EFFECT OF SERUM LEVELS OF FIBROBLAST GROWTH

FACTOR (FGF-23) AND INSULIN LIKE GROWTH FACTOR (IGF-1) ON BONE METABOLISM IN PRE-DIABETES AND TYPE 2 DIABETES PATIENTS

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in

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By

ABHILASHA SUWALKA

Registration Number: 11919675

Supervised By

Dr. Pranav Kumar Prabhakar

Professor

Department of Medical Laboratory Sciences

Lovely Professional University, Phagwara, Punjab

Co-Supervised by

Dr. Balram Sharma

Professor

Department of Endocrinology

SMS Medical College and Attached Hospital, Jaipur, Raj

LOVELY PROFESSIONAL UNIVERSITY, PUNJAB

2023

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DECLARATION

I, hereby declared that the presented work in the thesis entitled **Effect of serum levels of Fibroblast Growth Factor (FGF-23) and Insulin Like Growth Factor (IGF-1) on Bone Metabolism in Pre Diabetes and Type 2 Diabetes Patients** in fulfilment of degree of **Doctor of Philosophy (Ph. D.)** is outcome of research work carried out by me under the supervision **Dr. Pranav Kumar Prabhakar,** working as Professor and Head, in the Department of Medical Laboratory Sciences, School of Allied Medical Sciences, of Lovely Professional University, Punjab, India. And the co supervision of **Dr. Balram Sharma,** working as Professor in the Department of Endocrinology, of SMS Medical College & Attached hospital, Jaipur, Rajasthan. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.

(Signature of Scholar)

Name of the scholar: Abhilasha Suwalka

Registration No.: 11919675

Department of Medical Laboratory Sciences, School of Allied Medical Sciences,

Lovely Professional University,

Punjab, India

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CERTIFICATE

This is to certify that the work reported in the Ph. D. thesis entitled "**Effect of serum levels of Fibroblast Growth Factor (FGF-23) and Insulin Like Growth Factor (IGF-1) on Bone Metabolism in Pre Diabetes and Type 2 Diabetes Patients"** submitted in fulfillment of the requirement for the reward of degree of **Doctor of Philosophy (Ph.D.)** in the Clinical Biochemistry, is a research work carried out by Abhilasha Suwalka (11919675) is bonafide record of her original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.

(Dr. Pranav Kumar Prabhakar)

Professor

Department of Medical Laboratory Sciences,

Lovely Professional University, Phagwara, Punjab

ABSTRACT

A Chronic metabolic disease Type 2 Diabetes Mellitus (T2DM), which began with the insulin resistance phenomenon, is characterized by persistently increased blood sugar levels. It has an impact on the majority of people on the planet and will likely get worse in the near future. It is a long-term metabolic illness that starts with insulin resistance and progresses to altered tissue cells that do not react to insulin as they should. Numerous studies have given evidence that an increase in levels of glucose significantly alters bone production since glucose is a sort of nutrition for osteoblast cells. Peptides produced by the bone's osteoblast cells make up the hormone known as FGF-23, which circulates in the blood. Fibroblast Growth Factor-23 (FGF-23) is secreted by the bone's (osteoblast) cells because it shows a variety of physiological functions connected to bone metabolism. FGF-23 can exhibit physiological activity associated with bone metabolic processes such as hematopoiesis, calcification and Insulin signaling interaction, bone metabolism, and bone remodeling in bone cells are all impacted directly or indirectly by hyperglycemia. So, keeping in mind the various aspects of diabetes mellitus and pre-diabetes condition, the present study is designed to assess altered bone metabolism in FGF-23 and IGF-1 and other bone metabolic markers concentration in patients having pre-diabetes and diabetes. The present study is a cross-sectional comparative study where 75 pre-diabetic, 75 diabetic and 75 healthy non-diabetic individuals were recruited after the permission from Ethics Committee of SMS Medical College and Attached Hospital, Jaipur then drawned samples for assessment and associations of metabolic markers were evaluated in all the individuals with using chemiluminescence method. It was observed that the FGF-23 levels were found to be elevated in the diabetic group contrarily; the IGF-1 level in the blood samples was having the lowest IGF-1 levels in the blood. Thus, it can be concluded that the FGF-23 and IGF-1 are important bone metabolic diagnostic markers in determining the bone metabolism in Type 2 Diabetes Mellitus individuals.

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List of Abbreviations

Abbreviations	Full form
AGEP	Advance Glycation End Products
T2DM	Type 2 Diabetes Mellitus
FGF223	Fibroblast Growth Factor-23
HDL	High Density Lipids
LDL	Low Density Lipids
GDM	Gestational Diabetes Mellitus
IGF-1	Insulin Like Growth Factor-1
MODY	Maturity Onset Diabetes of the Young
BMD	Bone Mass Density
FRAX	Fracture Risk Assessment Tool
VDD	Viatamin D Deficiency
CDD	Cleidocranial Dysplasia
GH	Growth Hormone
IGFBP3	IGF Binding Protien 3
ILA	Insuline Like Activity
OC	Osteocalcin
BALP	Bone Specific Alkaline Phosphatase
P1NP	Procalcitonin Type 1 N Terminal Propeptide
CTX	C Terminal Propeptide
BMI	Body Mass Index
PTH	Para Thyroid Hormone
ALP	Alkaline Phosphatase
IGT	Impairment Glucose Tolerance
ADA	Americal Diabetes Association
BTM	Bone Turnover Marker
DXA	Dual Energy X-Ray Absorptiometry
BTM	Bone Turnover Marker
FPG	Fasting Plasma Glucose
IFG	Impaired Fasting Glucose
IGT	Impaired Glucose Tolerance
OGTT	Oral Glucose Tolerance Test

CHAPTER 1
INTRODUCTION

INTRODUCTION

Approximately 415 million individuals worldwide suffer from Type 2 Diabetes Mellitus (T2DM) as per the latest statistics, it has been estimated that its prevalence will adversely increase to 640 million people by 2040 and also hypothesized the rise in cases to 72.96 million among adults in India [1]. T2DM, more commonly referred to as diabetes, is a chronic condition that develops when the body is ineffectual to produce enough of the hormone insulin or unable to utilize it effectively, leading to an increase in blood sugar levels [2]. The human body's pancreas secretes insulin, a crucial hormone that carries glucose by the blood to the body cells, where it is transform into to energy. As the disease progresses, cells fail to respond to insulin properly and this insulin absence, or the incompetence of body cells to for the insulin response, causes higher amount of blood glucose the in body, or the hyperglycemia that is diabetes characteristic. When left uncontrolled for long time, it causes various health complications like hyperglycemia can damage a variety of body organs and can be life-threatening, including neuropathy, cardiovascular disease, nephropathy, retinopathy and disorders of eye that can cause blindness. On the contrary, proper diabetes management can delay or prevent these serious complications.

Over the years, there have been several discussions, arguments, and updates regarding the classification and detection of diabetes. However, as of recent years, diabetes mellitus is categorized into three main categories: Type 1 Diabetes (T1D), T2D, and gestational diabetes (GDM).

With advanced search over the topic, it was concluded that there are few usual variations of diabetes, such as diabetes of monogenic and secondary diabetes. Monogenic diabetes occurs due to a single mutation of gene in an autosomal dominant gene, in spite of various contributing factors of multiple genes and factors affecting environment, as is the case with diabetes of type 1 and type 2. Neonatal diabetes and (MODY) maturity-onset diabetes of the

young are the two conditions that fall within the category of monogenic diabetes. One to five percent of all diabetes patients have monogenic diabetes as the cause [3, 4, 5, 6, 7, and 8]. Secondary diabetes can result from the side effects of medicine, hormonal imbalance (such as Cushing's disease or acromegaly), pancreatic problems (such pancreatitis), or other conditions (e.g., corticosteroids). Diabetes Indicators: - [9,10]

Diagnosis of Diabetes:- If any one of the indication prevails	Impaired glucose tolerance (IGT):- When both the indication are met	Impaired fasting glucose (IFG): If both criteria are satisfied
≥7. 0 mmol/L (126mg/dL)- Plasma Fasting glucose	<7. 0 mmol/L (126 mg/dL)- Fasting plasma glucose	6. 1-6. 9 mmol/L (110 to 125 mg/dL)- Fasting plasma glucose
≥11. 1 mmol/L (200mg/dL) after 75 g oral glucose administration- Two hour plasma glucose	≥7. 8 <11. 1 mmol/L (≥140 to <200 mg/dL) after 75 g oral glucose administration- 2-hour plasma glucose	<7. 8 mmol/L (140 mg/dL) after 75 g oral glucose administration- 2-hour glucose for plasma
>11. 1 mmol/L (200 mg/dL) or HbA1c ≥ 48 mmol/mol- Random Glucose		

The cause of this disruptive process is not fully understood, but it has been identified that it involves a fusion of environmental as well as genetic susceptibility such as viral infections, some dietary factors and toxins [11]. The onset of a disease occurs at any age, although type 1 diabetes is most prevalent in children and adolescents. Type 1 diabetics must inject themselves with insulin every day to maintain healthy blood sugar levels; without this supplement, they would struggle to survive.

People suffering with T1D can better survive a healthy life and can delay many diabetes-related problems with adequate and controllable daily insulin therapy, routine blood glucose monitoring, and an acceptable healthy diet.

Detection of T1D is done when there is an increase in glucose level in blood which leads to various symptoms like dry mouth and abnormal thirst, very frequent urination, energy deficient (malaise), constant hunger, unexpected weight reduction, sickness, and blurred vision. Sometimes, Diagnosis of diabetes types is difficult and may need additional tests to differentiate among types of diabetes [12]. The occurrences of T1D are globally increasing, but it varies widely from one country to another country, where some parts of the world show much greater rates than others [11]. The reason for this is unknown, but the interaction of environmental as well as genetic factors is assumed [13].

90% of those with diabetes have T2D, which is the most common type. Diabetes is reported to be more common than ever, rising from 4% in 1995 to 5.4% in 2025, while the prevalence of the disease in adolescents is found to have been 4.0% in 1995 and is projected to rise to 5.4% in 2025. The evaluation for the percent increase in disease was done among various countries by researchers and it was hypothesized that major increase in cases will be in developing countries rather than developed countries [14, 15, 16, and 17]. The present approximation of occurrence of diabetes in India is 11.8 percent (as per the govt. Survey 2020) having broad regional alterations in relation to rural or urban dwellings. Urban populations in the country are 2-3 times more numerous than rural populations. In the metropolitan areas, it was discovered that poor socioeconomic groups had a higher chance of developing diabetes than their wealthy counterparts. A higher range of variations was in pre-diabetic patients in various states and cases for fasting glucose was higher in comparison to people suffering from reduced glucose tolerance. Diabetes in urban and rural areas was independent of several factors like sex, obesity, age, hereditary and hypertension. With coming times, it can take a face of for epidemic in India if not treated properly [18-22].

Osteoporosis and diabetes with Type 2 are known to be the common diseases highly related to an aging population. Due to the association with varied morbidity and mortality in societies, the disease causes a higher risk of health burden among people [23-25].

Although it has been seen that people with T2D have higher bone mineral density (BMD), there is growing indication linking to this condition to an elevated risk of fractures [26-28]. Rotterdam study on people with T2D shown that greater BMD in neck and lumbar spine, these individuals despite that they had a greater (69%) risk of fractures than without diabetes. Combining data from three larger observational prospective studies yielded the finding that patients with T2D have an elevated risk of femoral neck BMD T score fractures than without the disease [27, 29]. The Fracture Risk Assessment Tool (FRAX) of the World Health Organization has recently been illustrated to underestimate the risk of osteoporosis fractures in diabetes individuals. Diabetes is therefore seen as a risk factor for involvement in subsequent FRAX repetitions. These findings may explain changes other than BMD with the greater fracture risk seen in diabetic patients, and BMD assessments are in reality a true tendency for T2D individuals to acquire bone fragility. It implies that it is not reflective. In a recently published study comparing BMD in people with and without T2D, higher HbA1c was linked to higher BMD in all types of type 2 diabetic groups [30].

The pervasiveness in diabetes mellitus Type 2 has intensified due to the growth in epidemics of obesity, chiefly because of the modern changes in lifestyle. Prediabetes is a component of metabolic disorder with IFG and IGT that exists between normal glucose homeostasis and diabetes. Patients with pre-diabetes are expected to develop type 2 diabetes later on, while those with poorly controlled T2D mellitus are more likely to experience diabetic complications. These complications can cause bone health, such as osteoporosis and vitamin D deficiency (VDD), as well as macro vascular diseases, retinopathy, nephropathy, and

neuropathy. Recent research has shown that another prominent consequence of diabetes is an increased risk of fragility fractures [31].

Biochemical markers causing bone turnover when found in measured increased levels in serum are related with bone fragility and loss. Further more, hyperglycemia and Advanced Glycation End Products (AGEP) are conditions to cause osteocyte death and cortical bone deterioration. The major nutrition for osteoblasts is known to be glucose at the highest levels. When glucose levels are high, several in vitro experiments were conducted and showed significant alterations of bone formation. These modifications can cause low turnover of bones in pre diabetic and type 2 diabetic mellitus [32, 33].

Talking about the nature of osteoblast cells in bone metabolism, it’s been studied that; there is a Runx2 which is a trnscription factor and is a major indicator for differentiation of osteoblast [34, 35]. The production of this transcriptional factor in developing osteoblasts promotes osteoblast differentiation, its insufficiency results in cleidocranial dysplasia (CDD), also known as skeletal dysplasia, and its deactivation hinders osteoblast differentiation. CDD is categorized as hampering the differentiation of osteoblasts causing open fontanelles and hypoplastic clavicles. However, some features of Runx2 biology in not well known. For example, less knowledge was gathered about the character of the molecular events that cause Runx2 to accumulate in lineage cells of the osteoblast. The other findings which remain misunderstood are whether and how Runx2 supplies its role for formation of bone by differentiating osteoblasts. Certain features of osteoblast biology are going to raise this issue lately.

Type 1 collagen has been discovered to be the significant protein present in the extracellular matrix (ECM) of bone, and the production of this protein by osteoblast cells is frequently viewed as a biomarker in bone development. It is a heterotrimeric protein made up of 2 α1 (I) chains and 1 α 2 (I) chain that is encoded by two distinct genes [36]. When in vitro,

Runx2 has a capability to get bound to the collagen α1 (I) promoter and up regulate its activity [37]. However, in vivo, Runx2 first appears in osteoblasts before type 1 collagen is produced. As a result, the modulation of type 1 collagen production in osteoblasts is not fully known, and since type 1 collagen makes up the majority of the bone extracellular matrix, it is still unclear how osteoblasts control the creation of bones. Additionally, being involved in bone formation, osteoblasts also act as endocrine cells that secrete osteocalcin, a hormone that promotes glucose homeostasis [38]. The determination of bone as a controller of glucose metabolism is the determination of glucose function in osteoblasts, regardless of the molecular intricacy of this new regulation.

Diabetes-related hyperglycemia and hyperinsulinemia have been shown to impact bone remodeling in diabetic patients both directly and indirectly through the insulin receptors found on every bone cell. This interaction between insulin signaling and bone metabolism was demonstrated in several studies, where higher insulin signaling increases bone turnover while insulin resistance reduces remodeling of the bone. Although the term "insulin resistance of bone" is well understood and is associated with decreased bone metabolism, the association between pre-diabetes and risk of fracture is still uncertain [39-43].

Diabetes can affect the bone turnover process through biochemical markers. The 32 kDa glycoprotein known as FGF-23 is primarily produced in bone under normal conditions by osteoblast and osteocyte cells. The hormone FGF-23 was identified as bone-derived hormone that inhibits the kidney's ability to synthesize vitamin D hormone and reabsorb phosphate. It is widely known that people with healthy renal function experience phosphate wasting when serum level of intact fibroblast growth factor 23 is elevated. The primary effect of FGF-23 on mineral metabolism proved its role as a hormone and result of the hormone's decreased effect on the reabsorption of phosphate from urine [44-69].

