

## Study of Vitamin D Deficiency in Patients with Diffuse Body Pains

Authors

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### *Abstract:*

*Vitamin D deficiency continues to be worldwide threat, especially in developing countries. It is very much prevalent in the community. It is well known that Vitamin D deficiency causing Rickets in children and Osteomalacia in Adults. With the change in our life style and confining ourselves more to indoors the Vitamin D deficiency is again coming into the lime light . In the present study we have estimated various parameters like serum of vitamin D, calcium, phosphorus, alkaline-phosphatase and parathyroid hormone in normal healthy human volunteers as well as diffuse body pain patients (DBP). About 459 patients, 150 patients were identified as vitamin d deficiency with diffuse body pains and among them 101 were females is enrolled in the study.*

## INTRODUCTION

Vitamins are grouped into two categories i.e. fat soluble vitamins and water soluble vitamins. Fat soluble vitamins are stored in the body's fatty tissue. Water soluble vitamins must be used by the body right away (National Institute of Health – Vitamins 2005). Vitamin D is a fat-soluble

vitamin that is naturally present in very few foods, added to others, and available as a dietary supplement (Dietary supplement, National Institute of Health, 2008). Five forms of vitamin D have been discovered, vitamin D<sub>1</sub>, D<sub>2</sub>, D<sub>3</sub>, D<sub>4</sub>, D<sub>5</sub>. The two major forms of Vitamin-D that seem

to matter to humans the most are vitamins D<sub>2</sub> (ergocalciferol) and D<sub>3</sub> (cholecalciferol) which are collectively known as Calciferol (Nutrition/Diet;Public Health, 2009). Vitamin D is converted in the liver and kidneys to form an active chemical that actually functions as a hormone (StewartB.leavitt, 2008).

## ROLE OF VITAMIN D

Major role of vitamin D is to increase the flow of calcium into the blood stream, by promoting absorption of calcium and phosphorus from food in the intestines, and reabsorption of calcium in the kidneys; enabling normal mineralization of bone and preventing hypocalcemic tetany (Merck, Vitamin D 2007). Vitamin D is involved in a wide range of cellular activities, including differentiation, inhibition of cell growth, immunomodulation and control of other hormonal systems (Holick MF, Vitamin D deficiency 2007). It is also necessary for bone growth and bone remodelling by osteoblast and osteoclast (Dietary supplement, National Institute of Health, 2008). Vitamin D is also beneficial as it reduces inflammation and strengthens the immune system, thereby reducing the risk of rheumatoid arthritis (R.A) in women. It regulates normal cell differentiation & proliferation E.g: Prevention of Cancer. It promotes Insulin sensitivity & blood sugar relation (insulin secretion) (Admin, 2009).

### Vitamin D Deficiency

Vitamin D Deficiency (VDD) is very much prevalent and it has become a major health

problem in the community. It is well-known that Vitamin D deficiency causing Rickets in children and Osteomalacia in adults. With the change in our life style and confining ourselves more to indoors the Vitamin D deficiency is again coming into the lime light . **25-Hydroxy vitamin D [25(OH)D] Concentrations**

Deficient - < 20 ng/ml

Insufficient - 20 - 29 ng/ml

Optimal Range - 30 - 100 ng/ml

Potentially toxic - > 150 ng/ml

A Vitamin D deficiency can occur when usual intake is lower than recommended levels over time, exposure to sunlight is limited, the kidneys cannot convert vitamin D to its active form or absorption of vitamin D from the digestive tract is inadequate (Biser-R,et.al.,2001).

### Vitamin D Relationship with Pain

Vitamin D deficiency is wide spread and seems to be related to many health concerns like osteoporosis, depression, heart disease and stroke, cancer, diabetes, parathyroid problems, immune function, even weight loss specifically musculoskeletal pain. The pain may be felt in muscles, bone, joints, back, neck, shoulders, hips or knees. The process that links vitamin D to musculoskeletal pain is presumed to begin with a lack of circulating calcium (hypocalcemia) due to inadequate vitamin D as vitamin D

## Vitamin D and Health

- **Vitamin D and Cardio vascular system:**

People with Vitamin D lesser than 15ng/ml have a two fold increase risk of cardiovascular events like atherosclerosis with intima media thickness, Coronary artery disease (CAD), Myocardial Infarction, stroke and Congestive cardiac failure (CCF). Vitamin D is said to have a role in regulation of hypertension, vascular calcification and CAD. Vitamin D receptor has a broad tissue distribution that includes vascular smooth muscle cells, endothelial cells and cardiomyocytes. Deficiency of vitamin D leads to tonic up-regulation of renin-angiotensin aldosterone system with development of essential hypertension and left ventricular hypertrophy.

- **Vitamin D and Autoimmunity:** Vitamin D supplementation decreases the incidence and severity of autoimmune disease. The active vitamin D metabolite Calcitriol is an important modulator of the immune system. Calcitriol is able to suppress pro-inflammatory cytokines to enhance anti-inflammatory cytokines (Armin Zittermann, 2003). Vitamin D modulates the production of several neutrophins, upregulated IL-4 an inhibit differentiation and survival of dendritic cells resulting in impaired allo-reactive T cell activation. Vitamin D regulates T

helper cells Th<sub>1</sub> and dendritic cell function while inducing regulatory T-cell function.(Oliver Gillie, 2004).

- **Vitamin D and Reproduction:**

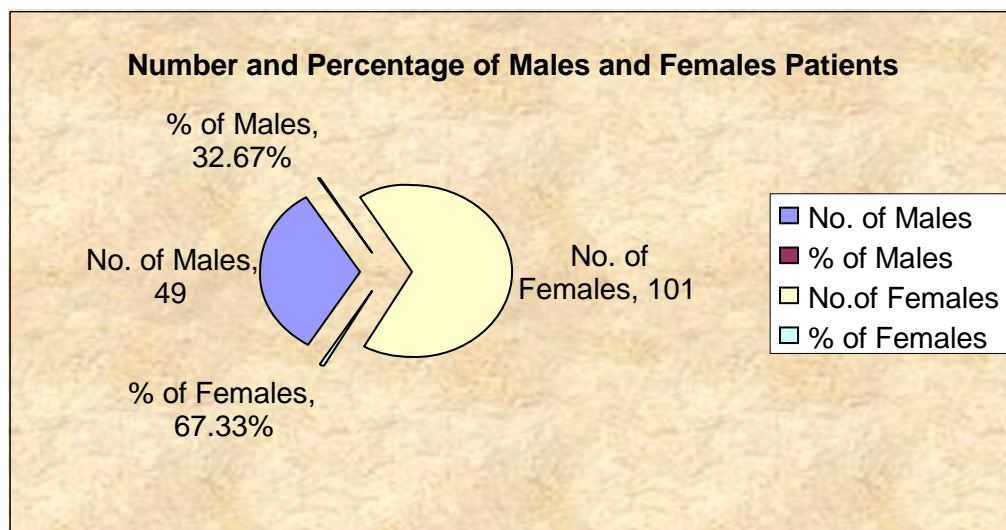
Experimental, clinical and epidemiological evidences suggests that the involvement of calcitriol in reproductive processes including onset of puberty, fertility, pregnancy, lactation and sexual behaviour. Nuclear receptors for calcitriol are seen in uterus, oviduct, ovary, mammary glands. Placenta and foetal membrane. Cooperative action between calcitriol and other steroid hormone especially estradiol has been noted.

