein level as compared to metformin monotherapy in Type2DM with vitamin D deficiency. A significant increase in serum 25(OH) D level is also observed in group III T2DM patients.

CONCLUSION

Vitamin D improves glycemic control in T2DM patients by lowering fasting blood glucose and HbA1c levels. An inverse relation between serum 25(OH)D and HbA1c level in T2DM patients has been observed. Vitamin D also reduces triglyceride level and improves HDL level in T2DM patients. Further Vitamin D promotes insulin synthesis and improves insulin sensitivity in T2DM patients. Hence Vitamin D supplementation improves the glycemic control and can reduce or prevent the development of insulin resistance in T2DM patients. Considering the multitude of diseases associated with vitamin D deficiency, increasing the population's awareness of beneficial effect of vitamin D on health will be an important strategy overall.

Group	Serialno	Age	Sex	Weightkg	Heightm	BMI_NEW	Weeks	HbA1C	FBS	PPBS	FastingSerumInsulinµlUml	SerumvitD	LIPIDPROFILE_TC	LIPIDPROFILE_TGL	LIPIDPROFILE_LDL	LIPIDPROFILE_HDL	IR
3.0	1.0	48.0	m	58.0	163.00	21.83	0.0	7	132.0	172.0	9	18	188	233	109	38	3.06
							4.0	7	128.0	168.0	9	22	185	227	107	39	2.78
							8.0	7	124.0	164.0	8	26	183	221	104	42	2.51
							12.0	6	118.0	158.0	7	32	180	216	102	43	2.13
	2.0	59.0	m	65.0	175.00	21.22	0.0	7	134.0	174.0	10	17	190	231	110	40	3.21
							4.0	7	130.0	170.0	9	20	187	225	108	40	2.92
							8.0	7	128.0	168.0	9	22	181	220	107	40	2.78
							12.0	7	120.0	161.0	8	30	179	215	104	41	2.25
	3.0	60.0	m	82.0	169.00	28.71	0.0	7	136.0	176.0	10	15	192	231	108	37	3.36
							4.0	7	132.0	172.0	9	18	188	225	106	38	3.06
							8.0	7	128.0	168.0	9	22	185	221	103	39	2.78
							12.0	7	122.0	162.0	8	29	181	217	101	39	2.38
	4.0	52.0	M	75.0	165.00	27.55	0.0	7	132.0	172.0	9	19	187	234	108	39	3.06
							4.0	7	128.0	168.0	9	30	185	228	107	39	2.78
							8.0	7	126.0	166.0	9	32	181	225	105	40	2.64
							12.0	6	118.0	157.0	7	40	179	220	101	40	2.13
	5.0	55.0	M	76.0	172.00	25.69	0.0	7	130.0	171.0	9	20	188	232	110	40	2.92
							4.0	7	126.0	167.0	9	30	186	229	107	40	2.64
							8.0	7	122.0	162.0	8	36	182	224	104	41	2.38
							12.0	6	116.0	155.0	7	40	180	218	103	42	2.00
	6.0	39.0	F	58.0	163.00	21.83	0.0	8	146.0	185.0	12	12	190	231	110	40	4.15
							4.0	8	142.0	182.0	11	19	187	226	109	40	3.82
							8.0	7	138.0	178.0	10	31	181	221	107	40	3.51
							12.0	7	132.0	172.0	9	38	180	216	104	41	3.06

Group	Serialno	Age	Sex	Weightkg	Heightm	BMI_NEW	Weeks	HbA1C	FBS	PPBS	FastingSerumInsulinµIuml	SerumvitD	LIPIDPROFILE_TC	LIPIDPROFILE_TGL	LIPIDPROFILE_LDL	LIPIDPROFILE_HDL	IR
	7.0	60.0	F	73.0	148.00	33.33	0.0	8	152.0	192.0	12	12	192	233	110	39	4.54
							4.0	8	146.0	185.0	12	18	184	266	106	40	4.15
							8.0	8	142.0	181.0	11	27	184	222	104	41	3.82
							12.0	7	138.0	179.0	10	39	181	221	102	42	3.51
	8.0	43.0	M	96.0	180.00	29.63	0.0	8	142.0	182.0	11	14	194	232	110	38	3.82
							4.0	7	138.0	178.0	10	20	188	225	106	39	3.51
							8.0	7	134.0	175.0	10	30	182	184	104	42	3.21
							12.0	7	128.0	167.0	9	38	179	212	101	42	2.78
	9.0	60.0	M	75.0	176.00	24.21	0.0	7	136.0	175.0	10	17	190	241	116	40	3.36
							4.0	7	130.0	171.0	9	23	187	233	105	38	2.92
							8.0	7	126.0	166.0	9	31	182	221	103	42	2.64
							12.0	7	122.0	161.0	8	38	180	220	101	44	2.38
	10.0	48.0	F	76.0	153.00	32.47	0.0	7	138.0	178.0	10	12	186	224	108	39	3.51
							4.0	7	132.0	172.0	9	18	182	218	106	40	3.06
							8.0	7	128.0	167.0	9	22	180	206	103	40	2.78
							12.0	7	124.0	163.0	8	27	178	204	100	42	2.51
	11.0	59.0	F	68.0	161.00	26.23	0.0	7	132.0	171.0	9	18	193	238	112	41	3.06
							4.0	7	128.0	168.0	9	22	189	232	109	43	2.78
							8.0	7	126.0	165.0	9	24	186	228	108	41	2.64
							12.0	6	118.0	157.0	7	33	183	224	105	45	2.13
	12.0	41.0	M	82.0	177.00	26.17	0.0	7	136.0	175.0	10	15	196	240	116	38	3.36
							4.0	7	132.0	172.0	9	18	193	235	112	40	3.06
							8.0	7	130.0	169.0	9	20	189	231	109	4	2.92
							12.0	7	122.0	161.0	8	29	186	226	109	43	2.38