Insulin-like growth factor, which is essentially formed in the liver in response to growth hormone (GH) stimulation, is another important anabolic bone regulator. It has been demonstrated that when IGF-binding protein 3 (IGFBP 3) is present in systemic circulation, osteoblast cells are typically bound to it. IGF-I exhibits nearly 50% amino acid sequence identity with insulin and implicitly exhibits the same hypoglycemia reaction. Numerous research has shown how IGF-I affects insulin sensitivity and its relation to T2D [70-75].

Physiological Functions of FGF in Diabetes

FGF-23 levels are closely related to diabetes. Increased FGF-23 has also been connected to obesity and insulin resistance. Unexpectedly, current investigations have identified the signaling for insulin-dependence on the synthesis of FGF-23. By stimulating the PI3K/PKB/Akt/FOXO 1 pathway, insulin and IGF-1 inhibit FGF-23 synthesis [76-78].

Metabolic functions of FGF-23 physiologically in bones

In the human skeleton, FGF-23 plays crucial physiological functions in hematopoiesis and the mineralization of bones. Additionally, it enacts crucial role information of bones and mineral imbalance. FGF-23 is a peptide hormone secreted through osteoblasts, or cells that produce bones into bloodstream. It has three primary physiological effects. First, it causes phosphaturia by decreasing phosphate reabsorption at the proximal tubules. Second, it decreases the activation and increases the breakdown of 25-hydroxyvitamin D (25(OH)), which lowers the levels of 1,25-dihydroxyvitamin D in the blood. Third, FGF-23 inhibits the parathyroid hormone (PTH) gene's transcription and PTH release phenomenon [47-55, 62, 63, 79].

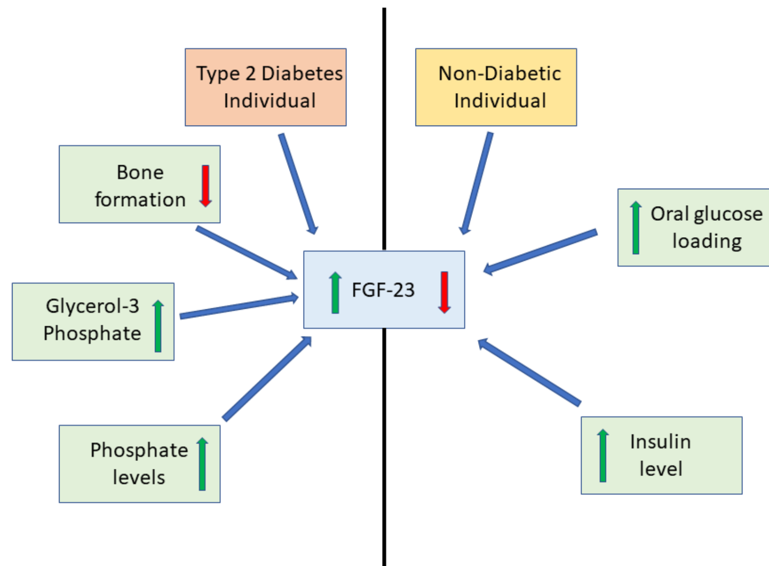


Figure 1. Functions of FGF- 23 in Diabetes

FGF-23 can play role as a physiological suppressor when hematopoietic (Blood) stem cells differentiate into bone microenvironment by the erythroid lineage. A significant amount of work has been intended on the pathophysiological effects of increased fibroblast growth factor-23 because the importance of disorders correlated with gain in FGF-23 function, such as phosphate-wasting diseases and chronic renal disease. In the distal renal tubules of the kidney, it is also a key activator of calcium and salt reabsorption. FGF23 is also beginning to have a function in the alkaline phosphatase expression and bone mineralization. The FGF-23 promotes alkaline phosphatase reduction in bone and Klotho-independent, in contrast to FGF-23 renal effects [50-54, 80-85].

Physiological Functions of IGF- 1 in Diabetes

IGF-I is also involved in controlling lipid and glucose metabolism. Early 1960s in vitro research found that not all the insulin-like activity (ILA) found in human serum is dependent on insulin. At the time, a large portion of ILA that could not be inhibited against insulin by targeted antibodies was attributed to a recently discovered molecule that was subsequently

given the designation IGF-I. According to laboratory evidence, IGF1 exhibits effects similar to that of insulin on the peripheral absorption of glucose and fatty acids. IGF-I shares structural similarities with insulin as well as downstream signaling pathways [86,88]. Exogenous administration of the recombinant human (IGF-I) improves insulin sensitivity in both T2D and healthy persons. IGF-I and its binding proteins have been linked to varying levels of insulin resistance states, and obesity, a major risk factor for T2D [89].

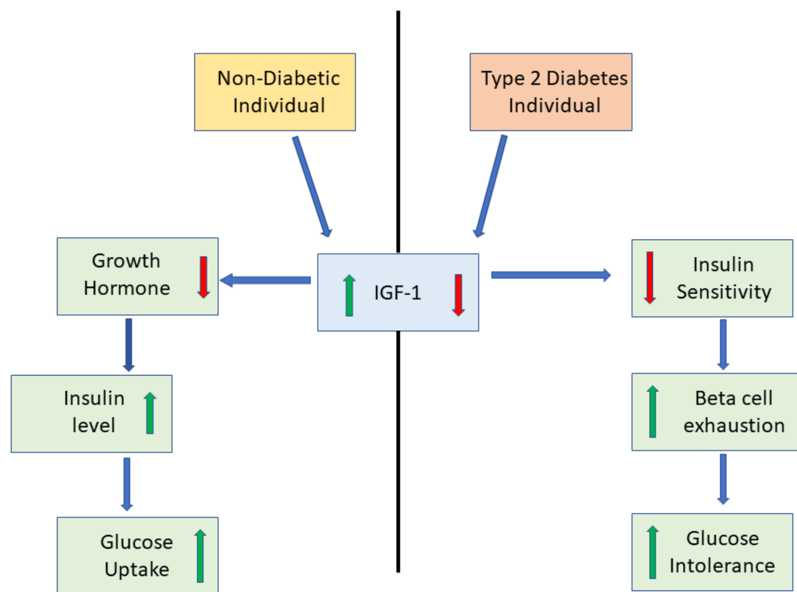


Figure 2. Functions of IGF- 1 in Diabetes

Physiological Functions of IGF- 1 in Bone Metabolism

IGF-1 hormone is a key mediator in bone formation, differentiation, cell growth survival, and progression of cell cycle. IGF-1 stimulates tissue, and appropriate acquisition of peak bone mass at age six requires that cells expand with even multiple functioning. IGF-1 promotes osteoblastogenesis and reduces apoptosis of osteoblast by stabilizing β-catenin, enhancing Wnt-dependent pathways [89].

Bone Turnover Markers (BTMs) & Bone Diseases

Bone and glucose metabolism are closely related to one another. A more proactive clinical diagnosis and treatment are now advised for diabetes associated with bone disease as a result of several experimental investigations that analyzed the mechanisms of the mutual link between glucose and bone homeostasis. Diabetes currently experiences major consequences in the form of bone diseases. In comparison to patients without diabetes, with those pre-diabetes and T2D have an elevated risk of hip fracture. A novel and intriguing perspective on the potential risk of metabolic bone disease and obesity in patients with diabetes has also been provided by research relating endocrine effects of bone metabolism as well as glucose metabolism [90-96]. In practically all research on diabetes mellitus, bone turnover indicators have been used more often in recent years for both diagnostic and monitoring purposes. Low levels of these markers have been seen in both type 2 and pre-diabetic individuals, and they suggest impaired bone production [97-98].

Determinative Bone Turnover Osteocalcin (OC), Bone-Specific Alkaline Phosphatase (BALP), and Procollagen type I N-terminal Propeptide (PINP) are markers for bone formation and C-Telopeptide (CTX), N-terminal Telopeptide (NTX), and Tartrate-Resistant Acid Phosphatase 5b (TRAcP5b) are commonly used for bone resorption, [99, 101-103]. In order to add to a bone-specific monitoring in T2DM care, osteocalcin showed effects in regular declines in T2DM patients, which may suggest further inter linkage between bone and other tissues [100-104].

The favorable impact of osteocalcin on glucose metabolism may be explained by the possibility that OC stimulates insulin secretion and vice versa. Improved glucose tolerance was also associated with higher OC concentrations [105].

Correlation of T2DM & BMI

Those people who are overweight are expected to get diabetes. The frequency of overweight and obesity has significantly increased, whereas under nutrition has significantly decreased,

particularly among India's urban populations, according to several national studies. Glucose generates fatty acids, which make up the majority of body fat. Blood glucose levels lead to an increase in BMI, which increases lipid biosynthesis and body weight [106]. Insulin has been discovered to be an anabolic hormone that causes energy conservation and signals the body to create fat. When a rise in BMI is present, insulin resistance increases blood glucose levels in the body. BMI and body weight are related, so it makes sense that BMI would be related to blood sugar levels. BMI was calculated as: weight (kg) divided by height squared (meter). Normal weight people were characterized as having a BMI between 18 to 22. 9 kg/m2, overweight people as having a BMI between 23 to 24. 9 kg/m2, and obese people as having a BMI between 25 or higher [107].

Regulation of PTH, ALP, Vitamin D, and Calcium with FGF-23 & IGF-1 in Bone Metabolism

Reduced vitamin D levels and increased levels of parathyroid hormone (PTH) and FGF-23 have a substantial role in the development of the metabolic syndrome, either directly through associations with insulin resistance or indirectly through specific metabolic syndrome components. Additionally, calcitriol promotes the generation of FGF-23 by increasing phosphate and calcium absorption in the intestine [108]. Both vitamin D and PTH are in charge of preserving extracellular calcium homeostasis and controlling blood calcium levels. A unique function of vitamin D is that it stimulates the promoter region of the FGF-23 gene by acting on its particular receptor VDR, regardless of the blood phosphate and calcium levels in the human body. When osteoid is mineralized, alkaline phosphatase (ALP) is also formed from bone cells (osteoblasts) and released into the bloodstream. However, only half of it is made primarily in the liver, with the other half coming from serum ALP levels that are derived from bone. However, high levels of a particular circulating bone ALP isoform (BALP) can be found using the assays that are now in use. Additionally, the local generation

of vitamin D in bone cells controls the local production of fibroblast growth factor 23. Intestinal calcium absorption can be improved by vitamin D, and osteoblast activity is stimulated by the PTH hormone, which is released in reaction to low calcium levels in the blood. PTH levels are elevated as a result of low vitamin D levels, which accelerate calcium loss from the skeleton at the expense of an elevated risk of fracture [109,111]. It is believed that hypocalcemia lowers circulating FGF-23 levels, prevents the production of calcitriol and exacerbates calcium shortage. Numerous studies results from clinical investigations have demonstrated that calcium and vitamin D supplements may have a positive impact on glucose metabolism. The metabolism of vitamin D and IGF-1 also interacts. Similar to how Vitamin D can directly stimulate IGF-1 and other organs, IGF-1 also activates the 1 alpha hydroxylase enzyme, which controls renal production of Vitamin D or calcitriol [110-113].

The subject of this study can aid in reviewing a summary of the most recent information on the altered bone turnover and biochemical markers in individuals with diabetes mellitus. Numerous factors contribute to the likelihood of bone turnover abnormalities in diabetic sufferers. As a result, we compared the levels of FGF-23 and IGF with biochemical indicators in the bone metabolism of both healthy individuals and those with pre-diabetes and T2D. Diabetes and bone health association is a developing new field for clinicians.

CHAPTER-2

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Due to the high occurrence of type 2 diabetes and associated complications, India has been dealing with a significant load on the global health care system in recent years. Epidemiological data from various parts of the nation reveal that diabetes is becoming more common in urban areas. According to national research conducted in 2000 AD, 12. 1% of urban individuals in the 20–year age group had diabetes. Indians experience the onset of diabetes symptoms earlier, giving vascular problems time to develop over time. Impairment in glucose tolerance (IGT), a precursor to diabetes, is also on the rise, particularly among younger people.

Diabetes is more common in urban areas than in rural ones, highlighting the significant contribution of urbanization to the disease's etiology. The major diabetes-related consequences include coronary artery disease; neuropathy, nephropathy, and retinopathy are also more common in the Indian population. Because of poor glycemic management, hypertension, and occasionally behavioral variables, problems are more common in low socioeconomic groups. The expense of treating a chronic illness, both directly and indirectly, is extremely high, particularly when vascular problems are present. Considering the current situation, it is vital to put preventative measures in place to lower the morbidity rates and death associated with diabetes and to reduce the financial burden of diabetes care on both patients and society.

The present study “Effect of serum levels of Fibroblast Growth Factor (FGF-23) and Insulin Like Growth Factor (IGF-1) on Bone Metabolism in Pre Diabetes and Type 2 Diabetes Patients” were reviewed with the following headings:-

- Diabetes and its Global Prevalence
- Bone Metabolism
- Bone Modulation in Glucose Metabolism
- FGF-23 role in bone metabolism and T2DM
- Insulin in production of FGF-23
- IGF-I role in the T2D
- Bone metabolic markers associated with T2DM.
- Vitamin D and calcium on Insulin
- Parathyroid gland in T2DM
- Sclerostin -The WNT/ ß CATENIN Pathway

2. 1 Diabetes and its Global Prevalence

According to research, diabetes is a component of metabolic illnesses indicating by the formation of persistent hyperglycemia brought on by defects in secretion and insulin action, or both. When insulin is found in lower levels, to attain proper responses and / or resistance to insulin of the liver to targeted tissues, primarily skeletal muscle, adipose tissue, and to some extent, insulin receptors, signaling systems and / or effector enzymes and genetic level dysfunctioning is responsible for the disorder. The extremity of the features depends on the type of diabetes and its duration. Few diabetics are asymptomatic in nature, principally those having T2D early in the disease, those having marked hyperglycemia, mainly in children showing absolute insulin inadequacy may have chances to exhibit, polyuria, polydipsia,

asymptomatic, polyphagia, loss of weight, and hazy eyes. Unconstrained diabetes can cause stupor, coma, and if left untreated, causes death from ketoacidosis or, rarely, non-ketoacidosis hyperosmotic syndrome [114-116].

The categorization of diabetes is an important aspect and it affects various treatment strategies, but it is not an easy function and many patients, especially young adults, [114,117-119] cannot fit into a single category while initial 10% needs further analysis and detection or revision [120]. T1D, T2D, gestational diabetes (GDM), and other categories of diabetes were suggested by the American Diabetes Association (ADA) in 1997 and continue to be the most generally used and recognized classifications of the disease [114]. Wilkin [121] suggested an accelerator hypothesis which claims that T1D and T2D from varied genetic backgrounds are the type of common insulin resistance disorders [122]. The difference between the two types of diabetes is dependent on pacing, with faster pacing reflecting a more sensitive genotype and obesity, an early indication where insulin resistance is key to the concept. Additional signs of type 1 diabetes include increased height [123,124] and impairment in β-cell glucose sensitivity [125]. Despite the inconsistent clinical researches, the utilization of antioxidants for the diabetes treatment procedures, the effects of higher free radicals, various metabolic stressors and oxidative stress, on the growth, etiology, and complications of diabetes is well documented [126-134]. The female hormone 17β-estradiol, which acts via the estrogen receptor α (ERα), has been shown to lead in destruction of β-cell ER-α knockout mice due to induced oxidative stress, leading to the development of pancreatic β-cell function. The ERα receptor activity prevents islets from glycolipid toxicity and thus protects β-cell dysfunctioning [135].