- **Vitamin D and Cancer:** Laboratory and animal evidence as well as epidemiological studies suggests that Vitamin D status could affect cancer risk (Davis CD, et.al.,2007). Vitamin D protects against many cancer forms or at least improves their prognosis (Armin Zittermann, 2003). Vitamin D and its metabolites reduce the incidence of many types of cancer by inhibiting tumor angiogenesis, stimulating mutual adherence of cells, and enhancing intercellular communication through gap junctions, thereby strengthening the inhibition of proliferation that results from tight physical contact with adjacent cells within a tissue (contact inhibition).

Vitamin D metabolites help maintain a normal calcium gradient in the colon epithelial crypts, and high serum levels of vitamin D are associated with markedly decreased proliferation of non-cancerous but high-risk epithelial cells in the colon. Calcitriol inhibits mitosis of breast epithelial cells. Pulsatile release of ionized calcium from intracellular stores, including the endoplasmic reticulum, induces terminal differentiation and apoptosis and calcitriol enhances calcium release. (Van den Berg H, 1997)

- **Vitamin D and Brain:** Epidemiological evidence shows association, between reduced sun exposure and mental illness

and remarkable improvement in its symptoms due to Vitamin D supplementation. Brain is the first organ which detects falling levels of vitamin D, which starts as fatigue and diffuse body pain progresses to psychomotor retardation and anhedonia. VDD can cause low mood, poor cognition, personality disorder, post traumatic stress disorder, bipolar disorder, seasonal affective disorder, non-specific mood disorder, major depressive disorder, premenstrual syndrome, parkinson’s disease and even schizophrenia. Low levels of vitamin D by decreasing calcium and magnesium levels can cause seizures.



• **Fig.1.1: Number and Percentage of Male and Female Patients**

**Total no. of patients N = 15**

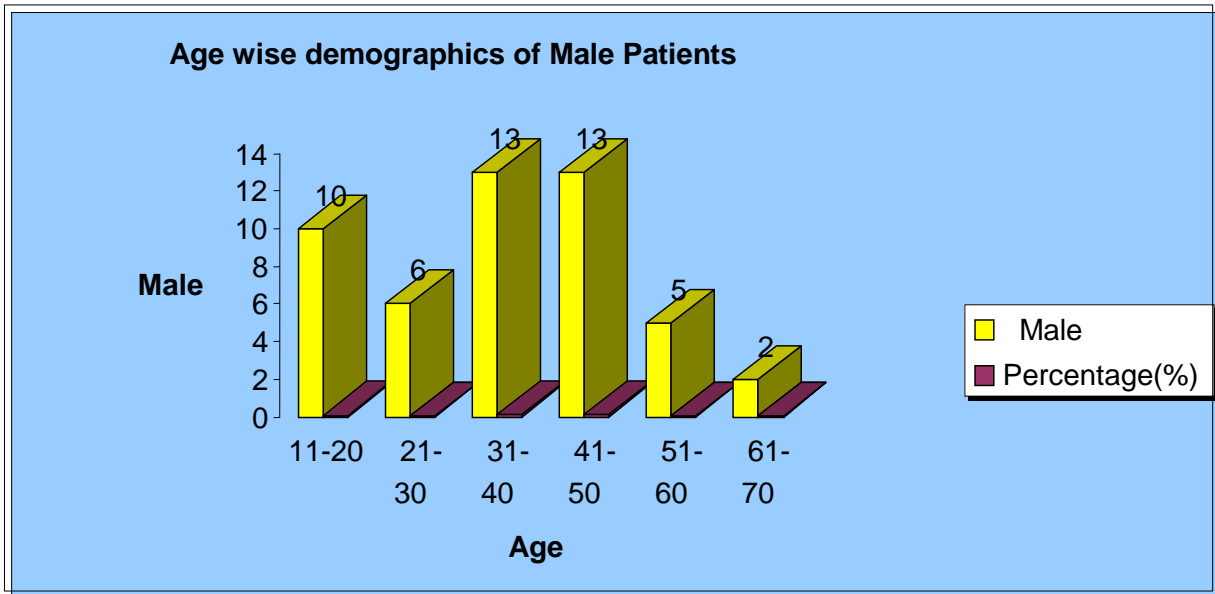


Figure 1.2: Age wise Demographics of Male Patients.

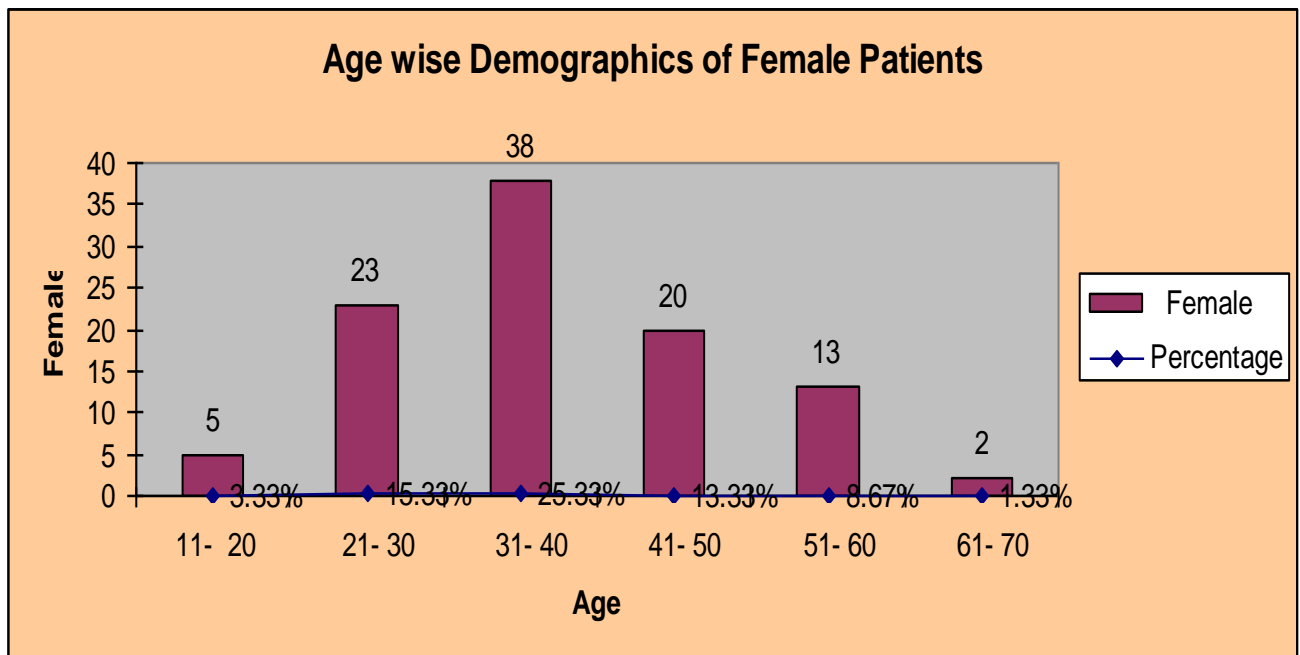


Figure 1.3: Age wise Demographics of Female Patients.