Group	Serialno	Age	Sex	Weightkg	Heightm	BMI_NEW	Weeks	HbA1C	FBS	PPBS	FastingSeruminsulinµIuml	SerumvitD	LIPIDPROFILE_TC	LIPIDPROFILE_TGL	LIPIDPROFILE_LDL	LIPIDPROFILE_HDL	IR
	13.0	38.0	M	69.0	168.00	24.45	0.0	8	140.0	151.0	11	11	197	242	114	37	3.66
							4.0	7	136.0	176.0	10	14	192	235	110	40	3.36
							8.0	7	132.0	172.0	9	18	198	230	106	40	3.06
							12.0	7	126.0	166.0	9	25	185	227	105	45	2.64
	14.0	47.0	F	70.0	156.00	28.76	0.0	8	142.0	181.0	11	10	198	244	118	39	3.82
							4.0	7	138.0	178.0	10	12	194	238	112	40	3.51
							8.0	7	134.0	174.0	10	16	191	234	108	42	3.21
							12.0	7	129.0	168.0	9	23	189	229	104	43	2.80
	15.0	49.0	F	68.0	160.00	26.56	0.0	8	148.0	189.0	12	7	197	241	112	39	4.31
							4.0	8	144.0	184.0	11	8	190	235	108	41	3.98
							8.0	8	140.0	181.0	11	11	183	228	104	41	3.66
							12.0	7	135.0	174.0	10	17	188	226	102	42	3.23
	16.0	52.0	M	79.0	170.00	27.34	0.0	8	146.0	186.0	12	8	194	246	116	40	4.15
							4.0	8	142.0	182.0	11	10	191	238	112	42	3.82
							8.0	7	138.0	178.0	10	12	188	234	109	44	3.51
							12.0	7	134.0	173.0	10	18	183	232	107	45	3.21
	17.0	52.0	F	58.0	170.00	27.00	0.0	8	142.0	181.0	11	10	200	247	118	41	3.82
							4.0	7	138.0	178.0	10	12	194	240	115	43	3.51
							8.0	7	132.0	171.0	9	19	181	235	113	41	3.06
							12.0	7	129.0	168.0	11	23	186	238	113	46	3.44
	18.0	39.0	F	65.0	156.00	26.71	0.0	8	144.0	183.0	12	9	202	248	120	43	4.34
							4.0	8	140.0	180.0	11	11	195	241	114	44	3.66
							8.0	7	136.0	176.0	10	14	188	236	115	45	3.36
							12.0	7	131.0	170.0	9	20	188	240	114	47	2.94

Group	Serialno	Age	Sex	Weightkg	Heightm	BMI_NEW	Weeks	HbA1C	FBS	PPBS	FastingSeruminsulinµlуml	SerumvitD	LIPIDPROFILE_TC	LIPIDPROFILE_TGL	LIPIDPROFILE_LDL	LIPIDPROFILE_HDL	IR
	19.0	54.0	F	68.0	163.00	25.59	0.0	8	155.0	195.0	13	5	204	249	122	39	4.86
							4.0	8	150.0	195.0	13	5	197	246	119	41	4.70
							8.0	8	146.0	187.0	12	8	194	243	117	43	4.15
							12.0	8	140.0	180.0	11	11	189	240	116	43	3.66
	20.0	52.0	F	86.0	166.00	31.21	0.0	8	148.0	187.0	12	7	203	251	121	39	4.31
							4.0	8	144.0	184.0	11	9	198	247	118	40	3.98
							8.0	8	140.0	180.0	11	11	193	242	117	41	3.66
							12.0	7	135.0	174.0	10	17	187	239	141	42	3.23
	21.0	53.0	M	88.0	172.00	29.75	0.0	7	134.0	173.0	10	16	206	254	124	37	3.21
							4.0	7	130.0	171.0	9	20	201	251	121	39	2.92
							8.0	7	126.0	167.0	9	24	196	246	118	40	2.64
							12.0	7	121.0	160.0	8	31	190	241	116	42	2.27
	22.0	58.0	M	85.0	173.00	28.40	0.0	7	134.0	173.5	10	17	195	238	113	38	3.21
							4.0	7	132.0	171.0	9	18	193	233	112	40	3.06
							8.0	7	124.0	165.0	8	26	199	230	111	42	2.51
							12.0	7	120.0	119.0	8	31	184	252	150	40	2.25
	23.0	55.0	M	69.0	169.00	24.16	0.0	7	132.0	172.0	9	18	192	240	116	38	3.06
							4.0	7	126.0	166.0	9	24	187	234	115	39	2.64
							8.0	7	120.0	161.0	8	30	187	232	113	41	2.25
							12.0	6	118.0	157.0	7	33	184	231	112	41	2.13
	24.0	55.0	F	66.0	158.00	26.44	0.0	8	142.0	181.0	11	10	209	248	119	29	3.82
							4.0	7	138.0	177.0	10	12	203	243	116	41	3.51
							8.0	7	134.0	173.0	10	16	203	240	115	42	3.21
							12.0	7	128.0	167.5	9	24	199	234	113	41	2.78