An interesting study was done by Hilary King, Ronald E. Aubert and William H. Herman in the year 1998 with an aim to estimate the “Global Burden of Diabetes, 1995-2025”. The aim

of this study was to estimate the incidence of diabetes and the number of diabetic patients aged 20 worldwide. The study was designed for calculating additional parameters like the sex ratio of the diabetic population, the ratio of urban to rural areas, and age composition at three points in the world: 1995, 2000, 2025. For the number of adults over the age of 20 in all nations throughout the world, the UN population estimates and forecasts have specifically used the parameter linked with prevalence of age-specific diabetes. In developing nations, urban and rural populations were taken into distinct consideration. The occurrence of diabetes mellitus among adult populations was found to be 4. 0% across the world in 1995 and have higher chances that it will achieve increased chance up to 5. 4% till the year 2025. According to estimates, affluent nations have a greater prevalence of diabetes than underdeveloped nations. Between 1995 and 2025, there would be an increase in the number of adults with diabetes from 135 million to 300 million. Most cases for diabetes have higher chances to increase in numbers in developing countries. In developed countries the chance of increasing is from 42% i. e. ranging from 51 million to 72 million population, and in developing countries there are chances in increment of cases by 170% i. e. from 84 million people to 228 million people. Therefore, by 2025, more than 75% of diabetic patients will live in developing countries when compared to 62% population of diabetic patients in 1995. By 2025, China, India, and the United States will have the highest diabetes patient prevalence rates. The majority of diabetes patients belong to the age group of 45-64 year in developing countries while in developed countries, majorly the diabetes associated patients are around 65 years old and it has been estimated that the increase in rising pattern will be even more pronounced by 2025. Especially in developed countries, there is more occurrence of diabetes among women than in men. In the near future, more and more cases of diabetes will rise in urban areas. This survey supports various previous predictions about global diabetes as an epidemic around the globe in the 21st century. It also evident a preliminary illustration of

epidemic features. It is generally recognized as a principle that monitoring diabetes globally is an essential first step in the control and prevention of diabetes [17].

A population based study on diabetes has been conducted by ICMR with an objective of the ongoing national ICMR Diabetes study to assess the prevalence of prediabetes and diabetes at the national level in India by analyzing state-level data.

The study uses a differentiated multistage layout to achieve a primarily community-based totally pattern of 57117 people samples who are of 20 years of age or older than them. The pattern populace represented 14 states of India`s total 28 states (Mainland side- Total 8states and Northeast side- 6 states of the country) along with contribution from one union territory of India. All the states had been sampled in a regulated manner: section I comprises of Jharkhand, Tamil Nadu, Maharashtra and Chandigarh which were sampled from November 2008 to April 2010; section II comprises of states Gujarat, Andhra Pradesh, Karnataka, Bihar, and Punjab, which were sampled from September 2012, till July 2013; and all the northeastern section were comprised of Arunachal Pradesh, Assam, Tripura, Mizoram, Manipur, and Meghalaya, where sampling was initiated from January 2012, till July 2015. According to WHO criteria diagnosis of diabetes and prediabetes were done through Capillary oral glucose tolerance examinations. These methods do not enable researchers to discriminate between T1D and T2D. When considering people's socioeconomic status, the incidences of diabetic syndrome in particular states are evaluated. To investigate the relationship between several factors and the development of diabetes and prediabetes, they employed a few logistic regression analyses.

Diabetes was reported to be prevalent overall in 7. 3% of India's 15 states. The incidence of diabetes were diversified ranging 4·3% to 10. 0% in Bihar and Punjab, respectively and become better in urban regions (11·2%) than in rural regions (5·2%) and even much better in mainland states (8·3%) than within the Eastern states (5·9%). Overall, 1862 (47·3%) from

3938 people recognized with presence of diabetes and were not investigated previously during the study. States having better GDP per-capita were regarded to have a better incidence of diabetes (e.g., Chandigarh, having best GDP of US$ 3433, with incidence of 13·6% diabetes). Among rural regions of all the states, diabetes becomes extra popular in people with good socioeconomic status. As a result, the incidence of diabetes has increased in metropolitan areas of some of the more rich states, such as Maharashtra, Chandigarh and Tamil Nadu. In all 15 states, the prediabetes prevalence increased to 10•3%. The prevalence of prediabetes varies from 6 percent in Mizoram to 14. 7 percent in the Tripura region, and when it becomes more prevalent, impaired fasting glucose is preferable to IGT. Obesity, age, hypertension, male sex, and familial records of diabetes were independent risk factors for diabetes in both rural and urban areas. The prevalence of diabetes varies greatly amongst Indian states. It is a topic of discussion and considerable concern that diabetes is spreading to economically disadvantaged parts of society, which calls for immediate preventative measures [22].

2. 2 Bone Metabolism

With an aim to inspect the effect of Bone Metabolism in diabetic patients several studies were analyzed and reviewed to come to certain conclusive points in contrast to the present study.

Research conducted in 2013 by Oei L et al. showed that despite having better bone mineral density (BMD), individuals with type 2 diabetes have a higher risk of hip fractures (BMD). The purpose of the study was to look at how glycemic management affects the complexity of the skeletal system. It was observed that the BMD for group of ICD is 1. 1-5. 6% greater, the cortex is thicker 4.6-5.6%, and the femur was found to be -1.2 to -1.8% narrower. Volunteers with ICD had a 47-62% greater risk for fracture than volunteers without diabetes (HR 1.47)

and ACD (HR 1.62), whereas subjects suffering from ACD have the same risk factors as subjects without ACD.

In summary, poor glycemic control in T2D is associated with an increased risk of fracture, a higher BMD, and a thick femoral cortex with narrow bones. It is hypothesized that the apparently "strong" bone fragility of the ICD may be due to the accumulation of micro cracks and / or the porosity of the cortex, considering impairments in bone repair [31].

Research by Beata Lecka –Czernik in the year 2017 showed that skeletal fragility is frequently linked to both T1D and T2D and is seen as a pathological challenge for the condition. People with type 2 diabetes have a high BMI despite having normal or high bone density whereas lower bone mass in T1D can significantly increase the risk of fractures. (As further discussed in, it was considered protective against fractures in non-diabetic subjects.) [136] Fractures as often the consequence of mild or moderate trauma cannot be explained by the increased decline due to comorbidity associated with diabetes [137]. Instead, in diabetic patients, despite high / normal BMD, bone has many structural features that are prone to fracture, such as increased porosity of cortex, decreased area for cortex, and decreased strengths of bones [138,139]. These characteristics suggest that the biomechanical bone quality of diabetic patients is impaired.

The reshaping of the bone, that is typical across all mammals, supports bone quality. Bone remodeling depends on ongoing bone resorption and remodeling to replace ageing tissue with freshly formed more functioning tissue. For the nourishment of bone mass, the balance among osteoclast-dependent resorption of bone and formation of bone (the latter depends on activity of osteoblast) is an important requirement. As a result, a mostly metabolic associated bone disorder, which includes postmenopausal as well as senile osteoporosis that is result from an imbalanced turnover of bone. Conversely, a decrease in bone turnover distinguishes diabetic bone (rather than imbalance). Surprisingly, the pelvic bone biopsy revealed a

decrease in the formation of the bone that was correlated with the duration of diabetes, with lower circulation levels of biochemical markers in the formation of the bone and a reduction in bone resorption in diabetic individuals [140, 141]. It has been hypothesized that low bone turnover among diabetic patients can result in incomplete repair of micro fractures, and thus their accumulation, leading to poor bone characters. In contrary, senile and postmenopausal osteoporosis, decreased strengths of bones in diabetes is linked to higher cortex porosity and is not associated with bone mass loss [142, 143]. With the finding of this research, it can be summarized that the characteristics of bones which are specific to diabetes can represent a new syndrome which may be categorized as diabetes-related to bone disease [43].

A recent review article by Ferrari S. L. et al. in the year 2018 correlates with our study. Where it was observed that people who have been diagnosed with diabetes are rational to suffer from brittle fractures. Although the pathogenesis of bone fragility in these people is not completely understood, it may be complex. Various longitudinal researches have shown that fracture prediction algorithms (FRAX) and BMD T-score anticipate fractures risk in patients with T2D, although both need to be adjusted for type 2 diabetes to minimize the risk. The ideal method has not been accepted yet, relying on clinical prospective studies in the treatment of diabetics. Therefore, the current preferred algorithm should be regarded as consent for some specialists that can change with time as additional authentication is collected. The data predicts that if patients have indications for treatment relying on criteria established for non-diabetic patients, those individuals should be handled with medications associated with osteoporosis. However, in non-occurrence of osteoporosis, the results of these medicaments for conditions where fragility of bones is primarily due to changes in quality of bones have not yet been thoroughly studied and should be used with caution. Looking at future aspects studies will need to continue to assess fracture structure factors (ultrastructure, material properties, etc.) and improve fracture prediction algorithms by

incorporating disease-specific fracture determinants like Complications related to microvascular which includes retinopathy & nephropathy, diabetes medicines, duration of diabetes and levels of HbA1c. New studies need to proactively evaluate the efficacy and precaution in treating osteoporosis among diabetic patients along with or without lower levels of BMD [98].

Another research article led by Sain S. Safarova in the present year 2019 emphasized "Alterations of Bone Metabolism in Patients with Diabetes Mellitus". The search was directed on a total of 235 patients for this study (98 participants for T1DM and 137 participants for T2DM). Eighty-nine participants were taken for control as non-diabetic patients. All 235 participants underwent tests that included measuring their BMD using a dual-energy X-ray absorption assay (DXA), measuring their levels of the markers for serum bone turnover (procollagen type I amino-terminal Propeptide (P1NP) and collagen type I at c-terminal (CTX) terrorpeptide), and measuring their levels of 25(OH)D and parathyrin. Our results evidenced that participants with T2DM have less amount of bCTx and amount of P1NP is higher, which reflects fewer noticeable alterations for metabolism in bones when compared with participants of T1DM, despite dependency on duration or age of illness. Osteoporosis was found in the majority among 50% of patients having T1DM in comparison to patients suffering from T2DM. In fewer cases, bone metabolism markers help in improving the evaluation of bone tissue status in the earlier phases of diabetes, but changes in microarchitecture of bone cannot be predicted by measurements of bone mineral density [144].

T2DM patients frequently have a higher risk of metabolic conditions that affect the bones. Bone turnover and BMD should be included when evaluating the metabolism of bone. In this study, founders analyzed bone turnover and BMD correlation in diabetes type 2 and the research is a cross-sectional based on hospital study which was evaluated on 1499 patients

who were admitted in hospital for uncontrollable type 2 diabetes. The link between bone metabolism markers (BTM) and BMD values was examined using a multivariate linear regression-based model, and a two-sided P-value less than 0. 05 was shown to be statistically significant. When assessed collectively for the lumbar spine, femoral neck, and total hip BMD, a negative connection between male and female for osteocalcin (OC) was discovered when confounding variables were taken into account. Alkaline phosphatase (ALP) and Type I collagen (P1NP) N-terminal propeptides were found to be negatively linked at 3-site BMD in men and in women for BMD of total lumbar, but among women the femoral neck and hip joints shows a positive relationship only with P1NP with total hips. In the study it was observed that patients suffering with T2DM, β-CTX, OC levels in serum, P1NP, and ALP were found to be correlated negatively with male BMD levels at three sites and female total lumbar BMD. In women, the correlation between the total hip arthroplasty and femoral neck differs [145].

Diabetoporosity- diabetes and the bone – a review article by Barbara Obermayer-Pietsch in 2018 over the relationship between the two clarified various uncertainties on our topic. In older people, osteoporosis and diabetes mellitus Type 2 (T2DM) commonly coexist, making them more susceptible. Elderly patients who have the condition evaluated by dual-energy bone mineral density measurement (DXA) have a higher risk of bone fractures while having normal to high levels of BMD. This coincidence has long been unrecognized as BMD levels measurements do not recognize patients with T2DM at threat of fracture. T2DM patients with large fractures have been identified with a higher threat of detained wound healing and higher mortality rates. Patients having T1DM have an even higher risk for bone fractures, up to 12 times more risk than individuals having normal metabolism for glucose. There are more chances that these patients are affected from their early childhood, and later on their glycemic control exacerbates fracture outcomes. Reduced fragility of skeleton and its

properties, including large and microvascular complications and chronic inflammation are the pathophysiological circumstances which can contribute to a higher threat of fractures among both T1DM and T2DM. A new generation of imaging techniques was developed in response to the urgent need for specific diagnostic methods, and numerous attempts were made by utilizing them. A new biomarker for diabetes was featured. The composite interactions of the newly recognized biomarkers were described in several articles. This study concludes about the report for the present state of knowledge for diabetes mellitus pathophysiology, diagnosis and bone management. The study focuses mainly on the growing global population of T2DM patients, who currently number around 415 million, as well as the 9 million fractures caused by osteoporosis each year among women over 200 million, and perhaps men around 120 million each year and growing group of 9 million T1DM- patients which is an important issue for futuristic medical care [146].

Another review article by Katrine Hygum, et al. in the year 2020 compares bone turnover rates between diabetes and non-diabetic populations. The study was designed as a meta-analysis and systematic review. 2881 total papers were recognized, among which 66 articles were analysed and included for this study. The amount of serum in C-terminal of bone resorption marker crosslinked telopeptide along with osteocalcin –formation marker for bones and diabetes patients had lower levels of type 1 propeptides of the amino terminal compared to control group. It was observed that individuals with diabetes have significantly lower levels of both bone resorption indicators and bone creation, which suggests that T2DM results in a slower turnover of bones, which can lead to bone fragility. It may be due to changes in the levels of sclerostin and osteoprotegerin [147].

Invasive research was done by Claudia Pinheiro Sanches in 2017 for assessing “The impact of type 2 diabetes on bone metabolism”. Osteoporosis-related fractures and diabetes complications are the two main causes of mortality and morbidity in elderly diabetic patients.

These two conditions describe a variety of traits, including vulnerability to genes, environmental factors, and molecular pathways. Type 2 diabetes mellitus (T2DM) induces functioning of bone cells abnormally and structure of matrix with higher rates of apoptosis for osteoblasts while it decreases osteoblastic differentiation, and increased bone resorption is caused by osteoblast cells, which also significantly alter the architecture of bone. The connection between these unfavorable situations raises the possibility that different anti-diabetic treatments will have an impact on bone quality. Both levels of blood glucose and homeostasis of bones in the body are regulated by many known regulatory factors and these factors includes insulin, highly glycated accumulated end products, gamma receptor activated by peroxisome growth factor, hormones of gastrointestine (such as glucose-dependent insulin-secreting peptides and peptides 1 and 2 for glucagon), and osteocalcin which is a bone derived from hormones. This diabetes related study induces various independent targets of pharmacological class of anti-diabetic therapy to influence bone quality through their indirect consequences on differentiation for cells of bones and bone remodeling process. In addition, it is very crucial to examine vulnerable fractures as a part of diabetic complication and further discuss the need for appropriate prevention and various preventive measures taken in time for this disease. This review focused on quickly examining how type 2 diabetes affects the mechanical and metabolic characteristics of bone as well as the risk of fractures [148].

2. 3 Bone Modulation in Glucose Metabolism

Glucose metabolism plays a very crucial role in diabetes and is an important factor which strongly gives evidence inducing diabetes in patients. Here in our topic we put great emphasis on glucose metabolism and came across various past researches associated with current topic.

In 2020 Ciprani C. et al. showed various functions of bones related to endocrine hormones apart from metabolism of minerals are glucose metabolism, energy metabolism regulation and sensitivity to insulin. Various in vitro and mouse studies examined the effects of osteoblasts and various molecules obtained from bone cells on metabolism associated with glucose. Furthermore, the result of glucose metabolism on cells of bones indicates an interaction between glucose metabolism and bone cells. Among humans, these processes are important determinants of fragility in skeleton and are linked with diabetes of both type 1 and 2. The main causes of metabolic malformation in diabetes disease include an increase in body fat, a reduction in fat loss, the effects of hyperglycemia itself, the production of end products of advanced glycation, chronic diabetes-related diseases like kidney disease, and impaired calcium-PTH-vitamin D metabolism. Ultimately, there are many on-going projects on anti-diabetes drugs that affect the skeleton, differences between various data collected from diabetes patients, and drugs of anti-osteoporosis disorder that affect glucose metabolism. The current review summarizes the linking feature of bone and glucose metabolism by providing insights into the latest human discoveries [149].

Tanaka K., et al. in the year 2015 find that the degradation of bone in individuals with DM is caused by the presence of advanced glycation end products (AGE) and higher glucose levels (HG). Regulation of the function of osteoblasts and osteoclasts are induced by producing nuclear factor-kB ligand (RANKL)- an activator of receptors and sclerostin by osteocytes. However, it is still unclear if HG or AGEs influences bone cells and induces the production of sclerostin and RANKL. The studies found that AGE and HG may prevent bone formation by causing bone cells to express more sclerostin, whereas AGE prevents bone resorption by down regulating RANKL expression. These processes can reduce the turnover of bone for Diabetes Mellitus. Additionally, AGE and HG are found to cause deterioration of cortical

bone by influencing osteocytosis of bone cells. Functioning of bone cell refinement and pathological processes are treated by parathyroid hormones [150].