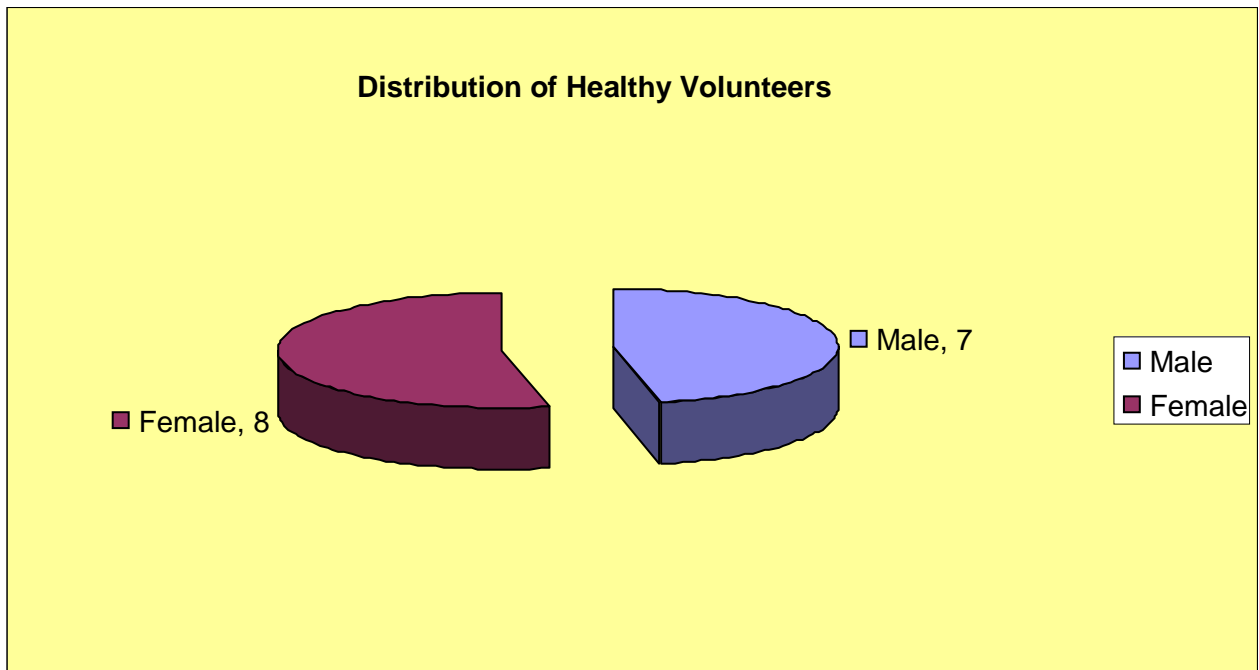


Fig. 1.4: Distribution of Healthy Volunteers

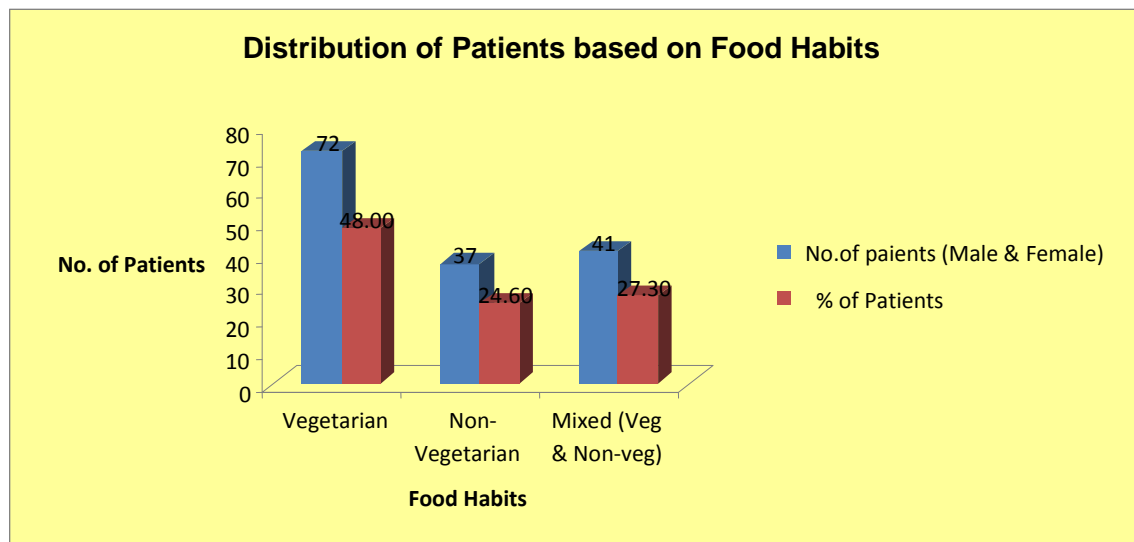


Fig 1.5: Distribution of Patients based on their Food Habits.

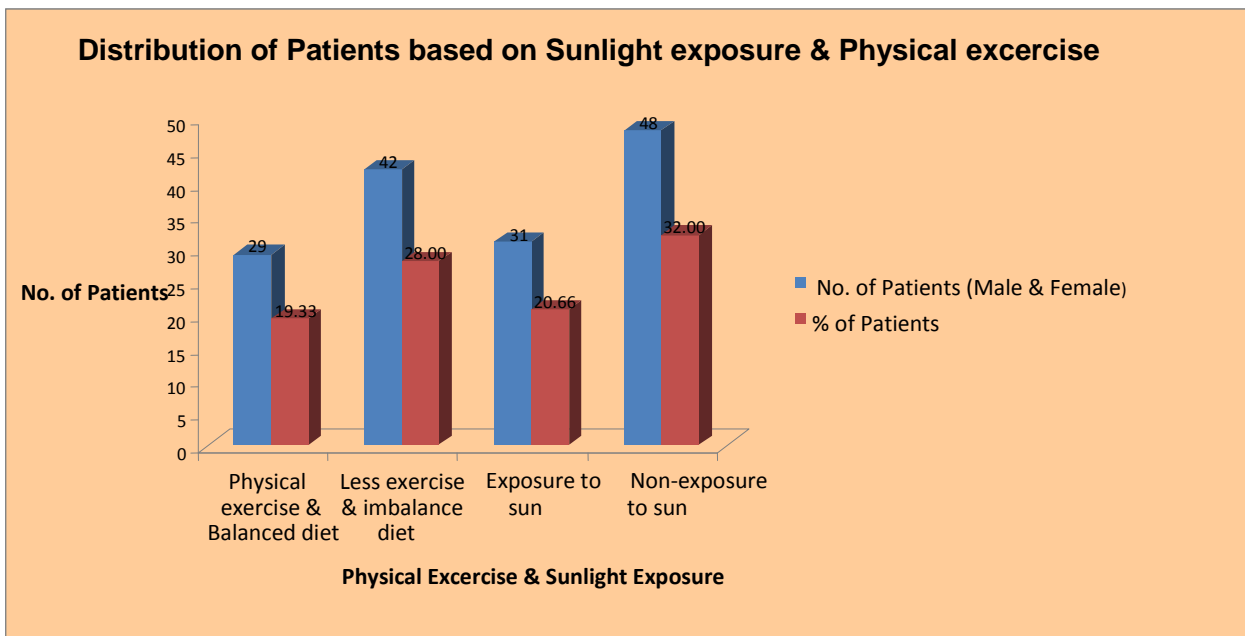


Fig 1.6: Distribution of Patients based on exposure to sunlight and physical-exercise