Group	Serialno	Age	Sex	Weightkg	Heightm	BMI_NEW	Weeks	HbA1C	FBS	PPBS	FastingSerumInsulinµIuml	SerumvitD	LIPIDPROFILE_TC	LIPIDPROFILE_TGL	LIPIDPROFILE_LDL	LIPIDPROFILE_HDL	IR
	25.0	48.0	F	76.0	160.00	29.69	0.0	8	144.0	185.0	11	8	212	250	121	38	3.98
							4.0	8	140.0	180.0	11	10	209	243	118	39	3.66
							8.0	7	136.0	176.0	10	15	206	232	115	41	3.36
							12.0	7	131.0	171.0	10	20	202	236	118	42	3.23
	26.0	43.0	M	86.0	170.00	29.76	0.0	7	134.0	174.0	10	15	191	235	101	39	3.21
							4.0	7	128.0	167.0	9	22	187	228	107	40	2.78
							8.0	7	124.0	163.0	8	26	183	230	105	42	2.51
							12.0	7	120.0	160.0	8	31	182	212	103	43	2.25
	27.0	48.0	M	75.0	178.00	23.67	0.0	7	133.0	173.0	10	15	192	235	118	38	3.18
							4.0	7	131.0	170.0	9	20	184	227	109	39	2.94
							8.0	7	126.0	165.0	9	24	187	218	107	40	2.64
							12.0	7	121.0	159.0	8	29	181	214	104	41	2.36
	28.0	58.0	F	72.0	158.00	28.84	0.0	7	138.0	177.0	10	13	207	206	117	39	3.51
							4.0	7	132.0	172.0	9	18	206	240	115	40	3.06
							8.0	7	126.0	167.0	9	24	203	237	113	42	2.64
							12.0	7	124.0	163.0	8	27	200	234	120	43	2.51
	29.0	59.0	F	78.0	156.00	32.05	0.0	8	140.0	179.0	11	10	206	247	120	37	3.66
							4.0	7	136.0	176.0	10	14	202	240	117	38	3.36
							8.0	7	130.0	171.0	9	20	199	237	115	39	2.92
							12.0	7	127.0	165.0	9	25	198	232	114	41	2.67
	30.0	46.0	M	86.0	159.00	34.02	0.0	7	136.0	176.0	10	14	190	238	110	39	3.36
							4.0	7	132.0	172.0	9	18	184	225	106	41	3.06
							8.0	7	126.0	165.0	9	24	183	210	123	42	2.64
							12.0	7	100.0	161.0	8	29	179	214	113	38	1.95

Group	Serialno	Age	Sex	Weightkg	Heightm	BMI_NEW	Weeks	HbA1C	FBS	PPBS	FastingSeruminsulinµlуml	SerumvitD	LIPIDPROFILE_TC	LIPIDPROFILE_TGL	LIPIDPROFILE_LDL	LIPIDPROFILE_HDL	IR
2.0	31.0	43.0	F	89.0	162.00	33.91	0.0	8	148.0	188.0	12	6	197	242	113	38	4.31
							4.0	8	146.0	186.0	12	6	196	241	111	39	4.15
							8.0	8	144.0	184.0	11	8	195	240	111	40	3.98
							12.0	8	142.0	182.0	11	9	194	238	110	40	3.82
	32.0	37.0	M	86.0	171.00	29.41	0.0	8	146.0	196.0	12	7	195	240	111	39	4.15
							4.0	8	144.0	194.0	11	8	194	239	110	40	3.98
							8.0	8	142.0	192.0	11	9	194	238	109	41	3.82
							12.0	8	140.0	190.0	11	10	193	237	108	42	3.66
	33.0	59.0	F	83.0	158.00	33.25	0.0	7	131.0	171.0	9	20	188	235	109	39	3.04
							4.0	7	129.0	169.0	9	22	187	234	109	39	2.80
							8.0	7	126.0	166.0	9	23	186	233	107	41	2.64
							12.0	7	124.0	154.0	8	25	185	231	107	42	2.51
	34.0	60.0	M	93.0	175.00	30.37	0.0	7	130.0	170.0	9	20	185	229	105	42	2.92
							4.0	7	128.0	168.0	9	22	185	229	105	42	2.78
							8.0	7	125.0	165.0	9	24	184	228	104	42	2.62
							12.0	7	123.0	163.0	8	25	183	228	104	43	2.49
	35.0	54.0	F	86.0	159.00	34.02	0.0	7	132.0	172.0	9	19	187	233	108	39	3.06
							4.0	7	130.0	170.0	9	20	187	232	187	41	2.92
							8.0	7	128.0	168.0	9	22	186	232	107	41	2.78
							12.0	7	125.0	165.0	9	23	184	230	103	42	2.62
	36.0	52.0	M	89.0	176.00	28.73	0.0	7	135.0	176.0	10	16	192	237	109	39	3.23
							4.0	7	133.0	173.0	10	17	190	237	108	39	3.15
							8.0	7	132.0	171.0	9	18	189	235	107	40	3.06
							12.0	0	129.0	169.0	9	19	193	239	110	39	2.90