Another influential study in the year 2015 by Wei Jianwen et al. proposed that the first determining element in osteoblast differentiation is the creation of collagen type I, which came before Runx2's articulation. This research assumed that glucose, the major nutrient for osteoblasts cells, is transferred with the help of Glut1, which has an expression that anticipates the expression of Runx2. Uptake of glucose promotes differentiation of osteoblast cells by repressing AMPK-dependent proteasome degradation for Runx2 and enhances formation of skeleton by hindering other outcomes of AMPK. Runx2 is unable to promote differentiation of osteoblasts as when upregulation of glucose is impaired, but increasing levels of blood glucose re-establish collagen production in Runx2 null osteoblasts and commences formation of bones in embryos deficient to Runx2. Additionally, Runx2 stimulates Glut1 expression. This feed-forward control between Runx2 and Glut1 regulates the extent of lifelong bone creation as well as the beginning of differentiation in osteoblast cells during development. These findings show a range of surprising complexities between metabolism of bone and glucose [151].

Zernik in 2017 found that bone turnover as well as energy metabolism are managed by complex procedures that shares various clues and results. Due to the skeleton size and the amount of energy absorbed in the mechanism of remodeling for bones, the bones of the body are strongly dependent on metabolism of glucose. Henceforth, it is interesting to know signaling of insulin has an important function in the management for turnover of bones. In particular, blood glucose is necessary for osteoblast cells to organize their development and bodily functions, and glucose and insulin cause them to express the bone-specific hormones

OC and RUNX2 [152, 153]. Additionally, osteoclast-protegerin, a decoy receptor for the osteoclast-promoting cytokine receptor activator of the nuclear factor kB ligand, is reduced in expression when insulin is present [154] Surprisingly, it has lately been engaged in the control of insulin manufacturing in beta cells of the pancreas, which offers additional proof that energy and bone metabolism are controlled by mechanisms that are interconnected. Given that insulin signaling and glucose metabolism have a positive association with bone production and turnover [155, 43].

2. 4 FGF-23 roles in bone metabolism and T2DM

With invasive research on our topic, we focused on the primary part of this thesis, which put greater emphasis on FGF-23 and its role for bone metabolism and T2DM.

In 2012, Kocelak P. et al. conducted research on the structure, function, and connection between kidney disorders and FGF-23. The FGF hormone family is made up of a number of different polypeptides that have a central region of homology. Osteocyte cells, which are a subfamily of FGF-19 and serve as the main phosphating produce osteoblasts and FGF-23. Both full-length FGF-23, which is biologically active intact FGF-23 (iFGF-23) and FGF-23 (cFGF-23) with inactive C-terminal, are present in circulation and can be found in serum. FGF-23, which has the co-receptor klotho protein, lowers the activity of the enzyme 1-hydroxylase (CYP27B1), which lowers vitamin D-dependent intestinal phosphate absorption. It also lowers calcium production and renal phosphate reabsorption. Consuming more phosphorus, PTH, and 1,25-dihydroxyvitamin D3, which are the three main factors that stimulate the release of the FGF-23 hormone. Impairment in metabolism of FGF-23 is associated with disorders of phosphate that manifest due to several disorders like osteomalacia or rickets or an increase in amount of tissue calcification. FGF-23 is also secreted by tumors and causes hypophosphatemia. When chronic kidney disease (CKD)

reaches its advanced stages, there is increased production of FGF-23, and concentrations of cFGF-23 and active iFGF-23 start to rise in correlation with a moderate deterioration in the glomerular filtration rate. A higher amount of FGF-23 secretion appears to represent a preventive mechanism in opposition to enhanced FGF-23 secretion as well as phosphate accumulation during early stages of chronic kidney disease. However, escalation of deficiency in calcitriol and secondary hyperparathyroidism can occur. Elevated FGF-23 levels are thought to be the only factor in increasing deaths in CKD patients. It is currently unclear if FGF-23 is negatively impacted or not, depending on whether this is due to just reduced calcium phosphate metabolism, decreased calcitriol synthesis, induced cell proliferation, or acting through cardiac or low-affinity klotho-independent receptors. There is not much proof of FGF-23's direct toxicity as of now [156].

Synthesis and Activity of FGF23 was studied by Noonan M. and White K. in the year 2019. The phosphorylation hormone known as FGF23 is mainly secreted in osteoblasts cells and is found to react to phosphate levels of serum and 1,25 $(OH)_2$ vitamin D increased levels. Recent research has revealed a novel FGF23 regulator that may aid in illuminating the pathophysiology of various disorders. The present discussion focuses on the latest studies which investigate the production and effects of FGF23. FGF23 synthesis in account to 1,25D is same in manner when compared to various other targeted steroidal hormones, but the responses at cellular levels for phosphate remain unclear. The intracellular processing gene's activity, which controls the glycosylation and phosphorylation of FGF23, has a significant role in regulating the serum level of physiologically active FGF23. FGF23 acts primarily through the αKlotho (KL) which is co-receptor under normal situations, whereas FGF23 exhibits activity and which is KL-independent under higher concentration conditions [157].

The effects of FGF23 on diabetic health and chronic renal disease were investigated by Fukagawa M. et al. in 2005. The biological function and molecular characteristics of the newly discovered phosphorylating factor, FGF23, are summarized in this work research. The important role of FGF23 has been discussed, especially with respect to its effect on the kidney, which is a major target organ. FGF 23 is a newly discovered phosphorylating factor in kidney diseases. Numerous animal studies using the overexpression or deletion of the FGF23 gene have revealed a major impact on renal phosphate excretion and vitamin D production. Although FGF23 was once thought to be the cause of various hypophosphatemic diseases, clear evidence points to its part in the physiological control of phosphate homeostasis. During chronic kidney disease, an important function is carried out in the secondary hyperparathyroidism etiology by FGF23. It is not yet known how FGF23 affects bones and the intestines in addition to other organs. In a physiological sense, phosphate homeostasis is known to be regulated by FGF23. Increased activity of FGF23 along with normally renal functioning causes hypophosphatemia, 1,25-dihydroxyvitamin D lower amount, rickets / osteomalacia. On the other hand, excessive FGF23 activity reduces 1,25-dihydroxyvitamin D synthesis, but it is insufficient to sufficiently clear the phosphate burden caused by reduced renal function, which aids in the onset of hyperparathyroidism [158].

In 2010, Nakai K. et al. addressed the most recent findings about the function of FGF-23 in chronic renal disease. It is found in bones and aids in controlling the metabolism of phosphate and 1,25-dihydroxyvitamin D. In the presence of FGF receptor-1 along with its co-receptor, FGF23 predominantly affects the kidney, where it stimulates urinary phosphate excretion and inhibits 1,25-dihydroxyvitamin D synthesis. In addition, various studies provide evidence of predictor for mortality in patients undergoing dialysis is FGF23, specifying FGF-23 having potential role as sensitive biomarker [159].

Study by Maria L. Mace et al. in the year 2015 emphasized on the higher circulating FGF-23 and its highest failure is articulated in kidneys which are yet to be understood. This study assessed earlier regulation on kidneys for FGF-23 during acute uremia which is persuaded by unilateral and bilateral nephrectomy. Bilateral nephrectomy gives rise to plasma higher levels within 15 minutes and is in stable state later on. As per the knowledge no any study showed the reason behind early gene expression of FGF-23 by bones has been seen. It has also been evident that higher amounts of phosphorus and calcium are responsible for normal FGF-23 amounts. By measuring levels of plasma FGF-23 in renal vein and renal artery it was demarcated that renal excretion is very significant. Hence, kidneys have an important functioning in homeostasis balance of FGF-23 in metabolism and in serum plasma [160].

Javier donates correa et al. in 2021 observed that higher amount of fibroblast growth factor is an important phosphate regulator, for diabetes mellitus development initially. These investigations suggest that FGF23 chronic hyper physiological levels, a condition that is common in people with end-stage renal illness (ESRD) and chronic kidney disease (CKD), may represent an unidentified mechanism that causes diabetes. These maladaptation and diabetes-inducing responses of FGF23 can occur at various levels which includes effects directly on pancreatic β-cells and effects indirectly resulting from simulation of the production in pro-inflammatory factors. The binding of FGF23 to an unconventional receptor complex and the resultant over activation of signaling pathways, which might have negative effects, are the mechanisms underlying both direct and indirect effects. Standard FGF23 hormone interaction with the complex of receptors created with the aid of FGFR1c and the co-receptor Klotho triggers Ras/MAPK/ERK signaling. Hyper physiological levels of FGF23 encourage non-Klotho-dependent coupling of the molecules to other existing

FGFRs, which is unfavorable for signaling of PLCy/CN/NFAT and results in over activation as shown in myocardium and hepatocytes. The development of effects is also aided by decreased Klotho expression, which increases non-specific activation of PLCy, CN, and NFAT and compromises the Klotho-dependent Ras, MAPK, and ERK signaling pathway. The creation of new therapeutic targets that can be affected by dietary modifications or altered by pharmacological intervention will be made possible by an understanding of these mechanisms [161].

Another evaluating study was done by H Komaba and M Fukagawa in 2009 identified That FGF23 is the hormone that regulates the metabolism of minerals and vitamin D. Chronic phosphate retention gradually raises circulating FGF23 levels in people with chronic kidney disease (CKD), which decreases the kidneys' capacity to produce 1, 25-dihydroxyvitamin D and, as a result, promotes parathyroid hormone secretion. This highlights FGF23's critical role in disturbed mineral homeostasis as a cause of CKD. Additionally, recent research has demonstrated that direct action of FGF23 on the glands of parathyroid and mediation of parathyroid hormone secretion in the even though these effects have not yet been verified in CKD patients, the existence of αKlotho as a cofactor, the development of refractory hyperparathyroidism disease and mortality can both be predicted using FGF23. The amount of FGF23 is noticeably elevated in patients with hyperparathyroidism who are receiving dialysis treatment, raised in response to aggressive vitamin D treatment for hyperphosphatemia. This brief analysis emphasizes the current understanding of FGF23's role in the etiology of mineral and bone disorders in CKD patients [162].

Hugo Diniz Joao M. Frazao published another article in 2013 that observed that the balance of phosphate in the body is regulated by the hormone FGF-23, which is made in bone. FGF-

23 levels are abnormally high in chronic renal disorders, and there is evidence that this hormone plays a pathogenic role in the emergence of CKD-Mineral and Bone Disorder (CKD-MBD). FGF-23 has also been connected in recent studies to the pathophysiology of CKD-MBD systemic issues. The perspective to the pathogenesis and bone alteration treatment and mineral metabolism treatment in CKD patients has changed as a result of mounting an indication that the effects of faulty mineral metabolism are not limited to diseases of bones. It has been suggested that FGF-23 serves as the body's first adopted response in the early stages of CKD to safeguard the organism from phosphate retention severe effects. Increased FGF-23 levels have been shown to induce direct, "off-target" damage to the heart and to be associated with elevated risk of death due to cardiovascular issues. The objectives of this paper are to review relevant aspects of FGF-23 physiology in bone biology and its mineralization homeostasis, as well as FGF-23's role in CKD-BMD and its clinical implications [163].

Reinhold G. Erben published yet another outstanding review article in 2018 that details his research on the physiological effects of FGF23. The hormone fibroblast growth factor-23, which is present in bones, prevents the kidney from producing vitamin D hormone and phosphate reabsorption. These endocrine effects of FGF23 on kidneys are Klotho dependent at physiological levels of hormone because co-receptor Klotho must be present on target cells for FGF receptors to bind to FGF23 with high affinity. It is widely known that people with adequate renal function experience phosphate squandering when their blood contains high levels of intact FGF23. A significant amount of study has concentrated on the pathophysiological effects of FGF23 excess due to the significance of diseases like chronic kidney disease and phosphate-wasting diseases that are linked to gains in FGF23 function. Less attention has been placed on FGF23's function in healthy physiology. Beginning to play a part in both bone mineralization and the regulation of alkaline phosphatase expression is

FGF23. The inhibition of alkaline phosphatase is Klotho-independent in bones and is mediated via FGF-23, apart from the renal effects of FGF23. Furthermore, the erythroid lineage development of hematopoietic stem cells in the bone microenvironment may be physiologically suppressed by FGF23. There is not much proof yet that FGF23 functions physiologically in organs except the kidney and bone. This mini-goal review's is to highlight what is currently known about FGF23's intricate physiological functions [164].

While going through review articles and knowing the importance of Fibrobalst growth factor Yu-Chen Guo and Quan Yuan in 2015 studied that osteoblasts and osteocytes largely release a hormone known as FGF23. The critical role of FGF23 in maintaining mineral ion homeostasis has since been well characterized for animal models after it was initially identified in human inherited and acquired rachitic diseases. Recent studies have demonstrated a considerable increase in FGF23 levels in the early stages of CKD, which may have a significant effect on patients' mineral abnormalities and bone metabolism. Additionally, our most recent articles have demonstrated that, rather than having a cumulative effect, FGF23 and its cofactor Klotho may independently regulate bone mineralization [165]. A study by Yongbo Lu and Jian Q. Feng in 2011 showed that the kidney is the target of the fibroblast growth factor 23 hormone, which is mostly produced by bone cells [166].

2. 5 Insulin in production of FGF-23

To assess the correlation between FGF-23 productions via insulin various articles were studied and following were the outcomes.

In 2018, Bar Ludmilla et al. found that the FGF23 generated by bone cells also controls phosphate levels of kidney and vitamin D metabolism. In contrast to disorders like kidney or cardiovascular disease, which have high plasma FGF23 levels, rapid ageing is caused by a

shortage of FGF23. It was found that there was a strong negative correlation between the plasma concentration of FGF23 in females and the elevated insulin level in human plasma following an oral glucose load [167].

Another study by Robledo A. et al in the year 2021. hypothesized that prolonged exposure of FGF21 is connected to metabolic irregularities, obesity, and lipodystrophy, as well as insulin resistance, T2D, and an increase in oxidative stress in adipose tissue. Both in patients and controls, blood levels of FGF21 were marginally higher in the overweight/obese groups. With a p-value of <0. 0001, FGF21 levels in HIV-infected participants demonstrated a substantial positive connection with lipids, insulin levels, and insulin resistance. FGF21 levels were significantly linked with the extent of the subjects' obesity in the seronegative group, but solely with weight and waist circumference.
In overweight-obese PLWH, elevated FGF21 and insulin resistance were seen. As FGF21 associations in the control group seemed limited for weight and waist circumference, FGF21 elevation may be seen as a compensatory mechanism. This can be explained by FGF21's role in encouraging glucose uptake for adipose tissue. Low levels of FGF21 in PLWH probably is result of an increase or decrease in adiposity that disrupted metabolism [168].

Kutluturk Y. et al. in the year 2019 studied that insulin resistance and dyslipidemia, obesity has been linked to altered bone mineralization. Fat has an unknown impact in FGF-23, which is essential for mineralization of bone. The study's goal was to determine whether FGF-23 levels and obesity-related dysmetabolism were correlated. It was found that there were detrimental correlations between fasting insulin and C-peptide levels in the obese participants with hepatic steatosis. Fat individuals' FGF-23 serum concentrations may decrease in the

current study due to hyperinsulinism linked to insulin resistance and/or decreased levels of 1,25(OH)2D3, which is present in children and adolescents who are obese [169].

2. 6 IGF-I roles in T2D

The prevalence of IGF1 was investigated to learn more about numerous features of T2DM, and the studies that were deemed to be necessary contrarian to our research are listed below.

Raj pathak et al. in the year 2009 showed that the IGF axis may be involved in both the normal homoeostasis of the glucose and in the pathogenesis of T2D. IGF-I may also help with pancreatic beta-cell function as well as systemic inflammation, which is a risk factor for T2D. IGF-I endogenous levels and its binding proteins range significantly between individuals, but the association between these factors and T2D prevalence was not adequately investigated. To evaluate this connection, substantial prospective research will be required [170].