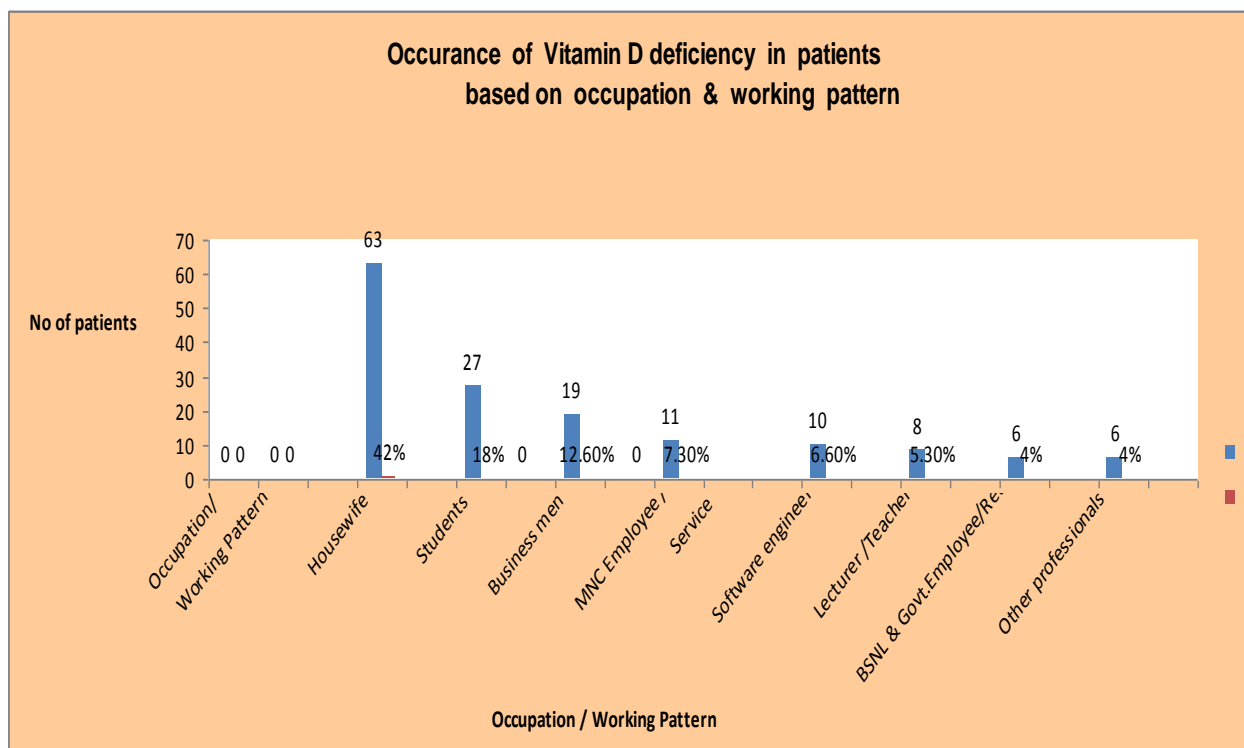
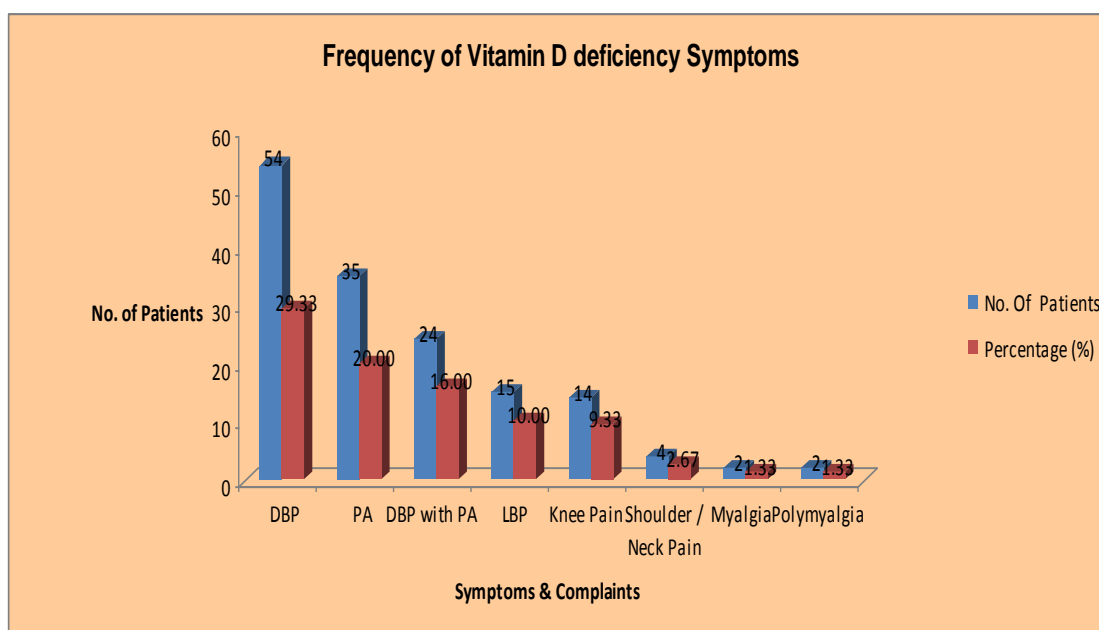


Fig. 1.7: Occurrence of Vitamin D deficiency in patients based on occupation & working pattern.



**Fig. 1.8: Frequency of Vitamin D deficiency symptoms**

## AIM

- The aim of the study is to know the true prevalence of vitamin D deficiency presenting as a diffuse body pains in Rheumatology Out-Patient's Department (OPD).
- To study the clinical symptoms and signs of vitamin D deficiency and their correlation with 25-hydroxy vitamin D [25(OH)D] levels.

## OBJECTIVE

- The primary objective is to study and assess the vitamin D levels in the patients suffering from diffused body pains with lack of vitamin D.

- The secondary objective is to monitor the treatment regimen at different visits and analyze the response of patients.

## STUDY DESIGN

Around 459 patients have been investigated, among them 150 patients were found with Vitamin D deficiency. The study conducted over the period of 9 months i.e. from October 2009 – July 2010. About 15 subjects were taken as control. The study was conducted in the department of Rheumatology with the consent of Head of the Department and consultant Rheumatologist, Department of Rheumatology, Krishna Institute of Medical Science (KIMS).



## INCLUSION CRITERIA

- All the patients irrespective of age and sex attending the Rheumatology Out-patient department in KIMS Hospital with complaints of diffuse body pains are taken into consideration.
- Estimation of serum calcium , phosphorus , Alkaline- phosphatase and 25(OH)D levels are checked in all the patients and healthy volunteers (Control).

## EXCLUSION CRITERIA

- Patients other than Rheumatology department.
- Patients who are hospitalized that is IN-PATEINTS.
- Patients with Inflammatory arthritis like Rheumatoid Arthritis and Systemic lupus erythromatus (SLE).

## METHODS

### Sample Collection

Around 459 patients have been investigated , among them 150 patients were found with Vitamin D deficiency. Samples were collected from patients from 1<sup>st</sup> visit. A patient proforma was prepared to record relevant details (appendix I, Proforma of patient profile with vitamin-D deficiency) such as age, sex, patient medication history, food habits, physical exercise and symptoms. A total of 15 healthy volunteers were taken as control in the study. Similar proforma for

these healthy subjects was prepared and recorded. About 10 ml of blood sample was collected from patients into clean dry test tube from ante-cubital vein labelled and preserved and later analysed for various parameters (serum levels of Vitamin D, calcium , phosphorus, alkaline-phosphatase, PTH) using different instruments and methods. The tests were performed in the hospital clinical laboratory using kits provided by the commercial manufacturer. Vitros chemistry Products slides for Calcium, phosphorus and alkaline phosphatase were used to estimate the serum levels of Ca, PO<sub>4</sub>, & ALP. Roche Cobas – Model No. e411 was used to estimate serum PTH and vitamin D levels.