Group	Serialno	Age	Sex	Weightkg	Heightm	BMI_NEW	Weeks	HbA1C	FBS	PPBS	FastingSerumInsulinµIuml	SerumvitD	LIPIDPROFILE_TC	LIPIDPROFILE_TGL	LIPIDPROFILE_LDL	LIPIDPROFILE_HDL	IR
	37.0	43.0	M	91.0	178.00	28.72	0.0	8	143.0	183.0	11	9	192	238	109	40	3.85
							4.0	8	141.0	181.0	11	10	191	236	109	41	3.76
							8.0	8	139.0	179.0	11	10	190	235	108	41	3.64
							12.0	7	137.0	177.0	10	10	188	233	107	42	3.48
	38.0	48.0	M	79.0	178.00	24.93	0.0	8	141.0	181.0	11	12	196	239	111	38	3.69
							4.0	7	138.0	177.0	10	12	195	239	110	39	3.51
							8.0	7	135.0	175.0	10	13	194	238	110	39	3.33
							12.0	7	133.0	173.0	10	15	193	237	109	39	3.18
	39.0	46.0	M	86.0	169.00	30.11	0.0	7	135.0	175.0	10	16	192	236	107	41	3.23
							4.0	7	133.0	173.0	10	16	192	236	107	41	3.15
							8.0	7	131.0	172.0	9	18	191	235	107	41	3.04
							12.0	7	129.0	169.0	9	18	191	235	106	42	2.90
	40.0	51.0	M	79.0	179.00	24.66	0.0	7	131.0	171.0	9	19	193	234	108	40	2.94
							4.0	7	129.0	169.0	9	20	193	233	107	41	2.87
							8.0	7	127.0	167.0	9	21	192	233	107	41	2.76
							12.0	7	125.0	165.0	9	23	192	233	106	41	2.62
	41.0	43.0	F	69.0	162.00	26.29	0.0	7	144.0	184.0	11	8	195	234	110	39	3.98
							4.0	8	142.0	182.0	11	9	194	234	110	39	3.82
							8.0	8	143.5	184.0	11	10	194	234	109	39	3.76
							12.0	7	142.0	183.0	10	11	193	233	109	40	3.61
	42.0	60.0	M	85.0	174.00	28.08	0.0	7	135.0	175.0	10	16	194	234	111	39	3.23
							4.0	7	133.0	173.0	9	17	193	234	110	40	3.09
							8.0	7	131.0	171.0	9	18	193	233	110	41	3.01
							12.0	7	129.0	169.0	9	19	192	232	108	41	2.90

Group	Serialno	Age	Sex	Weightkg	Heightm	BMI_NEW	Weeks	HbA1C	FBS	PPBS	FastingSerumInsulinµlUml	SerumvitD	LIPIDPROFILE_TC	LIPIDPROFILE_TGL	LIPIDPROFILE_LDL	LIPIDPROFILE_HDL	IR
	43.0	52.0	F	79.0	156.00	32.46	0.0	8	150.0	191.0	12	6	204	249	122	37	4.48
							4.0	7	148.0	188.0	12	6	201	248	120	38	4.31
							8.0	7	146.0	186.0	12	7	198	247	117	39	4.15
							12.0	6	143.0	183.0	11	8	196	246	115	39	3.95
	44.0	57.0	F	77.0	154.00	32.47	0.0	8	151.0	191.0	12	5	204	252	123	37	4.51
							4.0	8	148.0	188.0	12	6	203	251	122	37	4.31
							8.0	8	146.0	186.0	12	7	202	249	122	38	4.15
							12.0	8	145.0	185.0	11	8	201	248	121	39	4.08
	45.0	39.0	M	93.0	174.00	30.72	0.0	8	140.0	180.0	11	11	196	240	110	38	3.66
							4.0	7	137.0	177.0	10	12	196	240	110	39	3.48
							8.0	7	135.0	175.0	10	13	195	238	108	39	3.33
							12.0	7	132.0	173.0	10	15	194	238	109	41	3.16
	46.0	46.0	M	84.0	173.00	28.07	0.0	7	130.0	171.0	10	20	195	239	109	41	3.11
							4.0	7	128.0	168.0	9	21	195	239	110	41	2.78
							8.0	7	125.0	166.0	9	23	194	239	110	41	2.62
							12.0	7	123.0	163.0	8	25	193	239	107	41	2.49
	47.0	59.0	M	79.0	168.00	27.99	0.0	7	133.0	173.0	9	19	192	237	107	39	3.09
							4.0	7	130.0	170.0	9	20	194	238	108	40	2.92
							8.0	7	129.0	168.0	9	21	195	239	110	41	2.80
							12.0	7	127.0	169.0	9	22	195	243	104	41	2.73
	48.0	57.0	F	71.0	156.00	29.17	0.0	8	145.0	185.0	11	9	194	238	107	37	4.01
							4.0	8	143.0	183.0	11	8	194	237	107	38	3.88
							8.0	8	141.0	181.0	11	9	193	237	106	38	3.79
							12.0	8	139.0	179.0	11	10	193	236	106	39	3.64