Friedrich N., et al. in the year 2012 showed that about 50% of the amino acids in insulin and IGF-I are the same, and both trigger a comparable reaction in hypoglycemia. It was observed that the homeostasis model assessment of insulin resistance and the removal of participants with type 2 diabetes did not affect the statistical significance of the correlations. Insulin resistance and normal IGF-I levels are both correlated. For the sake of future risk classification, the molecular mechanism behind this complicated occurrence has to be clarified in greater depth [171].

An endocrinology society in the year 2006 published an article that states that on getting excellent control for concentration of glucose in people having type 2 diabetes mellitus (DM2) remains difficult for more than 50 years of contemporary research in diabetes, more of which has been devoted to studying this condition. This is partly because we don't fully comprehend the pathophysiology and natural history of DM2. Resolved the past three decades, there has been a heated dispute concerning the relative role of pancreatic failure and resistance to insulin in developing symptoms for type 2 diabetes. This debate is now mostly over. Both are obviously significant. Recombinant insulin should make it possible to supply enough insulin, or an insulin analogue, to satisfy even the most insulin-resistant person's needs. In addition to intrinsic insulin resistance, some persons and ethnic groupings are predisposed to this genetically [172].

IGF-I levels are raised by osteoprotegerin and RANK-ligand, which results in markers for osteoblastic activity and a decrease in bone resorption was studied by Menendez et al. in the year 2013. The important finding of the study was that the lower amount of IGF-I acted as a true replacement therapy by bringing serum levels of IGF-I back to normal, enhancing the expression of the majority of these proteins that are involved for formation of bones directly, and lowering resorption of bones through mechanisms involving osteoprotegerin, RANKL, and PTH receptor [173].

In a study, men over 65 will be examined to evaluate the association among BMD and insulin growth factor 1. Subjects for the trial included 41 patients, who are males, age about 65 to 88, without any history of drug consumption or a condition that is found to impair BMD. The control group was made up of 20 healthy men, aged 19 to 62. IGF-1 concentrations were assessed using an immunoradiometric technique. On the proximal femur and lumbar spine,

BMD was measured using dual-energy X-ray absorptiometry. According to the research, decreased blood levels of IGF-1 are associated with an increased incidence of hip osteopenia and are therefore at least largely to blame for the osteopenia seen in males 65 years of age or older [174].

In 2014, a study investigated the impact of IGF-1 and GH on osteoporosis and metabolism of bone. GH and IGF are essential for skeletal development throughout bone health and puberty throughout life. IGF-1 is an essential promoter of bone growth, while GH accelerates tissue creation by acting on target cells both directly and indirectly. GH and IGF-1 hormone use in clinical research reporting osteoporosis and fracture healing is described. In most studies, GH and IGF-1 injection significantly increased both bone growth and resorption. In patients with hip or tibial fractures, GH/IGF-1 injection accelerated clinical recovery and enhanced bone repair. There were several outcomes that were contradictory. Therapy with GH and IGF-1 hormone has a considerable anabolic impact. Clinical outcomes in treatment for osteoporosis and bone fractures can be significantly enhanced by GH therapy. In the anabolic process, GH and sex steroids interact. GH resistance process is taken into account [175].

The function of IGF-1 and insulin for skeleton physiology is discussed in this review, as well as how disturbances of this axis in diabetes may be the cause of many of the skeletal diseases that have been noticed [176].

IGF-I plays a critical role in controlling homeostasis of bones order to promote adequate longitudinal bone development and bone mass. Although growth hormones have the ability to directly affect skeletal cells, the majority of its functions are carried out via the systemic circulatory protein IGF-I, which is produced by peripheral tissues. IGF binding proteins

control how much IGF-I is available. The differentiation of osteoblastic function and bone production are improved by IGF-I. Adult GH insufficiency results in osteoporosis with lower bone turnover and a higher risk for vertebral and nonvertebral fractures; GH replacement therapy can partially correct the lower mass of bones. High bone turnover, a characteristic of acromegaly, can cause bone loss and vertebral fractures, especially in patients who also have coexisting hypogonadism. Growth hormone and IGF-I production declines with age, thus may have a role in the pathogenesis of osteoporosis brought on by anorexia nervosa and exposure to glucocorticoids [177].

Schnedl C. et al in 2015 studied FGF-23 levels during acute and chronic illness which shows us that the effects of insulin resistance (T2D) and deficiency (T1D) on FGF-23 are quite complex. In the majority of investigations, greater serum levels of FGF-23 were linked to diabetes. FGF-23 is a useful biomarker since various acute and chronic illnesses have higher plasma FGF-23 concentrations [178].

According to a 2017 study by Mera P., et al. titled "Regulation of Energy Metabolism by Bone-Derived Hormones," FGF-23 is a protein with a number of multifunctional actions. FGFs are categorised according to their action mechanisms as intracrine, paracrine, and endocrine FGFs [179]. As well as Renal Klotho in mineral metabolism and FGF-23 as a counter-regulatory phosphaturic hormone for Vitamin D, other studies focused on pathology, physiology, evolution, and pharmacology. Structure-wise, FGF-23 belongs to the FGF family, specifically the endocrine FGFs, which also comprise FGF19, FGF21, and FGF-23. FGF23, which is located on chromosome 12p13 and is made up of three exons, codes for a 32KD protein with 251 amino acids. However, it is functionally a member of the hormone class known as phosphatonins, which controls phosphorus metabolism [180].

Other studies conducted by Tuzun et al. in 2018 revealed that other tissues, including bone tissue, the thalamus, bone marrow vessels, the thymus, and lymph nodes, express FGF-23. Kidney is the main organ targeted since it controls 1,25(OH)2D3 synthesis and phosphate reabsorption. FGF-23 has developed over the past ten years into a potential marker for both diagnostic and predictive reasons as well as a therapeutic target for a variety of illnesses. Hereditary conditions include a) syndromes of excess or deficiency of fibroblast growth factor 23; and b) disorders of hypophosphatemia and hyperphosphatemia. FGF-23 has been suggested as a potential novel marker for gestational diabetes mellitus (DM) in people with the disease and is positively correlated with resistance in those with T2DM [181].

In 2019, Kutluturk Y. et al. demonstrated the connections between the FGF-23 level in serum and prediabetes, IR and dyslipidemia in children with obesity and adolescents. FGF-23 levels were found to be strongly inversely correlated with levels of fasting insulin and C-peptide in adolescents and children having obesity with hepatic steatosis [169].

According to a 2005 study by Clemmons et al., showed the engagement of more intricate mechanisms rather than the interaction between IGF-1 and insulin receptor and may represent the reported variations in insulin and IGF-I receptor distribution in tissues, but it may also reflect variations in post-receptor signaling pathways.

Pennesi P. et al. in 2006 showed that the insulin receptor, its corresponding ligands, and the insulin-like growth factor (IGF1) share structural similarities and share signal transduction pathways. Increased nocturnal gluconeogenesis causes hyperglycemia during the fasting

stage, which is a prevalent issue in type 2 diabetes and frequently unresponsive to bedtime insulin, along with the availability of insulin analogues that persist longer [183].

Juul A. et al. in 1995 studied that, hyposecretion of GH is linked to obesity, and GH hyposecretion is a key regulator of the liver's secretion of IGF-I. While the levels of total IGF-I are not found to be low in blood but it's possible that free IGF-I levels are even higher in obesity [185].

2. 7 Bone metabolic markers with type II diabetes mellitus

Our study assessed in identifying the metabolic markers for impaired bone metabolism in patients having diabetes and found that detection of FGF-23 levels in patients can be possible biological markers.

The clinical use of bone markers in various disorders was investigated by Vlog M. C. et al. in 2018. A crucial component of bone research in contemporary clinical medicine is the measurement in peripheral urine or blood for markers of bone (BMs). Since BMs can be used for diagnosis of bone related diseases, track course naturally, and even track the results of therapies, their use has significantly expanded during years recently. Use of BMs is still complicated, though, primarily because of the (pre)analytical variations of such substances, the difficulty in accessing assays, the diversified cut-off values used for different laboratories and countries, and the inconsistent clinical implications of BM measurement across different studies. The review provides a practical guide to clinicians based on the most recent research on when to test for various bone diseases, including, tumor-induced osteomalacia, Paget's disease van Bucher disease, primary osteoporosis, neoplasm/multiple myeloma, hypophosphatemic rickets, chronic kidney disease, T2DM and rheumatoid arthritis, While

ordering and interpreting measurements of BM, the doctor should take into account the following factors: recent fractures, fasting state, ageing, menopausal status, concurrent liver and renal illness. The study concludes that bone marrow samples are unquestionably useful for the current diagnosis of tumor-induced osteomalacia, Paget's disease, van Buchem disease, and hypophosphatemic rickets [105].

According to a 2015 study by Murali S. et al. showed the hormone FGF–23, which is secreted from bones, limits the kidney's capacity to absorb phosphate and creates the vitamin D hormone. It has long been a mystery why hypercalcemia and hyperphosphatemia coexist with a severe impairment in bone mineralization caused by a deficiency of FGF 23 or Klotho, which is the coreceptor for FGF 23 hormone. According to the study, 1,25(OH) D-induced bone upregulation of the mineralization-inhibiting osteopontin and pyrophosphate molecules is the only factor driving the deficiency in mineralization in Klotho animals. FGF23 or Klotho mice and mutant mice lacking both the function of FGF23 and Klotho and the vitamin D receptor are used to demonstrate this. The mineralization deficit in FGF23 animals consists of two parts: a component driven by 1,25(OH)D, comparable to that in Klotho mice, and another component due to deficiency of FGF23, which results in increased osteopontin buildup. We discovered that FGF23 acts via FGF receptor-3 in a manner which is Klotho-independent to limit transcription of alkaline phosphatase and phosphate synthesis for cells of osteoblasts, indirectly controlling osteopontin secretion. As a result, osteocytes which release FGF23 may create an autocrine/paracrine feedback loop for the regional control in bone mineralization [186].

In 2012, Angelin Bo. Et al conducted research on circulating fibroblast growth factors as metabolic regulator. A novel class of proteins called the circulating FGFs act like

conventional hormones. Researchers with a focus on human metabolism suggest that FGF23 in circulation is critical for regulating vitamin D and phosphate metabolism and can have additional systemic effects, especially for chronic kidney disease; that FGF19 signaling is critical for regulating bile metabolism, whereas FGF19 physiological role for promoting glucose and lipid metabolism is less clear; and that, even though there are some questions, FGF23 in circulation is crucial for regulating bile metabolism [79].

Using Support Vector Machine, Wang c. et al. conducted research on the subject of Metabolic Biomarkers of Bones based on Osteoporotic Diagnosis Caused by Diabetes Mellitus and proposed that Diabetes Significantly Alters the Metabolism of Bones. Osteoporosis and fractures can be brought on by diabetes in any form, type 1 or type 2. However, it is still challenging to detect osteoporosis in persons with T2D due to the absence of consistent increases in bone mineral density. Another way to look at it is that osteoporosis is caused by an imbalance in metabolism of bones, which is closely tied with diabetes. Here, an effective and straightforward model of SVM was developed related to various combinations in biochemical markers, including turnover makers of bones, calcium and phosphorus, etc., to aid doctors in identifying osteoporosis in type 2 diabetes. Multiple evaluations were used to gauge categorization performance. With the combination features of Age, Sex, TP1NP, BMI, and OSTEOC, the final model's predictive accuracy rate is above 88 percent. According to experimental findings, the model has predicted a result for daily monitoring and early identification of type 2 diabetic osteoporosis [188].

Sultan E. et al. conducted research where according to the study's data, both well-controlled and poorly controlled female patients with T2D had altered bone metabolic markers, with the uncontrolled group exhibiting more pronounced anomalies. This might be as a result of the enormous impact glycemic control has on diabetics' bone turnover [189].

2. 8 Vitamin D and calcium on Insulin

Levels of insulin modifications are seen in volunteers with diabetes mellitus. The altered parameters were assessed in diabetes patients and calcium and Vitamin D concentrations were studied. Llaurado G. et al. in 2015 showed that AS is associated with plasma concentrations of FGF-23 (positively) and serum concentrations of vitamin D (negatively) in people with T1DM and a history of cardiovascular events [190]. Asbaghi O. et al. in 2019 showed that supplementing with calcium and taking high-dose vitamin D for a brief period of time had positive effects on blood sugar, insulin, and insulin resistance; Consequently, larger RCTs with sufficient and varied dosing regimens are necessary [110].

In 2004, Chiu K., et al. conducted research which showed a negative correlation between vitamin D concentration and insulin sensitivity and a detrimental effect of hypovitaminosis D on cell activity. Metabolic syndrome and insulin resistance are more likely to occur in people with hypovitaminosis D. More investigation is required to comprehend the underlying mechanics better [191].

2. 9 Parathyroid glands in type II Diabetes Mellitus

Relationship for altered parathyroid hormone concentrations in diabetes mellitus patients were also analysed and the following studies were taken into account.

Reis J. et al. in 2007 suggested that older men with greater PTH levels are more likely to develop metabolic syndrome, and that there are no variations in vitamin D levels between the sexes but why there is a sex difference in the PTH-metabolic syndrome link is unknown [192]. Hussain A. et al 2018 study showed that vitamin D levels were decreased in both groups, with no obvious difference between diabetic patients and control individuals. Both severely vitamin D deficient diabetes patients and healthy controls had elevated PTH levels. PTH levels were normal in the greater proportion of diabetic and healthy participants who

had modest vitamin D insufficiency. Healthy subjects of study having inadequate vitamin D levels had normal PTH levels. PTH levels were low in about 10% of diabetes individuals with significant vitamin D insufficiency. Regardless of diabetes mellitus, the population in our study had a general lack of 25-OHD, pointing to a larger requirement for vitamin D supplementation. This finding approaches the development of criteria for insufficiency of vitamin D, diagnosis, and treatment and emphasizes the significance of PTH testing for this context. It is not evident that vitamin D deficient patients have higher PTH levels [193].

2. 10 Sclerostin -The WNT/ ß CATENIN Pathway

Another outbreaking relationship was tried to find out with the modified levels of sclerostin altered in impaired bone metabolism and following studies were undertaken.

Gennaro L. et al in the year 2012 studied showed that sclerostin concentration were greater for men than in women across the board in the cohort, and they both significantly rose with age. Patients with DM1 but not DM2 retained the age-sclerostin positive connection. Furthermore, DM2 patients had greater sclerostin levels than DM1 patients or controls, and the persistence of this difference after accounting for body mass index and age. Regardless of age or gender, sclerostin was found to have an inverse relationship with PTH in patients with non-diabetes (r= -0. 30; P<0. 01), which is consistent with previous clinical and experimental results. The findings suggested that DM2 has higher sclerostin levels than normal. Additionally, both DM1 and DM2 may have problems with the transcriptional inhibition of sclerostin synthesis by PTH [194]. A study in 2012 showed that in T2DM patients, sclerostin of serum is found to be in relation to T2DM duration, glycated hemoglobin, indicators of bone turnover, and BMD. To determine how sclerostin affects bone metabolism in this population, more research is required [195].

CHAPTER-3

HYPOTHESIS FOR RESEARCH

HYPOTHESIS FOR RESEARCH

The goal of the current study is to compare type 2 pre-diabetes and diabetic mellitus patients to healthy controls to assess the functioning of FGF-23 and IGF-1 levels as potential metabolic biochemical markers in bone metabolism. To the best of our knowledge, there has not yet been any research on the role of FGF-23 and IGF-1 in bone metabolism.

India is known as the most populated Diabetic country of the world because of the major barrier to socio-economic development of the country. The alarming situation in the country is the increasing number of Diabetes patients which indicates the higher need to raise the control measures for the disease. Control of this disease largely depends on early detection of disease and treatment of patients. The present study provides an insight for current knowledge in altered bone turnover & biochemical parameter for patients with Diabetes Mellitus.

Despite advances in medical science technology, the diagnosis of T2DM is still primarily relied on clinical symptoms of the diabetic patient. For many clinicians around the world, the association between bone health and diabetes is a new topic of study. The goal of the current study is to compare pre-diabetic and diabetic patients with healthy controls to determine the impact of Insulin like Growth Factor (IGF-1) and Fibroblast Growth Factor 23 (FGF-23) and other metabolic markers in diabetic patients.