### Estimation of Vitamin D<sub>3</sub> (25-OH)

#### Reagents

M - Streptavidin-coated microparticles (transparent cap ), 1 bottle, 6.5ml : Streptavidin-coated microparticles 0.72mg/mL; Preservative .

R1 – Reaction buffer (gray cap), 1 bottle, 8mL: Acetate buffer approx. 220mmol/L, pH 3.9; albumin(human) 2.g/l; preservative

R2 - Anti-25-OH vitamin D<sub>3</sub>-Ab ~Ru(bpy)<sup>2+3</sup> ( black cap); 25-OH vitamin D derivative ~ biotin (black cap), 1 bottle, 9ml: Monoclonal anti-25-OH vitamin D<sub>3</sub> antibody (mouse) labeled with ruthenium complex 1.5 mg/L; biotinylated 25-OH vitamin D 0.15mg/l; phosphate buffer 20mmol/L, pH 6.5; preservative

## Principle and Procedure

The Elecsys Vitamin D<sub>3</sub> (25-OH) assay is used as an aid in the assessment of Vitamin D<sub>3</sub> sufficiency in adults for the determination of 25-hydroxy vitamin D<sub>3</sub> in human serum & plasma. The electrochemiluminescence immunoassay “ECLIA” is intended for use on Elecsys and cobas e immunoassay analyzers.

The Test principle involved is Competition Principle. Total duration of assay is 18 minutes.

**First Incubation:** 25-OH vitamin D<sub>3</sub> in the sample (35µL) competes with the biotin labeled vitamin D in the complex contained in R2 (biotin-vitamin d & monoclonal 25-OH vitamin D<sub>3</sub>-specific Rutenium labeled antibody). The remaining amount of complex (biotin-vitamin d & monoclonal 25-OH vitamin D<sub>3</sub>-specific Rutenium labeled antibody) is dependent upon the analyte concentration in the sample.

**Second Incubation:** After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. Results are determined via a calibration curve which is instrument-specifically

generated by a 2-point calibration and a master curve provided via the reagent barcode.

## Estimation of calcium

**Reagents:** Arsenazo III dye, buffer, pH 5.6, Vitros Chemistry Products Calibrator Kit 1 on Vitros Chemistry Systems, Vitros Ca slides-multilayered, analytical element coated on polyester support.

## Principle and Procedure

When a drop of sample (10µL) is deposited and spread evenly on calcium slide the bound calcium is dissociated from binding proteins. This dissociated calcium forms a complex at pH 5.6 with Arsenazo III dye, results in shift in the absorption maximum. After 5 min incubation at 37°C, the reflection density of the colored complex is measured using UV spectrophotometer at 680nm. The amount of colored complex formed is proportional to the calcium concentration in the sample.

## Estimation of Phosphorus

### Reagents:

- p-Methylaminophenol, ammonium molybdate, buffer, pH 4.5, Vitros Chemistry Products Calibrator Kit 1 on Vitros Chemistry systems.
- Vitros Phosphorus slide-multilayered, analytical element coated on polyester support.

**Principle**

The analysis is based on the reaction of inorganic phosphate with ammonium molybdate to form an ammonium phosphomolybdate complex at acidic pH (4.2),

p-Methylaminophenol sulfate an organic reducing agent, reduces the complex to form stable heteropolymolybdenum blue chromophore.

**Procedure:**

A drop of sample (10 $\mu$ L) is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers and incubated for 5 minutes at 37°C. Phosphorus in the specimen forms complex with ammonium molybdate. This complex is reduced by p-Methylaminophenol sulfate to give a blue complex. The concentration of phosphorus in the sample is determined by measuring the heteropolymolybdenum blue complex by reflectance spectrophotometry at 670nm.

**Estimation of Alkaline-phosphatase****Reagents**

- Vitros alkaline-phosphatase slide and Vitros Chemistry Product Calibrator Kit 3 on Vitros chemistry systems.
- Vitros Alkaline-phosphatase slide - multilayered, analytical element coated on polyester support.

**Principle and Procedure**

A drop (11 $\mu$ L) of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers and incubated for 5 minutes at 37°C. The spreading layer contains the p-nitrophenyl substrate and other components required for the reaction. The Alkaline-phosphatase in the sample catalyzes the hydrolysis of the p-nitrophenyl phosphate to p-nitrophenol at alkaline pH. The p-nitrophenol diffuses into the underlying layer and it is monitored by reflectance spectrophotometry at 400nm. The rate of change in reflection density is converted to enzyme activity.

**Estimation of Parathyroid hormone (PTH):Reagents –working solutions**

M - Streptavidin-coated microparticles (transparent cap), 1bottle,6.5Ml: Streptavidin-coated microparticles 0.72mg/mL; Preservative .

R1 - Anti-PTH-Ab-biotin (gray cap), 1 bottle, 7mL: Biotinylated monoclonal anti-PTH antibody (mouse) 2.3mg/Ml; Phosphate buffer 100mmol/L, pH 7.0 ; preservative .

R2 - Anti-PTH-Ab ~Ru(bpy)<sup>2+3</sup> ( black cap), 1 bottle, 7ml: Monoclonal anti-PTH antibody (mouse) labeled with ruthenium complex 2.0mg/mL; phosphate buffer 100mmol/L, pH 7.0; preservative.

**Principle and Procedure**

The Elecsys PTH assay can be used intraoperatively for quantitative determination of

intact parathyroid hormone in human serum and plasma for the differential diagnosis of hypercalcemia and hypocalcemia. The electrochemiluminescence immunoassay “ECLIA” is intended for use on Elecsys and cobas e immunoassay analyzers.

The Elecsys assay for determining intact PTH employs a sandwich test principle in which a biotinylated monoclonal antibody reacts with the N-terminal fragment(1-37) and a monoclonal antibody labeled with a ruthenium complex reacts with the C-terminal fragment ( 38 -84 ). The antibodies used in this assay are reactive with epitopes in the amino acid regions 26-32 and 37-42.The Test principle involved a sandwich principle; total duration of assay is 18 minutes.

**First Incubation:** 50µL of sample, a biotinylated monoclonal PTH –specific antibody and monoclonal PTH-specific antibody labeled with a ruthenium complex form a sandwich complex.

**Second Incubation:** After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with Procell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.Results are determined via a calibration curve which is instrument-specifically

generated by 2-point calibration and a master curve provided via the reagent barcode.

## PLAN OF WORK

It is evident from the data presented in the introduction that the incidence of vitamin D deficiency is much prevalent in the community. The present work has been described under the following sections.

- 1) Estimation of serum 25(OH) D levels.
- 2) Estimation of serum calcium.
- 3) Estimation of serum phosphorus.
- 4) Estimation of serum alkaline-phosphatase.
- 5) Estimation of serum parathyroid hormone (PTH).
- 6) Monitoring vitamin D levels in patients with proper drug therapy during dosage regimen.
- 7) Comparative analysis of vitamin D levels of serum calcium, phosphorus, alkaline phosphatase and parathyroid hormone of patients suffering with diffuse body pains with that of normal healthy human volunteers.

## RESULTS

The present investigations were carried out to study and monitor the vitamin D for the assessment and therapy in diffused body pain patients. In the present study we have estimated various parameters like serum of vitamin D, calcium, phosphorus, alkaline-phosphatase and parathyroid hormone in normal healthy human

volunteers as well as diffuse body pain patients (DBP). About 459 patients, 150 patients were identified as vitamin d deficiency with diffuse body pains and among them 101 were females (Table & Fig. 1.4).