Group	Serialno	Age	Sex	Weightkg	Heightm	BMI_NEW	Weeks	HbA1C	FBS	PPBS	FastingSerumInsulinµlUml	SerumvitD	LIPIDPROFILE_TC	LIPIDPROFILE_TGL	LIPIDPROFILE_LDL	LIPIDPROFILE_HDL	IR
	49.0	46.0	M	85.0	178.00	26.83	0.0	7	135.0	175.0	10	16	195	239	110	37	3.23
							4.0	7	133.0	173.0	9	17	194	238	109	38	3.09
							8.0	7	131.0	171.0	9	19	194	239	108	39	2.94
							12.0	7	129.0	169.0	9	20	193	239	108	39	2.87
	50.0	52.0	M	89.0	180.00	27.47	0.0	7	133.0	173.0	9	19	193	234	107	40	3.09
							4.0	7	130.0	170.0	9	19	192	234	107	40	3.02
							8.0	7	128.0	168.0	9	21	192	238	106	41	2.78
							12.0	7	126.0	165.0	10	23	191	233	106	41	3.11
	51.0	55.0	M	86.0	175.00	28.08	0.0	7	137.0	177.0	10	11	197	240	111	39	3.35
							4.0	7	135.0	175.0	10	14	195	235	109	39	3.23
							8.0	7	133.0	173.0	9	15	195	238	110	39	3.09
							12.0	7	131.0	171.0	9	17	194	238	128	40	2.97
	52.0	55.0	M	78.0	160.00	30.47	0.0	7	120.0	163.0	8	28	196	236	105	41	2.31
							4.0	7	118.0	160.0	8	30	195	235	104	42	2.21
							8.0	6	117.0	158.0	7	31	193	234	104	42	2.11
							12.0	6	116.0	156.0	7	33	192	233	103	42	2.00
	53.0	48.0	F	57.0	159.00	22.55	0.0	8	145.0	185.0	11	8	198	241	108	38	4.01
							4.0	8	143.0	183.0	11	9	198	239	107	38	3.92
							8.0	8	141.0	181.0	11	9	197	238	107	39	3.79
							12.0	8	139.0	179.0	11	10	196	236	106	40	3.64
	54.0	36.0	M	78.0	177.00	24.90	0.0	7	137.0	179.0	10	13	198	240	111	38	3.48
							4.0	7	135.0	177.0	10	13	197	240	110	39	3.37
							8.0	7	133.0	175.0	11	14	196	239	109	40	3.51
							12.0	7	129.0	172.0	10	15	195	240	109	40	3.09

Group	Serialno	Age	Sex	Weightkg	Heightm	BMI_NEW	Weeks	HbA1C	FBS	PPBS	FastingSerumInsulinµIuml	SerumvitD	LIPIDPROFILE_TC	LIPIDPROFILE_TGL	LIPIDPROFILE_LDL	LIPIDPROFILE_HDL	IR
	55.0	48.0	M	85.0	168.00	30.12	0.0	7	127.0	169.0	9	22	194	240	109	38	2.76
							4.0	7	125.0	177.0	8	23	194	239	109	39	2.50
							8.0	7	123.0	165.0	9	23	193	238	108	40	2.58
							12.0	7	128.0	163.0	8	25	193	237	107	40	2.56
	56.0	40.0	M	96.0	174.00	31.71	0.0	8	147.0	187.0	12	7	200	244	111	36	4.17
							4.0	8	145.0	185.0	11	8	198	243	110	37	4.08
							8.0	8	143.0	183.0	11	8	197	242	112	38	5.30
							12.0	8	141.0	181.0	11	9	196	241	109	39	3.79
	57.0	38.0	M	88.0	175.00	28.73	0.0	8	141.0	181.0	10	10	196	241	111	38	3.62
							4.0	7	143.0	189.0	11	11	196	241	110	39	3.71
							8.0	7	139.0	179.0	10	11	195	240	110	39	3.54
							12.0	7	134.0	175.0	10	13	194	239	109	40	3.31
	58.0	56.0	M	77.0	170.00	26.64	0.0	7	133.0	173.0	9	19	195	240	110	39	3.09
							4.0	7	131.0	171.0	9	19	194	240	110	39	2.94
							8.0	7	128.0	169.0	9	20	193	239	129	40	2.75
							12.0	7	126.0	163.0	8	21	193	239	108	41	2.55
	59.0	48.0	F	83.0	159.00	32.83	0.0	8	127.0	156.0	12	21	200	245	111	35	3.67
							4.0	8	147.0	184.0	11	22	198	247	111	36	4.07
							8.0	8	147.0	187.0	11	23	197	243	111	37	3.92
							12.0	8	139.0	179.0	10	23	197	238	107	38	3.54
	60.0	58.0	F	96.0	172.00	32.45	0.0	7	139.0	179.0	10	24	196	237	107	37	3.54
							4.0	7	137.0	177.0	10	13	196	237	106	38	3.38
							8.0	7	135.0	175.0	10	14	195	237	106	38	3.30
							12.0	7	133.0	172.0	10	15	195	238	107	39	3.18