CHAPTER-4

OBJECTIVES OF THESIS

Objectives of Thesis

1. To assess and compare the serum levels of Fibroblast Growth Factor 23 (FGF-23), Insulin Like Growth factor 1 (IGF-1), and other metabolic bone markers in apparently healthy controls, prediabetes and type 2 diabetes mellitus patients

2. To find out the correlation between the serum levels of Fibroblast Growth Factor 23, Insulin Like Growth factor 1 with metabolic bone markers (e.g Osteocalcin, Parathyroid Hormone,Vitamin D, Calcium, Phosphorus, Alkaline Phosphatase) among healthy controls , pre diabetes , Diabetes Mellitus Type 2 patients

3. To evaluate the putative role of Fibroblast Growth Factor 23 and Insulin Like Growth factor in Bone metabolism as a possible metabolic biomarkers in Pre & Type 2 Diabetes Mellitus Patients

CHAPTER-5

MATERIALS AND METHODS

Materials used and Methodology.

75 individuals with prediabetes, 75 diabetes and 75 apparently healthy controls participated in the current cross sectional comparative research study in the age group of above 60 years attending the OPD (Out Patient Department) wards of Department of Endocrinology of SMS Medical College and attached groups of hospital, Jaipur (Rajasthan).

The Institutional Review Board of SMS Medical College Jaipur gave their approval to this study. According to pre-planned Proforma the cases were examined after only describing full explanation regarding the study in details and all criteria for inclusion and exclusion were applied on case and control. Then following evaluation was performed: -

- Detail Clinical history
- Demographic information and a general physical examination, duration of diabetes, alcohol use, physical activity, smoking, and BMI (Body mass index), arterial pressure, added indication of malnutrition (if present)
- Laboratory investigations (Routine & Specific).

5. 1 STUDY DESIGN:

The current study is a cross sectional type of observational study which is designed as a comparative study and conducted in the Department of Endocrinology with a 3 year plan study.

5. 2 SAMPLE SIZE:

Sample size was calculated (through statistical tool) at 95% confidence level assuming the absolute allowable error (precision) of 8 %, 225 patients of Diabetes mellitus,pre diabetes and healthy controls were required as sample size while considering feasibility for study purpose.

The patient's demographic data and history of illness was recorded in self-designed semi structured Proforma.

The following were assessed in the current investigation, which involved 150 people with Type 2 Diabetes and 75 controls that were matched for age and gender. :-

1. Diabetes duration, age, gender physical activity, BMI, alcohol consumption, smoking, HbA1c, fasting plasma glucose, blood pressure, and body lipid profiles.

2. Complications of microvascular and macrovascular diseases include myocardial infarction, stroke, retinopathy, and neuropathy.

3. The amount of FGF-23 in blood and the metabolic parameters of the minerals phosphate, calcium, parathyroid hormone (PTH), and 25-hydroxyvitamin D (25(OH) D) in older patients who visited the endocrinology department outpatient clinic at S. M. S. Hospitals in Jaipur were used to diagnose diabetes mellitus in both sexes. All the obtained results were comprised of age & sex matched 75 healthy control subjects having no history or symptoms of Type 2 Diabetes Mellitus.

5. 3 Criteria for Diagnosis of Diabetes

The American Diabetes Association's Standards of Care on Diabetes – Jan 2023 criteria for the Diabetes Guidelines are as follows:-

Degree of Glucose Control

Evaluation based on HbA1c (%)

- ✓ **Non-Diabetic** ≤ 5. 6
- ✓ **Pre-Diabetic** 5. 7 to 6. 4
- ✓ **Diabetic** ≥ 6. 5

- ✓ Patients with HbA1c levels <5. 6% are considered to have good metabolic control (Healthy control)
- ✓ A1C ≥6.5% (48 mmol/mol). The test should be performed for the diagnosis of diabetes in a laboratory with NGSP method certified and standardized to the DCCT (Diabetes Control and Complications Trial) assay.

OR

Criteria for the Diagnosis of Diabetes based on Glucose level -

- ✓ Fasting is defined as FPG (Fasting Plasma Glucose) ≥126 mg/dL (7.0 mmol/L). And no caloric intake for at least 8 h. OR
- ✓ 2-h PG ≥200 mg/dL (11.1 mmol/L)
- ✓ The test should be performed as described by WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water during OGTT (Oral Glucose Tolerance Test).
- ✓ Random plasma glucose ≥200 mg/dL (11.1 mmol/L) in a patient with classic symptoms of hyperglycemia or hyperglycemic crisis.

Criteria for the Diagnosis of Pre Diabetes-

- ✓ Prediabetes are defined by the existence of FPG 100 mg/dL to 125 mg/dL
- ✓ (Impaired Fasting Glucose) OR
- ✓ 2-h PG during 75-g OGTT 140 mg/dL to 199 mg/dL

5. 4 SELECTION CRITERIA:

1. Group A (n=75): Non Diabetic apparently healthy control.
2. Group B (n=75): Pre diabetic patients.
3. Group C (n=75): Type 2 diabetic patients

Inclusion criteria:

- Non-Diabetic apparently healthy controls.

- The current study involves voluntarily diagnosed cases with Pre diabetes and Type 2 Diabetes Mellitus patients (Othewise healthy).
- Age group – More than 60 years.
- Either male or female patients with T2DM.

Exclusion criteria:

- Patient with macro and micro vascular complications of diabetes.
- Patients with preexisting CKD with renal failure.
- Patients with previous cardiovascular events significant cardiac or neoplasm.
- Patients with severe inflammation derived from severe trauma or surgical infection.
- Hyperparathyroidism or any other endocrine or genetic disorder.
- Patients using any hormonal therapy, iodine containing drugs or therapy effecting hormone level.
- Known case of osteoporosis or therapy effecting hormone level e.g patient taking anti oesteporotic therapy or medication directly affecting studied parameters. (Patients on drugs that interfere in vitamin-D metabolism/ bone metabolism like alcohol Steroid or calcium preparation, zoledronic acid, teriparatide, denosumab or other anti-resorptive therapy).

5. 5 BLOOD SAMPLE COLLECTION AND ANALYSES FOR BIOCHEMICAL PARAMETERS:

- Blood sample was collected at the time of arrival of volunteer in outdoor patient room (OPD) after onset of symptoms before initiation of treatment by using aseptic technique.

- After an overnight fast, patients with Type 2 Diabetes Mellitus had venous blood samples, which ranged in size from 5 to 10 ml, drawn in the morning. The samples of patients were left in standing position for an hour; Blood serum was centrifuged at 2500 rpm and was fully analyzed by automated analyzer. After it was subjected for nutritional and endocrinal assessment.
- Commercially available kits using standard laboratory techniques/methods was performed for following investigations

5. 6 ROUTINE INVESTIGATION:

S. No.	PARAMETER	METHOD /REAGENT KIT
1	Blood Glucose Fasting /PP/R	Hexokinase and GOD-POD (Precision biomed Pvt. Ltd)
2	Serum Urea	Kinetic-Urease (Precision biomed Pvt. Ltd)
3	Serum Creatinine	Modified Kinetic-Jaffe (Precision biomed Pvt. Ltd)
4	Serum Alkaline Phosphatase (ALP)	Modified IFCC kinetic. (Meril Diagnostic)
5	HbA1c	HPLC (High-Performance Liquid Chromatography) (BioSystem, Barcelona,).
6	SGOT(AST)	Optimized UV IFCC (Precision biomed Pvt. Ltd)
7	SGPT(ALT)	Optimized UV IFCC (Precision biomed Pvt. Ltd)
8	Total Protein	Biuret Method (Precision biomed Pvt. Ltd)
9	Serum Albumin	Bromocresol green (BCG) Method (Precision biomed Pvt. Ltd)

10	Total Calcium	Arsenazo III (Precision biomed Pvt. Ltd)
11	Serum Phosphorus	UV Molybdate method (Precision biomed Pvt. Ltd)
12	Serum Cholesterol	CHOD –Trinder (Meril Diagnostic)
13	Triglyceride	GPO/PAP- Trinder (Precision biomed Pvt. Ltd)
14	HDL Cholesterol	Selective Detergent/Enzymatic Method (Meril Diagnostic)
15	LDL Cholesterol	Selective Detergent/Enzymatic Method (Meril Diagnostic)

5. 7 SPECIFIC INVESTIGATIONS:

S. No.	P ARAMETER	METHOD/ REAGENT KIT
1	Serum Fibroblast Growth Factor 23(FGF-23)	Chemiluminesence (Immunotopics Inc. San).
2	Serum Insulin Like Growth factor1(IGF-1)	Chemiluminesence (Immunotopics Inc. San).
3	Serum Osteocalcin	Chemiluminesence (Immunotopics Inc. San).
4	Vitamin D/Calcitriol	Chemiluminesence (Immunotopics Inc. San).
5	Parathyroid Hormine (PTH)	Chemiluminesence (Immunotopics Inc. San).

5. 8 STATISTICAL ANALYSES:

The collected data were transformed into variables, coded and entered in Microsoft Excel. Data were analyzed and statistically evaluated using SPSS-PC-20 version.

Normal distribution of different parameters was tested by the Shapiro-Wilk normality test. Quantitative data was expressed in mean±standard deviation and depends on normality difference between mean of more than two groups were compared by ANOVA test or Kruskal Wallis H test followed by posthoc test. Qualitative data were expressed in frequency and percentage and statistical differences between the proportions was tested by chi square test. Spearman correlation was used to see correlation between two quantitative variables. p value less than 0.05 was considered statistically significant. Correlation between FGF-23& IGF-1 with Controls results was found out by using Spearman correlation co-efficient. Significant levels were undertaken as P-value < 0. 05.

TEST METHODOLOGY -BLOOD SUGAR

Method of Hexokinase and GOD-POD

PRINCIPLE:

By releasing hydrogen peroxide, the enzyme glucose oxidase (GOD) in serum/plasma oxidizes glucose to gluconic acid, which is then broken down by the enzyme peroxidase into water and oxygen (POD).

BLOOD SUGAR STABILITY AND STORAGE:

- The reagents were stable up until the labelled expiration date while being stored at 2 to 8°C.
- When reagents were opened then contamination should be avoided.

SPECIMEN:

- Serum or fluoride plasma.
- Stability 24hrs. at 2-8°C

(When made within 30 minutes of collection, serum or plasma). Discard contaminated specimens.

PREPARATION OF REAGENT:

Ready to use reagents should be used.

PROCEDURE:

Label and pipette in the test tubes as Blank (B), Standard (S), and Test (T) as follows:

S. No.	Reagent	Procedure		
		B	**S**	**T**
1.	Glucose Reagent (1)	1. 0 ml	1. 0 ml	1. 0 ml
2.	Glucose Standard (2) (Conc^{n}. :100mg/dl)	--	10 µl	--
3.	Specimen	--	--	10 µl

After thoroughly combining them, incubate them for either 15 or 10 minutes at 25°C or 37°C.

Read the absorbance for the S and T after the reagent B at 505 nm or using a green filter after thoroughly mixing (500-540nm).

FORMULA:

Glucose Conc^{n}. (mg/dl) = Abs. of Test/Abs. of Standard X 100

NORMAL RANGES:

Serum/plasma (fasting) : 75-115 mg/dL

Serum/plasma (post prandial): 90-140 mg/ dL

It is advised that each lab create its own reference/normal range [196,197].

UREA

Method of Kinetic-Urease

PRINCIPLE:

In the presence of urease, urea is transformed to ammonia. Ammonia is then coupled to alpha ketoglutarate in glutamate dehydrogenase (GLDH) presence, resulting in the conversion of the NADH analogue to a NAD. The amount of urea present in the patient sample directly correlates with the rate of NADH analogue consumption. Following these reactions, for enzymatic determination:

COMPOSITION OF REAGENT:

Reagent 1: Buffer Reagent

Reagent 2: Enzyme Reagent

Urea Standard: 40mg/dl

MATERIALS REQUIRED BUT NOT PROVIDED:

- Clean & Dry Glassware.

- Micropipettes & Tips.

- Colorimeter or Bio-Chemistry Analyzer.

SAMPLES:

Hemolysis free serum.

Dilute the urine in ratio of 1:100 with distilled water. Anticoagulants containing fluoride or ammonium ions should not be used.

REAGENT STABILITY:

Reagents were kept stable up to the date of expiry specified as on the label when stored and tightly closed with 2-8°C temperature, shielded from light, and any other contaminations to be prevented throughout their usage.

Avoid being under direct sunlight.

WORKING REAGENT:

Mix 1 part (200 µl) of the enzyme reagent with 4 parts (800 µl) of the buffer reagents. The reagents were stable at 2-8°C for 30 days.

ASSAY PROCEDURE:

Mix it well, and take initial reading after 30 secs of incubation as absorbance A1. Exactly after a 60 second interval read absorbance A2.

Determine Δ Absorbance.

Δ Abs. = A2-A1

FORMULA:

Urea Conc. (mg/dl) = Δ Abs. of Sample/ ΔAbs. of Standard X Conc. of Standard [198]

CREATININE

Modified Kinetic-Jaffe Method

PRINCIPLE:

Picric Acid and Creatinine combine to generate an orange-colored complex with alkaline picrate in an alkaline media. The amount of creatinine present in the sample directly correlates to the color intensity that develops over time.

STABILITYAND STABILITY:

Reagent is stable till the expiry date listed on the labels and stored at 2 to 8 °C.

SPECIMEN:

- Preferred Specimen is the Serum/Plasma.
- Stability: 24 hours at 2-8°C for serum & plasma.
- Urine sample should be freshly used.

REAGENT COMPOSITION:

Picric acid -> 5. 0mMol/L

Sodium Hydroxide -> 150mMol/L

Activators and Stabilizers

REFERENCE VALUES:

Serum		**24-hour collection of urine**
Males	0. 6-1. 5mg/dl	1.1 - 3. 0gm
Females	0. 6-1. 4mg/dl	1.0 - 1. 5gm

PROCEDURE:

The test tubes with pictures are labelled Standard (S) and Test (T).

Reagent	(S)	(T)
Creatinine Reagent	1. 0ml	1. 0ml
Creatinine Standard (Conc. 2mg/dl)	100µl	-
Specimen	-	100µl

They should be thoroughly combined before reading the Standard (S) and Test (T) absorbances against distilled water at 520 nm:

Initial absorbance A_0- exactly after 30 sec (37°C is the Reaction Temperature)

Final absorbance A_1 - exactly 90 sec. after A_0

Determine ΔA for Standard (S) and Test (T)

$\Delta AS = AS_1 - AS_2$

$\Delta AT = AT_1 - AT_2$

CALCULATIONS:

Serum Creatinine (mg/dl) = ΔAT/AS X 2

Urine Creatinine (gm/dl) = ΔAT/AS X 2

Urine Creatinine/24hours = Urine Creatinine in gm/L X Vol. of Urine in 24 hours collected in Liters [199, 200, 201]

SERUM ALKALINE PHOSPHATASE (ALP)

Modified IFCC kinetic Method.

Principle:

Alkaline phosphatase hydrolyzes 4-nitrophenol phosphate to produce 4-nitrophenol in an alkaline environment. Alkaline phosphatase activity can be determined by spectrophotometrically measuring the 4-nitrophenol produced at 405 nm.

COMPONENTS OF KIT:

Composition

R1 – Alkaline Phosphatase Reagent : Buffer of AMP 700mmol/l, salts of magnesium 2. 7mmol/l, zinc salt 1. 36mmol/l, HEDTA 2 mmol/l : 2. 69 mmol/l, pNPP 19. 51 mmol/l.

REQUIRED MATERIALS BUT NOT PROVIDED:

Spectrophotometer UV/VIS, Laboratory instrumentation with holder of thermostatic cuvette or clinical chemistry analyzer: semi-automated, calibrated micropipettes, glass or high quality polystyrene cuvettes, test tubes/rack, heating bath, controls, saline.

STORAGE, REAGENT PREPARATION& STABILITY:

Reagents ought to be prepared for use. Avoid sources of direct light.

Stability: at 2 to 8°C, up to the label's expiration date.

REAGENT DETERIORATION:

If the agent's absorbance against distilled water at 405 nm is greater than 1. 25, discard it.