The patients were between the age group of 13 to 70 years. The higher percentage of males were in the age group of 31-50 years (17.34%) and higher percentage of females were in the age group of 31-40 years (25.33%).(Table & Fig.1.4.1&1.4.2).About 15 healthy volunteers were selected, among them 46.6% (n=7) were males and 53.3% (n=8) were females. The prevalence of low 25 (OH) D levels in such patients was showed in Table No. 1.4.6 The frequency of various Vitamin D deficiency symptoms is tabulated in Table & Fig .1.4.7 shows graphical representation of the same.

Diffused body pain was present in over 54 cases (36 %). The major cases were of DBP, PA & DBP with PA with 36 %, 23.33 % & 16 % respectively. Taking the normal range of Vitamin D level as 30-100ng /ml the patients were classified into 2 grades. The grades were deficient (< 20 ng/ml) and insufficient (20-29 ng/ml) levels of 25(OH)D. The distribution of severity of 25(OH)D level amongst the patients is shown in Table and Fig.6.1 Vitamin D levels in the patients were monitored before and after administration of vitamin D supplements at different stages like first, second and third visit and compared the levels with normal healthy human volunteers.

It was observed that serum 25(OH) D levels were increased on treatment. This could be observed from the increased levels of 25(OH) D in 1<sup>st</sup> and 2<sup>nd</sup> visit shown in Table no.6.2 The significance of drug therapy could be revealed from statistical data of vitamin D levels. There is significant rise ( $P<0.0001$ ,  $P<0.05$ ) in mean and standard deviation of vitamin D in patients on subsequent visits in diffused body pain patients and it is compared with the normal ie. control group shown in Table and Fig 6.3. The serum levels of vitamin D before treatment was found to be  $10.18 \pm 6.26$ ng/ml and after the treatment with vitamin D supplements it has been increased to  $24.41 \pm 7.5$ ng/ml in 1st visit and  $33.23 \pm 7.52$ ng/ml on 2<sup>nd</sup> visit (Table and Fig.6.3). Similarly, Calcium Phosphorous, Alkaline phosphatase and PTH were estimated in patients. The normal value of Calcium is 8.4 -10.2mg/dl. The level of Ca in normal (control group) is in the mean and standard deviation of  $9.42 \pm 0.42$ mg/dl before treatment the level was  $7.35 \pm 2.51$ mg/dl and after treatment the level was improved to  $9.30 \pm 0.69$ mg/dl and  $9.67 \pm 0.45$ mg/dl ( $P<0.0001$ ,  $P<0.05$ ) (Table and Fig.6.4). The normal value of Phosphorus is 2.5-4.5mg/dl. The level of Phosphorus in normal (control group) is in the mean and standard deviation of  $4.14 \pm 0.308$  before treatment it was  $3.59 \pm 0.74$ mg/dl and after treatment the level was improved to  $3.71 \pm 0.49$ mg/dl and  $4.39 \pm 5.13$ mg/dl ( $P<0.0001$ ,  $P<0.05$ ) (Table and Fig.6.5)

The normal value of Alkaline phosphatase is 39-117U/L in adults and 117.0-390.0U/L in children.

The level of ALP in normal (control group) is in the mean and standard deviation of  $89.58 \pm 10.66$  U/L and before treatment it was  $77.34 \pm 26.38$  U/L and after treatment the level was increased to  $81.75 \pm 18.88$  U/L and  $84.27 \pm 17.77$  U/L ( $P < 0.05$ ) (Table and Fig.6.6) The normal value of Parathyroid hormone is 10-65ng/ml. The level of PTH in normal (control group) is in the mean and standard deviation of  $60.32 \pm 3.72$  ng/ml and before treatment it was found to be  $61.80 \pm 22.48$  ng/ml and after treatment the level was

$50.06 \pm 11.62$  ng/ml and  $47.04 \pm 9.26$  ng/ml ( $P < 0.0001$ ,  $P < 0.05$ ) (Table and Fig.6.7). Comparative study of mean and Standard deviation of bone profile of serum levels of control and standard indicated significant rise ( $P < 0.0001$ ,  $P < 0.05$ ) in Vitamin D, calcium, phosphorus, levels of standard when compared to constant values of control (Table & Fig 6.8).