Group	Serialno	Age	Sex	Weightkg	Heightm	BMI_NEW	Weeks	HbA1C	FBS	PPBS	FastingSerumInsulinµlUml	SerumvitD	LIPIDPROFILE_TC	LIPIDPROFILE_TGL	LIPIDPROFILE_LDL	LIPIDPROFILE_HDL	IR
1.0	61.0	46.0	M	89.0	178.00	28.09	0.0	7	123.0	163.0	8	28	194	237	107	38	2.40
							4.0	7	120.0	160.0	8	30	194	236	106	39	2.25
							8.0	6	1,118.0	158.0	7	32	193	235	105	40	20.15
							12.0	6	115.0	155.0	7	36	195	237	107	40	1.99
	62.0	37.0	M	88.0	172.00	29.75	0.0	7	121.0	161.0	8	30	195	236	106	38	2.27
							4.0	6	118.0	158.0	7	32	194	235	105	39	2.13
							8.0	6	116.0	156.0	7	34	193	234	104	40	2.00
							12.0	6	114.0	154.0	7	36	194	235	105	40	1.89
	63.0	39.0	F	68.0	155.00	28.30	0.0	6	119.0	159.0	7	32	194	234	105	40	2.14
							4.0	6	114.0	156.0	7	34	193	233	104	40	1.97
							8.0	6	114.0	154.0	7	36	191	230	103	41	1.89
							12.0	6	111.0	151.0	6	38	195	237	105	41	1.75
	64.0	37.0	M	85.0	180.00	26.23	0.0	6	117.0	157.0	7	34	193	236	104	42	2.02
							4.0	6	114.0	154.0	7	36	192	234	103	42	1.89
							8.0	6	112.0	152.0	6	41	189	229	101	43	1.77
							12.0	7	109.0	149.0	6	26	195	239	109	38	1.64
	65.0	42.0	M	94.0	176.00	30.35	0.0	7	125.0	165.0	8	28	194	238	108	39	2.53
							4.0	7	122.0	162.0	8	30	193	237	107	40	2.38
							8.0	7	119.0	159.0	8	32	193	236	106	41	2.23
							12.0	6	117.0	157.0	7	30	191	234	104	41	2.11
	66.0	37.0	M	82.0	172.00	27.72	0.0	6	115.0	155.0	7	36	192	234	104	41	1.90
							4.0	6	113.0	153.0	6	38	190	232	103	42	1.79
							8.0	6	110.0	150.0	6	40	189	231	102	42	1.66
							12.0	6	107.0	147.0	6	42	187	230	101	43	1.53

Group	Serialno	Age	Sex	Weightkg	Heightm	BMI_NEW	Weeks	HbA1C	FBS	PPBS	FastingSerumInsulinµIuml	SerumvitD	LIPIDPROFILE_TC	LIPIDPROFILE_TGL	LIPIDPROFILE_LDL	LIPIDPROFILE_HDL	IR
	67.0	46.0	F	74.0	163.00	27.85	0.0	6	113.0	153.0	6	38	190	231	103	41	1.79
							4.0	6	111.0	155.0	6	40	188	230	102	42	1.67
							8.0	6	108.0	148.0	6	42	186	230	101	43	1.55
							12.0	6	105.0	145.0	6	45	185	226	100	44	1.50
	68.0	39.0	M	77.0	176.00	24.86	0.0	7	121.0	161.0	6	30	195	238	108	39	1.64
							4.0	7	119.0	159.0	8	31	194	237	106	40	2.20
							8.0	6	117.0	157.0	7	33	193	236	105	41	2.11
							12.0	6	114.0	154.0	7	36	192	235	104	42	1.89
	69.0	47.0	M	92.0	177.00	29.37	0.0	7	123.0	163.0	8	28	196	240	109	38	2.40
							4.0	7	121.0	161.0	8	29	196	239	108	39	2.33
							8.0	6	118.0	158.0	7	32	193	236	106	41	2.13
							12.0	6	115.0	155.0	9	34	192	234	104	41	2.41
	70.0	42.0	F	76.0	158.00	30.44	0.0	7	127.0	167.0	8	24	198	242	111	37	2.57
							4.0	6	124.0	164.0	8	26	197	241	110	38	2.42
							8.0	7	122.0	162.0	8	28	196	240	109	39	2.29
							12.0	7	120.0	160.0	7	31	194	239	107	39	2.07
	71.0	59.0	M	83.0	164.00	30.86	0.0	6	117.0	157.0	7	34	191	234	104	42	1.99
							4.0	6	115.0	155.0	7	35	190	233	103	42	1.90
							8.0	6	113.0	153.0	6	37	189	232	102	43	1.70
							12.0	6	109.0	149.0	6	40	187	230	101	44	1.56
	72.0	46.0	M	90.0	174.00	29.73	0.0	6	119.0	159.0	7	31	193	236	106	39	2.17
							4.0	6	117.0	157.0	7	33	192	234	106	40	2.02
							8.0	6	115.0	155.0	7	35	191	232	105	41	1.93
							12.0	6	112.0	15.0	6	38	196	238	106	42	1.77