SPECIMENS:

Saline, plasma (heparinate only). When stored at room temperature, sera typically exhibit a small increase in activity, ranging from 1% over six hours to 3-6% over one to four days. Even in sera kept in the refrigerated, activity rises gradually. After the serum is thawed,

activity in frozen sera declines and then gradually increases. EDTA, Citrate, and Oxalate are not suitable because of inhibition of ALP activity.

TEST PROCEDURE:

Put the reagent in the tube	1000µl
Sample	20 µl
Mix, take a preliminary absorbance reading after one minute, and incubate at 37 °C. Perform the additional 2 readings at intervals of 60 seconds. Determine the ΔA/min.	

RESULT CALCULATION:

Serum/plasma: ALP U/I = ΔA/min x 2764

SI conversion factor: 1U/I x 0. 017 = 1µkat/l [202]

HbA1c

HPLC (High-Performance Liquid Chromatography)

Principle:

A latex turbidimetric assay is used to measure the hemoglobin A1C (HbA1C) concentration after the hemolysate has been prepared. The various hemoglobin's found in the hemolysate are unspecific ally adsorbed in a ratio corresponding to their concentration on the surface of the latex particles. Agglutination caused by HbA1C anti-human antibody is proportional to amount of hemoglobin A1C.

Composition:

A. Latex particle suspension, reagent, sodium azide 0. 95 g/L, pH 8. 0.

B. Anti-human HbA1C antibody, reagent, stabilizers, and p/h 6. 0.

Storage:

Store them at 2-8ºC temperature.

Testing of the human blood used to create the standard revealed that it did not contain either HBs antigen or anti-HIV antibodies. The standard should be treated with caution, though, as it may be contagious.

Reagent Preparation:

Reagents are offered in a usable state.

Samples:

EDTA was used as an anticoagulant throughout the typical methods used to collect venous blood.

Blood HbA1C is constant for 7 days at 2-8ºC.

Method:

Preparation of Hemolysate

1. Into a test tube, pipette out:

Blood	50 µL
Distilled water	5. 0 mL

2. Mix gently. Avoid the formation of foam. Incubate for 5 minutes at room temperature.

Calculation:

The HbA1C results can be linked to the IFCC Reference Method.

%HbA1C-NGSP.

DCCT (%) is 0. 0915 times HbA1C-IFCC (mmol/mol) plus 2. 15.

Reference Values:

The following cut-off values.

IFCC mmol/mol	NGSP. DCCT (%)	Reference Values/Degree of control
20 - 48	4. 0 - 6. 5	Non-Diabetic
42 - 53	6. 0 - 7. 0	Goal
53 - 64	7. 0 - 8. 0	Good Control
>64	>8. 0	Action Suggested

Only for reference [203-204].

SGOT-AST

Optimized UV IFCC Method

PRINCIPLE:

The reaction is catalyzed by Aspartate transaminase between L-aspartate and alpha-ketoglutaric acid, which yields oxaloacetate and glutamate (GOT-AST). When Malate Dehydrogenase (MHD) is present, Oxaloacetate and NADH combine to create Malate and NAD. The photometric measurement of NADH oxidation is inversely associated with the GOT activity of the sample.

REAGENTS:

Reagent 1 includes Buffer Reagent

Reagent 2 includes Enzyme Reagent.

SAMPLES:

Serum free of hemolysis. Heparin or EDTA plasma.

WORKING REAGENT PREPARATION:

One part of Reagent 2 should be mixed with four parts of Reagent 1.

GENERAL SYSTEM PARAMETERS:

ASSAY PROCEDURE:

Working Reagent	1000 µl
Sample	100 µl

After 60-second incubation, check the absorbance reduction every minute for 3 minutes at 37°C.

Determine the ΔA/min.

CALCULATION:

At 340 nm with 1cm Light Path

SGOT Activity (U/l) = ΔA/min. x 1746

REFERENCE NORMAL VALUE:

0 to 35 U/L

Each lab should define its own normal ranges [205].

SGPT-ALT

Optimized UV IFCC Method

PRINCIPLE:

L-Alanine and 2-oxoglutarate are converted by ALT at the optimum pH. Lactate dehydrogenase (LDH) transforms the pyruvate in the presence of NADH/NAD+ coenzyme, into L-lactate while the NADH/NAD+ oxidoreductive process leads to lower the absorbance at 340 nm. Differences in serum ALT activity and absorbance are related.

REAGENTS:

Reagent1: Buffer Reagent

Reagent 2: Enzyme Reagent

SAMPLES:

Serum free of hemolysis. Heparin or EDTA plasma.

PREPARATION OF WORKING REAGENT:

Reagents 1 and 2 should be combined in a ratio of 4:1.

Working Reagent is stable between 2 and 8 °C for 30 days.

ASSAY PROCEDURE:

Working Reagent	1000µl
Sample	100µl

Quantify the decrease in absorbance every 60 seconds for three minutes at 37 degrees Celsius after mixing and after a 60-second incubation. Determine the Δ A/min.

CALCULATION:

At 340 nm with 1 cm Light path

SGPT Activity (U/I) = Δ A/min. $\times$ 1746

REFERENCE NORMAL VALUE:

0 to 40 U/L

Normal value for infants is higher than adults.

The reference values are merely illustrative. Each lab should define its own normal ranges [205].

TOTAL PROTEIN

Biuret Method

PRINCIPLE:

1. Dehydration, multiple myeloma, and chronic liver disorders all have higher levels of total protein than healthy individuals, but renal diseases and advanced liver failure have lower levels.
2. Total Protein is a reagent kit that uses the biuret method to quantify human serum and plasma total protein content.

COMPONENTS OF REAGENT:

Components Concentration

Component	Concentration
Cupric Sulphate	7mmol/l
Potassium Iodide	6mmol/l
Sodium Hydroxide	700mmol/l
Na^+/K^+ tartrate	40mmol/l

PREPARATION:

Total Protein is a reagent that is ready for use.

The Kit is still stable after the labelled expiration date.

SPECIMEN COLLECTION AND PRESERVATION:

Blood was collected in a sterile tube. Plasma with EDTA or heparin can be used. Because hemoglobin reacts as a protein in the total protein assay, avoiding hemolysis. The stability of total protein in serum and plasma is at 2–8 °C for 3 days and at -20 °C for 6 months.

ASSAY PROCEDURE:

Parameters.

Procedure:

Before usage, the reagent should be warmed up to room temperature.

The reagent used was 1. 0 ml.

Standard used was 0. 02 ml (20µl).

The assay was incubated at 37 °C for 10 minutes.

The absorbance was taken at 546 nm (530-570).

If not exposed to direct light, the final color remains stable for eight hours.

FORMULA:

Total protein (gm/dl) = Abs of sample / Abs of standard x 5

REFERANCE VALUE FOR NORMAL PEOPLE:

Adults :6. 2-8. 5 gm/dl [206-208]

ALUBMIN

Bromocresol green (BCG) Method

PRINCIPLE:

In cases of severe liver illness, malabsorption, diarrhea, eclampsia, and nephrosis, albumin levels are reduced. The degree of dehydration has increased.

A single reagent kit called albumin is used to quantitatively measure albumin levels in human serum and plasma using the BCG technique.

REAGENT COMPONENTS:

Succinate buffer at pH 3. 6 was used at the concentration of 100 mmol/l.

Bromocresol green was used at the concentration of 0. 15 mmol/l

PREPARATION, STORAGE AND SRABILITY:

A single reagent that is ready for usage is albumin.

The reagent kit should be kept between 2 and 8 degrees Celsius and is stable through the labelled expiration date.

COLLECTION of SPECIMENS AND ITS PRESERVATION:

Blood should be drawn into a clean, dry tube. We can use EDTA plasma, heparinized plasma, or serum. Avoid stagnant veins.

The stability of albumin in serum or plasma is 2 days at 2-8 °C and 6 months at -20 °C.

PROCEDURE:

Reagent should be pre warmed at RT.

Assay should be performed as given below:

The reagent used was 1. 0 ml.

Specimen /Standard used was 0. 01 ml (10µl)

The test mixture at was incubated at R. T. for 60 seconds. After that, abs. was measured at 600 (600-650) nm against a blank surface. The final hue remains constant for 10 minutes. if not directly exposed to light.

FORMULA:

$$\text{Albumin (gm/dl)} \; \frac{\text{Abs of sample}}{\text{Abs of standard}} \times 4$$

REFERENCE VALUE:

3. 5 -5. 2 g/dl [209-210]

CALCIUM

Arsenazo III Method

PRINCIPLE:

The complex that Ca^{2+} and Arsenazo III create at a neutral pH is directly correlated with the amount of calcium present in the sample in terms of color intensity.

***COMPOSITION OF REAGENT*:**

Reagent 1: Arsenazo III Reagent

Calcium Standard: 10mg/dl

MATERIAL REQUIRED BUT NOT PROVIDED:

- Clean and Dry glassware
- Micropipettes and Tips
- Colorimeter or Bio - chemistry Analyzer

SAMPLES:

Free hemolysis serum.

Distilled water is used to dilute urine in a 1:3 ratio, and 0. 1N HCL is used to alter the pH to 3–4.

WORKING REAGENT:

The reagent is ready for use.

GENERAL SYSTEM PARAMETERS:

ASSAY PROCEDURE:

After combining, incubate at R. T. for 5 minutes, and then measure the optical density (A).

CALCULATION:

Calcium Conc. (mg/dl) = O. D of sample / O. D of S × Conc. Of S

LINEARITY:

Up to 25 mg/dl, the reagent is linear.

If the sample's calcium concentration is higher than 25 mg/dl, properly dilute it and perform another test. Divide the outcome by the dilution factor.

REFERENCE NORMAL VALUE:

8. 8- 10. 2 mg/dl [211]

PHOSPHOROUS

UV Molybdate Method

PRINCIPLE:

Molybdic acid and inorganic phosphate combine in an acidic environment to generate an unreduced phosphomolybdic acid complex that gives absorbance at 340 nm. The relationship between the absorbance and phosphorus content of the sample is straightforward.

REAGENT COMPOSITION:

Reagent 1 : Molybdate reagent

Phosphorous standard: 5. 0 mg/dl

COLLECTION AND PRESERVATION* OF *SAMPLES:

Blood was drawn into a clean, dry container. We can use EDTA plasma, heparinized plasma, or serum. Avoid stagnant veins.

24-hour serum plasma. distilled water and urine diluted 1:20.

PREPARATION OF REAGENT:

Ready to use reagents.

REAGENT STORAGE AND STABLITY:

When kept at 2-8˚C, reagents are stable until the expiration date.

PARAMETERS:

VOLUME IN TEST TUBES

	B	**S**	**Sample**
SAMPLE	-	-	20 µl
S	-	20 µl	-
REAGENT	1000 µl	1000 µl	1000 µl

5

minutes at 37 °C after thoroughly blending. Measure the sample's (Ac)and the standard's final absorbance (As) against the blank reagent.

CALULATION:

Ac / As × Conc. S. = mg/dl Serum
Ac /As × Conc. S ×20 = mg/dl Urine

LINEARITY:

The process is linear up to 12. 0 mg/dl of concentration.

REFERANCE INTERVAL:

Adults: 2. 5 -4. 5 mg/dl = 0. 81- 1. 45 mmol/L

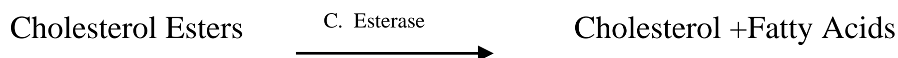
Children: 4. 0 – 7. 0 mg/dl = 1. 29 – 2. 26 mmol/L

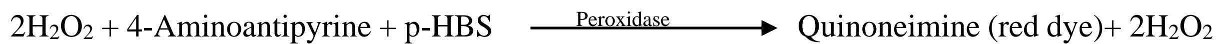
Urine: 0. 4 – 1. 3 g/24hr. Urine = 12. 9- 42 mmol/24hr [212]

CHOLESTROL

CHOD –Trinder Method

PRINCIPLE:





When the sample is read at 520 nm, the amount of total cholesterol is directly proportional to the intensity of the red color produced.

REAGENT AND COMPOSITION:

Reconstituted reagent is what is meant by concentration.

4-Aminoantipyrine, 0.6 mM; Sodium Cholate, 8.0 mM; Cholesterol Esterase, ≥150 u/L; Cholesterol Oxidase, ≥200 u/L; Horseradish Peroxide, 1500 u/L; p-Hydroxybenzene Sulfonate, 20 mM; Surfactant Buffer, pH 7. 2; non-reactive stabilizers and filters; and sodium azide (.01%) as a preservative.

WORKING REAGENT PREPARATION:

Reagent is ready to use.

STABLITY AND STORAGE:

2-8˚C storage

It should not be used if:

1. The vial has been clogged with moisture and should not be used.

2. The functioning reagent does not operate according to expectations.

Precautions

1. This reagent is only to be used for in vitro diagnosis.
2. The reagent includes sodium azide. Avoid digestion. Metal azide, which is highly explosive, may occur when lead and copper pipes react. Flush with a lot of water after disposal to avoid azide buildup.

SPECIMEN COLLECTION AND STORAGE:

It's advised to use non hemolyzed serum. Serum cholesterol is said to be stable for seven days at room temperature (between 18 and 25 °C) and six months when stored and well covered from evaporation.

INTERFERENCES:

The levels of cholesterol are impacted by a variety of medications and substances.

MATERIALS PROVIDED:

Cholesterol Reagent

CALCULATIONS:

Abs. (Patient)/Abs. (S) $\times$ Conc. of S (mg/dl) = Cholesterol(mg/dl)

EXPECTED VALUES:

A Recommended Range of the NCEP is :

Desirable Cholesterol: <200 mg/dl

Borderline – High Cholesterol: 200-239 mg/dl

High Cholesterol:> 240 mg/dl [213,214]

TRIGLYCERIDES

GPO/PAP- Trinder Method

PRINCIPLE:

Lipoprotein lipase breaks down the samples' triglycerides into glycerol and fatty acids (LPL). Then, Glycerol Kinase (GK) phosphorylates glycerin in the presence of ATP and Mg++ ions. Following that, in the presence of molecular oxygen, Glycerol-3-Phosphate Oxidase (GPO) oxidizes Glycerol-3-P. (O2). Hydrogen peroxide, 4- amino antipyrine, and phenol derivative interact in the presence of peroxidase to create a colorful product that absorbs well at 505 nm (490-550 nm) (POD).

COMPOSITION OF REAGENT:

Reagent 1: Enzyme reagent

Triglyceride standard: 200 mg/dl

MATERIAL REQUIRED BUT NOT PROVIDED:

- Clean and Dry Glassware
- Micropipettes and Tips.

- Colorimeter or Bio-Chemistry Analyzer.

SAMPLES:

Heparin or EDTA Plasma, free of Hemolysis serum.

STABILITY OF REAGENT:

When kept tightly covered, at a temperature between 2 and 8 degrees Celsius, shielded from light, and protected while being used, reagents are stable until the labeled expiration date.

WORKING REAGENT:

Ready for use reagents

GENERAL SYSTEM PARAMETERS:

ASSAY PROCEDURE:

	B	**S**	**Sample**
Reagent	1 ml	1 ml	1 ml
S	-	10 µl	-
Sample	-	-	10 µl

After a five-minute incubation, combine and check the optical density (A).

CALCULATION:

$$\text{Triglyceride Conc. (mg/dl)} = \frac{\text{OD of sample}}{\text{OD of S}} \times \text{Conc. of S}$$

LINEARITY:

Up to 1000 mg/dl, the reaction is linear.

If the triglyceride concentration surpasses 1000 mg/dl, adequately dilute the sample and do a new analysis. Divide the outcome by the dilution factor.

REFERENCE NORMAL VALUE:

Female: 40-140 mg/dl

Male: 50-165 mg/dl [215]

HDL CHOLESTEROL

Selective Detergent/Enzymatic Method

PRINCIPLE:

The HDL Cholesterol test uses a homogeneous two-reagent system to assess HDL-Cholesterol in serum or plasma while accounting for other lipoprotein particles. The test involves two basic steps. To produce a colorless end product in step one, cholesterol oxidase, peroxidase, and A chromogen system, cholesterol esterase, cholesterol oxidase, and the release of HDL cholesterol produce a blue color complex that may be detected dichromatically at 546/670 nm. The increase in absorbance that follows is inversely correlated with the sample's HDL-C content.