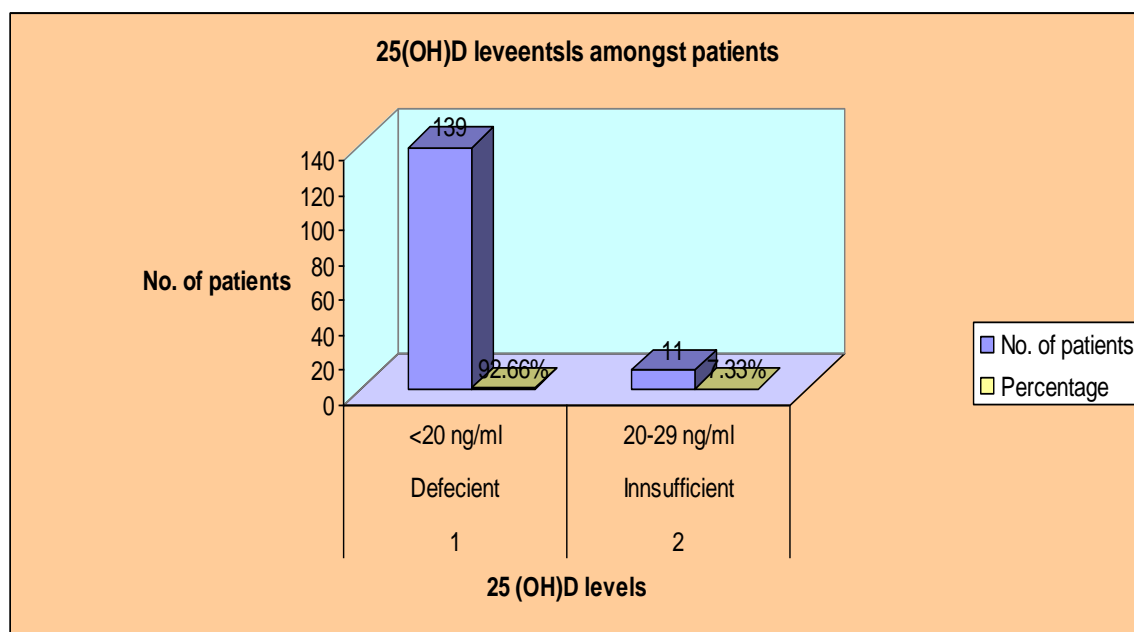
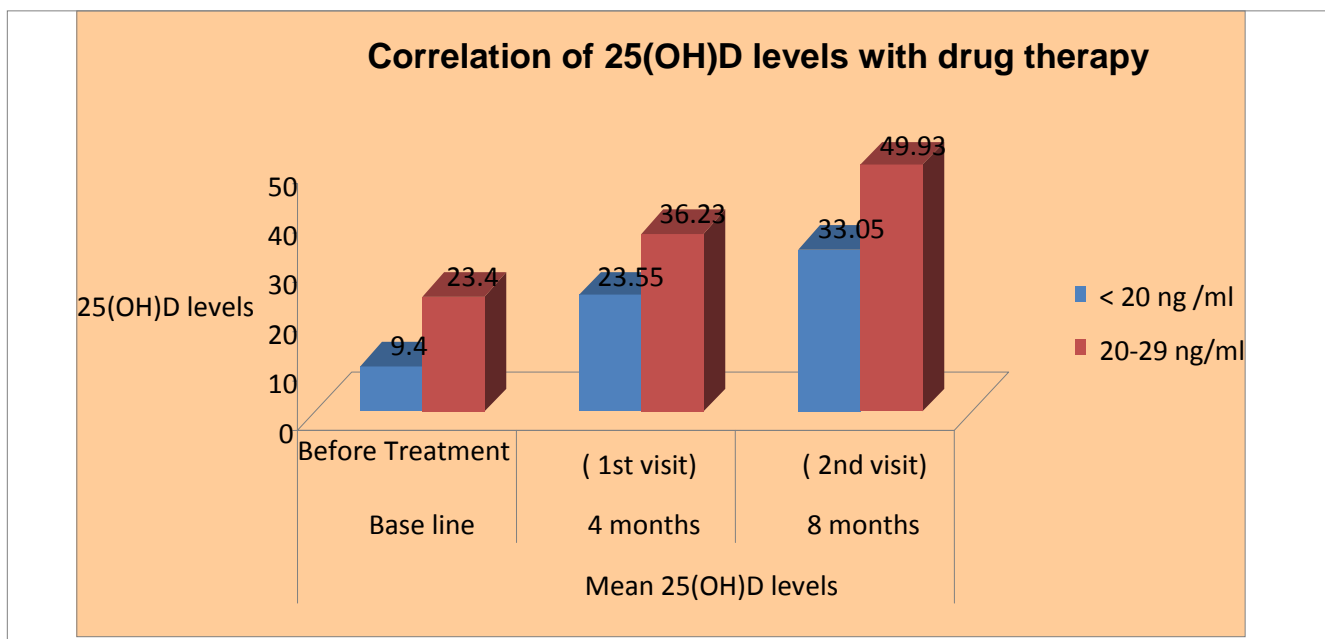
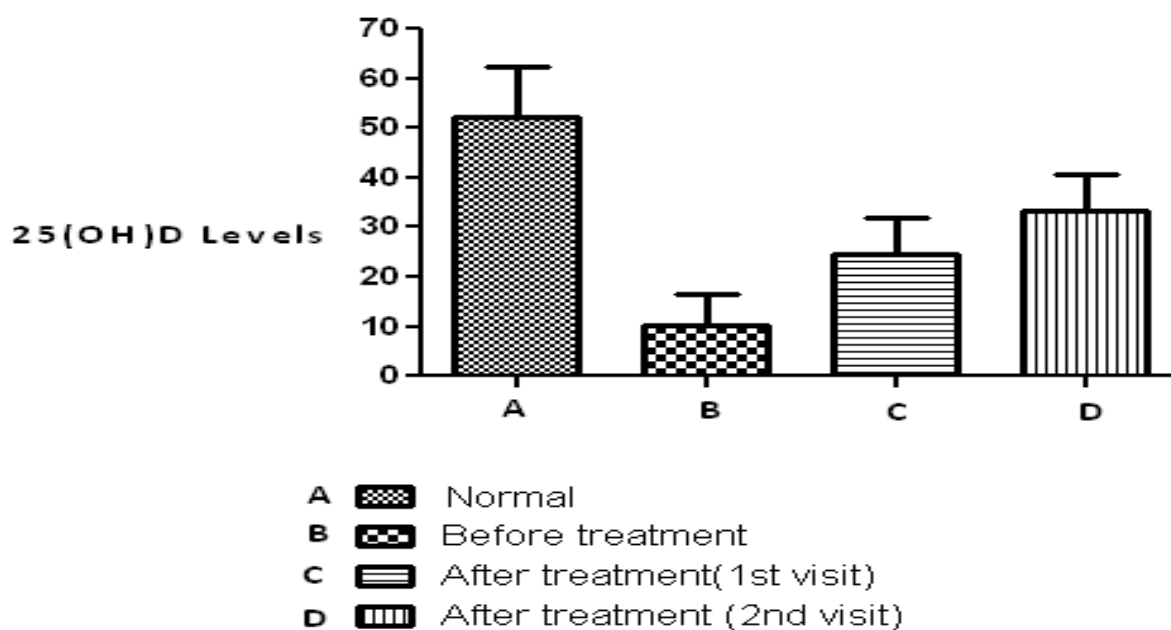


Figure 6.1 : 25(OH)D levels amongst Patients



**Fig 6.2: Correlation of 25(OH) D levels with drug therapy**

There was a significant rise ( $P < 0.0001$ ) in vitamin D levels on drug therapy, indicating that the patients showed the positive response to the treatment given to them.