Group	Serialno	Age	Sex	Weightkg	Heightm	BMI_NEW	Weeks	HbA1C	FBS	PPBS	FastingSerumInsulinµlUml	SerumvitD	LIPIDPROFILE_TC	LIPIDPROFILE_TGL	LIPIDPROFILE_LDL	LIPIDPROFILE_HDL	IR
	73.0	57.0	F	82.0	162.00	31.25	0.0	7	123.0	163.0	8	28	195	236	105	40	2.40
							4.0	7	121.0	161.0	8	30	194	235	104	41	2.27
							8.0	7	118.0	158.0	7	32	194	234	103	42	2.13
							12.0	6	116.0	155.0	7	33	193	232	102	42	2.03
	74.0	60.0	F	72.0	158.00	28.84	0.0	6	121.0	161.0	8	30	193	233	104	41	2.27
							4.0	6	118.0	158.0	7	32	192	232	103	42	1.98
							8.0	6	115.0	155.0	7	34	191	231	102	43	1.90
							12.0	6	113.0	153.0	7	35	192	232	103	44	1.81
	75.0	42.0	M	81.0	168.00	28.70	0.0	6	118.0	155.0	7	36	190	230	102	40	1.95
							4.0	6	113.0	153.0	6	38	189	229	101	41	1.79
							8.0	6	110.0	150.0	6	40	187	227	100	41	1.71
							12.0	6	108.0	148.0	6	41	185	225	98	42	1.63
	76.0	39.0	M	82.0	170.00	28.37	0.0	6	110.0	150.0	6	40	188	229	101	44	1.66
							4.0	6	107.0	147.0	6	42	187	228	99	45	1.53
							8.0	6	105.0	145.0	6	45	186	226	98	45	1.40
							12.0	6	103.0	142.0	5	46	185	223	96	46	1.29
	77.0	58.0	F	78.0	160.00	30.47	0.0	7	127.0	167.0	9	24	197	240	110	37	2.67
							4.0	7	125.0	165.0	8	26	196	238	108	38	2.53
							8.0	6	122.0	162.0	8	29	195	236	107	39	2.29
							12.0	6	120.0	159.0	7	31	193	233	105	40	2.19
	78.0	59.0	F	80.0	157.00	32.46	0.0	7	126.0	166.0	9	24	197	241	111	37	2.64
							4.0	7	124.0	164.0	8	26	196	239	109	38	2.51
							8.0	7	121.0	161.0	8	28	195	237	107	39	2.36
							12.0	7	120.0	160.0	8	29	194	234	104	40	2.25

Group	Serialno	Age	Sex	Weightkg	Heightm	BMI_NEW	Weeks	HbA1C	FBS	PPBS	FastingSeruml nsulinµIuml	SerumvitD	LIPIDPROFILE_TC	LIPIDPROFILE_TGL	LIPIDPROFILE_LDL	LIPIDPROFILE_HDL	IR
	79.0	52.0	M	82.0	169.00	28.71	0.0	6	118.0	168.0	7	32	193	233	103	41	2.13
							4.0	6	116.0	156.0	7	34	192	232	102	42	2.00
							8.0	6	113.0	153.0	7	36	191	231	103	43	1.87
							12.0	6	110.0	151.0	7	38	190	229	101	43	1.77
	80.0	46.0	M	79.0	164.00	29.37	0.0	7	127.0	167.0	9	24	196	237	108	38	2.67
							4.0	7	124.0	164.0	8	26	194	236	106	40	2.51
							8.0	7	122.0	162.0	8	28	192	235	105	41	2.38
							12.0	7	119.0	159.0	7	30	190	233	103	42	2.12
	81.0	56.0	M	82.0	162.00	31.25	0.0	7	120.0	160.0	8	30	191	234	103	41	2.28
							4.0	7	118.0	158.0	7	32	190	233	102	42	2.13
							8.0	6	116.0	156.0	7	34	189	231	101	42	2.00
							12.0	6	113.0	153.0	7	36	190	228	101	43	1.87
	82.0	38.0	F	66.0	154.00	27.83	0.0	6	119.0	159.0	7	32	189	232	100	41	2.14
							4.0	6	117.0	157.0	7	34	188	231	99	42	2.02
							8.0	6	116.0	154.0	7	36	187	230	99	43	1.92
							12.0	6	112.0	152.0	6	38	185	227	98	43	1.77
	83.0	60.0	M	89.0	171.00	30.44	0.0	6	123.0	163.0	8	28	193	235	105	44	2.40
							4.0	7	120.0	160.0	8	30	190	234	104	45	2.25
							8.0	6	118.0	158.0	7	32	189	234	103	45	2.13
							12.0	6	115.0	155.0	7	34	187	230	99	46	1.99
	84.0	52.0	F	77.0	163.00	28.98	0.0	6	114.0	154.0	7	36	186	228	98	38	1.89
							4.0	6	112.0	158.0	6	38	185	227	98	38	1.77
							8.0	6	110.0	147.0	6	40	184	226	97	39	1.66
							12.0	6	107.0	143.0	6	42	181	220	96	40	1.53

Group	Serialno	Age	Sex	Weightkg	Heightm	BMI_NEW	Weeks	HbA1C	FBS	PPBS	FastingSerumInsulinµlUml	SerumvitD	LIPIDPROFILE_TC	LIPIDPROFILE_TGL	LIPIDPROFILE_LDL	LIPIDPROFILE_HDL	IR
	85.0	48.0	M	86.0	166.00	31.21	0.0	6	116.0	154.0	7	34	188	230	100	42	2.00
							4.0	6	114.0	152.0	8	36	186	229	98	43	2.17
							8.0	6	112.0	149.0	6	38	184	228	97	44	1.77
							12.0	6	109.0	145.0	6	40	180	226	96	45	1.64
	86.0	47.0	F	71.0	159.00	28.08	0.0	7	125.0	162.0	8	26	196	237	107	39	2.53
							4.0	7	122.0	160.0	8	28	195	235	106	40	2.38
							8.0	7	120.0	158.0	8	30	193	234	105	41	2.25
							12.0	6	118.0	153.0	7	33	186	229	98	42	2.13
	87.0	46.0	M	78.0	166.00	28.31	0.0	6	113.0	150.0	6	38	185	227	97	40	1.79
							4.0	6	110.0	146.0	6	40	183	226	96	40	1.66
							8.0	6	108.0	143.0	6	42	181	224	95	41	1.55
							12.0	6	106.0	140.0	6	44	179	220	93	42	1.44
	88.0	57.0	F	69.0	159.00	27.29	0.0	6	117.0	157.0	7	34	188	231	101	41	2.02
							4.0	6	115.0	155.0	7	36	187	230	100	42	2.20
							8.0	6	113.0	153.0	6	38	186	228	99	42	1.79
							12.0	6	110.0	150.0	6	41	184	224	97	42	1.66
	89.0	54.0	M	76.0	161.00	29.32	0.0	6	114.0	154.0	7	36	186	229	100	42	1.89
							4.0	6	112.0	152.0	6	38	184	227	99	43	1.77
							8.0	6	110.0	150.0	6	40	183	225	98	44	1.66
							12.0	6	107.0	147.0	6	42	180	221	96	44	1.53
	90.0	59.0	F	77.0	155.00	32.05	0.0	7	128.0	168.0	9	22	198	243	111	41	2.78
							4.0	7	126.0	166.0	9	24	197	239	109	41	2.64
							8.0	7	124.0	164.0	8	26	196	238	108	42	2.51
							12.0	7	121.0	161.0	8	28	194	236	106	42	2.36

CASE SHEET

Registration no: **Date:**

Name: **Age:** **Sex:**

Address:

Occupation: **Marital Status:** **Weight (Kg):**

Height (Cm): **BMI:**

Complaints:

H/o Present illness:

Past History

Personal History:

Family History:

Obstetric History:

General Examination:

Systemic examination:

Investigation:

1. Plasma glycosylated hemoglobin (HbA1C)
2. Fasting Blood Sugar
3. Post Prandial blood Sugar
4. Serum insulin
5. Serum 25(OH)D
6. Serum Calcium
7. Blood Urea
8. Serum Creatinine
9. Lipid profile - Total cholesterol, Triglycerides, High density lipoprotein, Low density lipoprotein
10. Liver function tests - SGOT,SGPT
11. Urine albumin
    Sugar
    Deposits

Treatment:

Signature of the Investigator

CONSENT FORM

(To be obtained from subject)

Introduction:

You are requested to participate in a study conducted in **department of Pharmacology**, Chennai Medical College Hospital & Research Centre, Irungalur, Tiruchirapalli, Tamil Nadu entitled.

"A study on the prevalence of Vitamin D deficiency and the effect of Vitamin D on the Glycemic control and lipid profile in type II Diabetes Mellitus patients in a Rural Tertiary Care Hospital."

Your participation in this study is Voluntary. You at liberty to participate/ withdraw from the study. Please read this consent form carefully and ask the Consultant, any questions you may have about the study before signing.

Explanation of Procedure:

If you agree to participate in this study, we will ask some question to you and collect relevant information/ Blood sample. You may be examined by the investigator, data from the study will be used for research purposes only. The results of the study will not to be given to you directly. There will be no cost to you participating in this study.

Potential benefits:

Your participation will help us to know the risk factor of this problem and results of this study will be beneficial for future generations.

Assurance of Confidentiality:

The information concerning your participation in the study will be kept confidential to the full extent permitted by law and used only for scientific purposes. No one except members of the research team will have access the results. Your name will not be disclosed in any report or released in any way.

Patient Consent:

I have read the explanation about this study and have been given an opportunity to discuss it and to ask questions. I have not received any money for participating in this study.

Signature of Subject Signature of Witness Date

Signature of Researcher

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