**Fig 6.3 : Serum Levels of 25(OH)D in Control and Standard group**

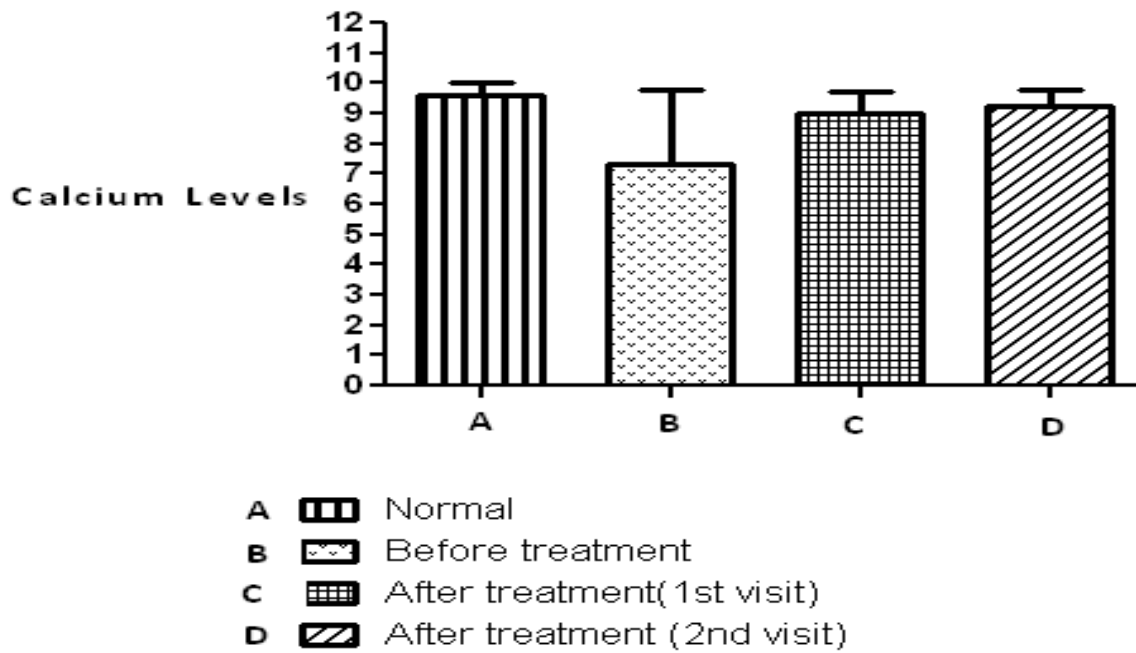


Fig 6.4 : Serum Levels of Calcium in Control and Standard group

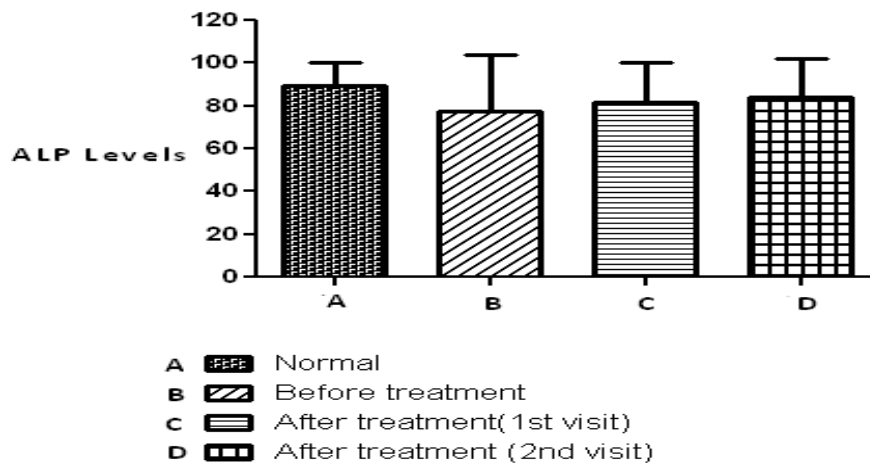


Fig6.6:Serum Levels of Alkaline Phosphatase (ALP) in Control and Standard group



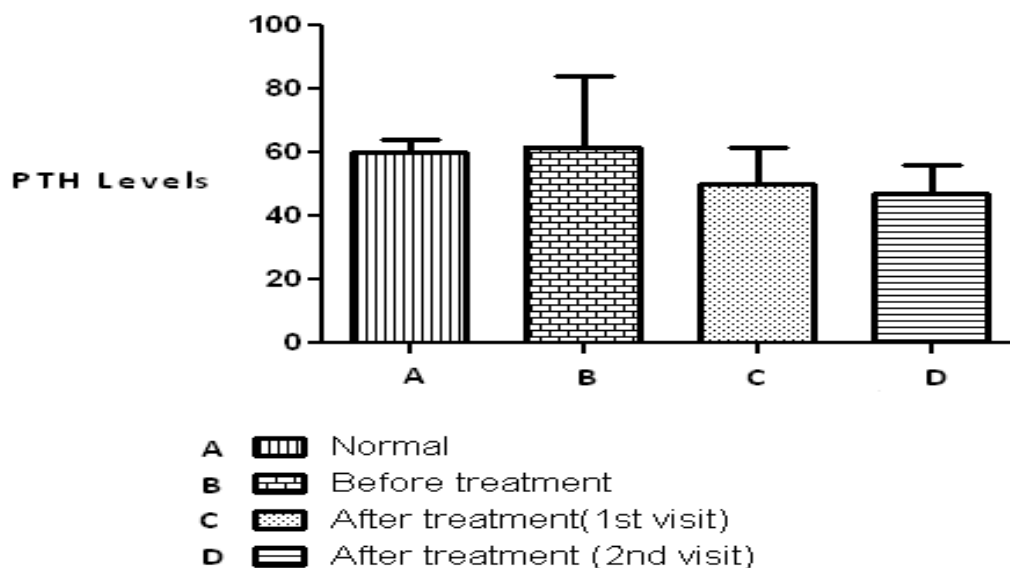


Fig 6.7: Serum Levels of PTH in Control and Standard group

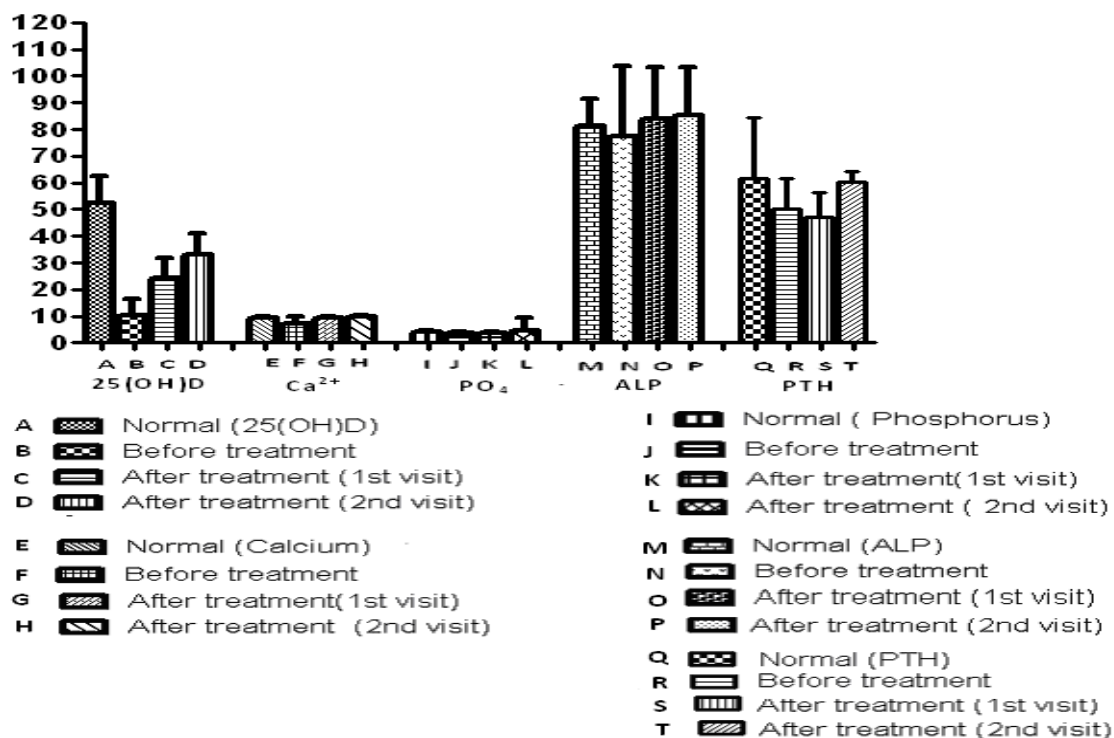


Fig 6.8 : Comparative study of Mean and Standard deviation of Bone profile of Serum levels of Control and Standard

**SUMMARY AND CONCLUSION:**

The present study conducted on 150 patients of all the social classes. This showed that Vitamin D deficiency is very common in developing countries. Majority of the cases had the complaints of diffuse body pains, polyarthralgia. Based on the results obtained of 25(OH)D levels following conclusions can be drawn from this study. Vitamin D deficiency is observed in all sections of society irrespective of age, sex and socio-economic status. It affects individual in the prime of their life. Preponderance of female patients compare to male patients suffering from Vitamin D deficiency possibly reflects social factors like avoidance of milk and milk products and staying indoors. Studying and working in Air conditioner environment for long hours which affects vitamin D synthesis in body there by depleting Vitamin D levels slowly. Thus to summarize that various factors such as age, sex, working pattern, lifestyle, diet are major factors that affects vitamin D levels and alters serum levels of calcium, phosphorus, alkaline-phosphatase and parathyroid hormone resulting in diffused body pains associated with various symptoms. In our investigations we have observed that the supplementation of vitamin D therapy for about 4-8 months showed improved levels of Vitamin D, thereby not only significantly reducing symptoms like diffused body pains but also improved serum levels of calcium, phosphorus, alkaline-phosphatase. Lastly to conclude health awareness is important and active intervention is

required in the form of a National policy for vitamin D food fortification programme in our country as diffused body pain patient's in our study were from all sections of society.

**ACKNOWLEDGEMENT**

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