KIT COMPONENTS:

Composition:

R1 – HDL Direct Reagent: Good’s Buffer 30mmol/l, 4-AAP 0. 9 mmol/l. POD 2400U/l, Ascorbate Oxidase 2700 U/l

R2 – HDL Direct Reagent: Goods buffer 30 mmol/l, CHE 4000U/l, CO 20000 U/l, DAOS 0. 8mmol/l

R3 – HDL Direct Calibrator: Concentration is lot specific, see vial label.

R4 – HDL Direct Lipid Control: Concentration range is lot specific, see pack insert/instructions for use.

MATERIALS REQUIRED BUT NOT PROVIDED:

Laboratory equipment, such as a clinical chemistry analyzer or a spectrophotometer UV/VIS with thermostatic cuvette holder: semi-automated, glass, calibrated micropipettes, or high quality polystyrene cuvettes, test tube/rack, heating bath, saline.

REAGENT PREPARATION, STORAGE AND STABILITY:

Reagents 1 and 2 are prepared for usage.

By adding the specified amount of distilled or deionized water from the vial label, Calibrator and Liquid Control should be reconstituted. After sealing the vial, leave it stand for five minutes. By gently whirling the contents of the vial, you can dissolve them without creating foam. Do not tremble.

Unopened reagents, calibrators, and lipid controls are stable at 2 to 8 °C up until the expiration date on labels. Keep the reagents from freezing.

Reagents are stable at 2 to 8 °C for up to 4 weeks once opened.

At 2–8 °C, the calibrator and lipid control remain stable for several weeks after reconstitution. The reconstituted calibrator and lipid control can be divided into smaller amounts and kept at -20°C for one month.

REAGENT DETERIORATION:

Reagents should not be used after the printed expiration date on the label. Avoid thawing and freezing the liquid control and calibrator that were aliquoted.

SPECIMEN:

Use EDTA plasma or hemolyzed serum. Avoid using heparin or citrate as an anticoagulant. When frozen once, serum and plasma are stable at 2-8°C for 7 days or for 1 month at -20°C.

TEST PROCEDURE:

Dispense	**Blank**	**Calibrator**	**Sample**
Reagent 1	360 µl	360 µl	360 µl
Calibrator	-	5 µl	-
Sample	-	-	5 µl
Mix and incubate at 37^{0} C for 5 min.			
Reagent 2	120 µl	120 µl	120 µl
Mix and incubate at 37 ^{0}C for 5 minutes. At 546/670 nm, compare the absorbance of the calibrator (Ac) and sample (Ax) to the reagent blank.			

RESULT CALCULATION:

Serum/Plasma:

HDL Cholesterol mg/dl = Ax/Ac x Concentration of calibrator

SI conversion factor: 1 mg/dl x 0. 0259 = 1 mmol/l

Expected Values	**Male**	**Female**
Low Risk	>50 mg/dl	>60 mg/dl
Moderate Risk	35 – 50 mg/dl	45-60 mg/dl
High Risk	< 30 mg/dl	<45 mg/dl

Each laboratory should verify this range or determine a reference interval for the community it serves [216].

LDL CHOLESTEROL

Selective Detergent/Enzymatic Method

PRINCIPLE:

The assay comprises two separate reaction steps:

1. Chylomicron, HDL and VLDL cholesterol are eliminated by cholesterol esterase, cholesterol oxidase, and then catalase.
2. Particular assessment of LDL-Cholesterol following detergent-induced LDL-Cholesterol release in Reagent 2.

When measured at 600 nm, the intensity of the quinonimine dye generated is exactly proportional to the cholesterol concentration.

SAMPLE COLLECTION AND PREPARATION:

Samples should be taken from a person who is fasting. Samples may be taken from a non-fasting person, but the results should be carefully interpreted. It is advised to use serum, heparinized plasma, or EDTA plasma as samples. Reduced outcomes are brought on by EDTA plasma. 6 days of serum stability between +2 and +8 ^{0} C. The samples shouldn't be frozen. Centrifuge is any sample that contains precipitates before utilization.

STABILITY AND PREPARATION OF REAGENTS:

R1 should be Enzyme Reagent 1

Ready for usage supply when kept at +2 to +8 ^{0} C, it is stable until its expiration date.

R2 should be Enzyme Reagent 2

Ready for usage supply when kept at +2 to +8 ^{0} C, it is stable until its expiration date.

MATERIALS PROVIDED:

Direct LDL-C Reagent

INTERFERENCES:

Icteric samples (bilirubin <25 mg/dl), hemolytic samples (Hb <1. 0 g/dl), and lipemic samples (triglycerides <600 mg/dl) have no effect on the assay. Before assay, triglyceride samples from lipemic patients should be diluted 1 + 9 with 0. 9% (w/v) NaCl. The equivalent outcome needs to be multiplied by ten [217-219].

EXPECTED VALUES:

mg/dl	**mmol/l**	
<100	<2. 59	Optimal
100-129	2. 59-3. 35	Near or above optimal
130-159	3. 36-4. 12	Borderline High
160-189	4. 13-4. 89	High
>190	>4. 90	Very high

FGF-23 (FIBROBLAST GROWTH FACTOR-23)

Chemiluminescence Method

PRINCIPLE:

An immunoassay with three steps that uses sandwich chemiluminescence is used to quantify FGF23. The magnetic particles (solid phase) are coated with a certain monoclonal antibody, and fluorescein is coupled to a separate monoclonal antibody against FGF23 molecule (fluorescein-antibody conjugate).

FGF23 that is present in calibrators, samples, or controls binds to the antibody conjugate during the initial incubation. The solid phase is added to the reaction following the initial incubation. Only when both antibodies are bridged by FGF23 molecules does a sandwich develop. After the second incubation, a monoclonal anti-FGF23 antibody tagged with fluorescein already coupled to the solid phase reacts with a monoclonal anti-fluorescein antibody conjugated to an isoluminol derivative. At the third incubation, the unattached substance is eliminated.

After that, the starter chemicals are added, which starts a flash chemiluminescence process. In order to determine the amount of isoluminol-antibody conjugate, a photomultiplier measures the light signal as relative light units (RLU), which are a measure of the FGF23 concentration in calibrators, samples, or controls.

MATERIALS PROVIDED:

- Magnetic particles (1. 5mL)
- Buffer A (6 mL)
- Assay buffer 1 (13. 5 mL)
- Conjugate (13. 5 mL)

STORAGE AND STABILITYOF REAGENT:

- Sealed: Up until the expiration date, stable at 2°-8°C.
- Stability when opened on board or at 2°–8°C for up to 12 weeks.
- For a reagent integral that has already been opened, always utilize the same analyzer.
- To store reagent integral upright, use the analyzer's included storage rack.

- Avoid freezing.

- Store upright to aid in the appropriate resuspension of magnetic particles later.

- Steers clear of bright light.

SPECIMEN COLLECTION AND PREPARATION:

Utilizing collecting containers requires careful adherence to the manufacturer's instructions. Venipuncture should be used to draw blood aseptically, and following centrifugation, Serum should be separated from red blood cells, clots, or gel separator.

Conditions for 10 minutes of centrifugation range from 1,000 to 3,000 g. Depending on the suggestions made by the tube maker, conditions may change. The laboratory should assess and validate the use of alternative centrifugation conditions. (Bowen et al., Clinical Biochemistry, 43, 2010).

Regarding storage restrictions, samples should be aliquoted and maintained deep-frozen (-20°C or below) unless the assay is run within 8 hours after sample collection.

To increase the consistency of results, samples taken from red cells, clot or gel separators that contain fibrin, turbidity, lipaemia, or erythrocyte debris, or specimens that have been frozen and thawed, must be clarified by additional centrifugation (10,000 g for 10' is advised). Transferring specimens with a lipid layer on top requires using a secondary tube and being careful to only transfer the cleared material. It is not advisable to examine materials that are severely haemolyzed, lipemic, contain particulates, or clearly show microbial contamination. Prior to assaying, look for and eliminate air bubbles.

A minimum of 300 µL of specimen (150 µL of material plus+ 150 µL of dead volume) is needed for the determination.

ASSAY PROCEDURE:

The bar codes on the reagent integral label of the LIAISON® Analyzer allow for the identification of each test parameter. The integral cannot be applied if the analyzer cannot

read the bar code label. Please contact your local DiaSorin technical support for guidance before discarding the reagent component.

INTERPRETATION OF RESULTS:

For the unidentified samples, the Analyzer automatically determines the FGF23 concentrations. Consult the appropriate Analyzer Operator's Manual for more information.

On LIAISON® and LIAISON® XL, calibrators and controls may produce differing RLU or dosage results, but patient outcomes are comparable.

Assay range: From 5 to 5000 pg./mL, the Analyzer directly determines FGF23 concentrations. Samples with FGF23 concentrations over the assay range may be pre-diluted using the instrument's Dilute function before being retested (the suggested dilution factor is 1:10; on the LIAISON® Analyzer, the recommended dilution would be 360 µL diluent + 40 µL patient sample). For the purpose of determining the FGF23 concentration levels of the neat specimens, the values automatically multiplied by the dilution factor. Up to five sample pre-dilutions can be carried out using the specimen diluent excess that is available in the reagent component.

EXPECTED VALUES:

For the population under consideration, each laboratory should determine its own range of predicted values. A research using EDTA plasma samples from 910 seemingly healthy European people aged 18 to 89 with an estimated GFR (determined by the CKD epi formula) > 60 mL/Mn was conducted to determine the normal range of LIAISON® FGF 23 [220-222].

Subjects	Median FGF-23	Observed Ranges 1.5 ºC to 97. 5 ºC Percentile
N= 910	57. 5 pg./mL	23. 2 – 95. 4 pg./mL

INSULIN LIKE GROWTH FACTOR-1 (IGF-1)

Chemiluminescence Method

PRINCIPLE:

After separating IGF-I from other binding proteins, the method for the quantitative measurement of IGF-I is a one-step sandwich chemiluminescence immunoassay (CLIA). Magnetic particles (solid-phase) are coated with a monoclonal antibody, and an isolation derivative is coupled to a different monoclonal antibody (isolation-antibody conjugate).

IGF-I that is present in calibrators, samples, or controls binds to both the conjugate and the solid phase during incubation. Following incubation, a wash cycle is used to remove the unbound material.

The starter reagents are then introduced, which triggers a flash chemiluminescence reaction. A photomultiplier measures the light signal as relative light units (RLU), which is an indication of the quantity of isolation conjugate present in the calibrators, samples, or controls.

MATERIALS PROVIDED:

- Magnetic particle suspension (2. 3 mL)
- Conjugate (12. 0 mL)
- Neutralization buffer (18. 0 mL)
- Acidification solution (28. 0 mL)

STABILITY AND STORAGE OF REAGENT:

Till the expiration date, sealed items are stable at 2 to 8 °C.

Opened: at 2 to 8 °C or onboard: Four weeks of stability.

If the controls are discovered to be within the expected ranges after this time, it is still possible to continue utilizing the reagent integral.

For a reagent integral that has already been opened, always use the same LIAISON® Analyzer.

To enable subsequent appropriate resuspension of magnetic particles, store upright.

Use the storage rack that comes with the LIAISON® Analyzer family to store reagent integral upright. Avoid being under direct sunlight.

SPECIMEN COLLECTION AND PREPARATION:

The only sample material validated is human serum.

Collect serum according to established methods. Avoid hemolysis. Before testing, carefully defrost the samples, combine them, look for air bubbles, and get rid of them before assaying. Thawed samples kept at 2-8 °C must be used within 6 hours.

If the test is not performed on the day of sample collection, the serum should be separated from the sediment and be stored in a separate tube at -20 °C.

It is not recommended to test materials that are severely haemolyzed, lipemic, contain particulates, or clearly show microbial contamination.

Avoid using clotted samples.

Steer clear of frequent freeze-thaw cycles. Get rid of any remaining aliquot volume.

A single determination requires a minimum of 170 µL of specimen (20 µL of specimen plus 150 µL of dead volume).

The acidification solution provided in the integral is validated for predilution only. No diluent is provided for specimens with concentrations above the assay range.

ASSAY PROCEDURE:

Correct assay performance is ensured by strict adherence to the analyzer operator's instructions.

Each test parameter on the LIAISON® Analyzer is identifiable by a barcode on the reagent integral label. The integral cannot be applied if the analyzer is unable to read the barcode label. Please contact your local DiaSorin technical support for guidance before discarding the reagent component.

Analysis tool LIAISON® XL: Information contained in the reagent integral Radio Frequency Identification Transponder is used to identify each test parameter (RFID Tag). The integral is useless if the analyzer is unable to read the RFID Tag. Please contact your local DiaSorin technical support for guidance before discarding the reagent component.

INTERPRETATION OF RESULTS:

For the unidentified samples, the analyzer automatically determines the IGF-I values in ng/mL. Consult the LIAISON® operator's manual for more information.

On LIAISON® and LIAISON® XL, calibrators and controls may produce differing RLU or dosage results, but patient outcomes are comparable [223-225].

OSTEOCALCIN

Chemiluminescence Method

PRINCIPLE:

A direct, two-site sandwich type chemiluminescence immunoassay is used to quantify osteocalcin (CLIA). Mouse anti-human osteocalcin synthetic antibody has been affinity-purified and coated. A derivative of isoluminol is conjugated to the second mouse antibody that was affinity purified. Osteocalcin attaches to the solid phase during incubation, and isoluminol conjugated antibody then binds to it. The unbound material is eliminated after

incubation using a wash cycle. After the initial reagents have been added, a flash chemiluminescent reaction begins. Relative light units, which are proportional to the amount of osteocalcin present in the calibration standards, controls, or test samples, are used by a photomultiplier to measure the light signal.

REQUIREMENT OF MATERIALS:

- Magnetic particles (2. 4 mL)
- Conjugate (20 mL)
- Assay Buffer (20 mL)

REAGENT STORAGE:

The Reagent Integral must be kept upright after delivery in order to allow for magnetic particle re-suspension. After the date of expiry reagent integral should not be utilized. The Reagent Integral is stable once seals are removed for 4 weeks within the analyzer or when stored between 2 and 8 degrees Celsius.

SPECIMEN COLLECTION AND PREPARATION:

Human serum must be utilized. When compared to serum, EDTA and lithium heparin plasma exhibit bias; however, these samples can be tested with this kit as long as a unique reference range is established for each kind of sample used. Fasting samples are advised but not necessary. Venipuncture should be used to draw blood aseptically, then the serum should be extracted as soon as the blood has had a chance to clot. The sample can be preserved without the use of additives or preservatives. Before testing, samples with erythrocyte debris, lipemia, turbidity, or particulate matter may need to be clarified by filtration or centrifugation. Samples that are severely microbially contaminated, grossly hemolyzed,

lipemic, or include particle matter shouldn't be examined. Prior to assaying, look for and eliminate air bubbles.

ASSAY PROCEDURE:

Follow the Analyzer's operating instructions to the letter if you want your tests to go smoothly. Each test parameter was identified by a specific barcode on the reagent integral using the LIAISON® Analyzer. The integral cannot be applied if the analyzer is unable to read the barcode label. Each test parameter is identifiable by data encoded in the reagent.

RESULTS INTERPRETATION:

The LIAISON® Analyzer calculates automatically osteocalcin concentration in the sample. Concentration was expressed in ng/mL. To convert results to SI units: ng/mL × 0. 171 = nmol/L.

EXPECTED VALUES:

Serum samples from 66 male, 73 pre-menopausal female, and 66 post-menopausal female subjects in apparent good health and with 25 OH Vitamin D levels ≥ 20 ng/mL were analyzed by the LIAISON® Osteocalcin assay [226-233].

Population (N)	**Median Osteocalcin**	**Observed 95% Range**
Males (66)	18. 4 ng/mL	4. 6 – 65. 4 ng/mL
Females		
Pre-Menopausal (73)	17. 6 ng/mL	6. 5 – 42. 3 ng/mL
Post-Menopausal (66)	21. 4 ng/mL	5. 4 – 59. 1 ng/mL

VITAMIN D

Chemiluminescence Method

PRINCIPLE:

The LIAISON® 25 OH Vitamin D test is a direct CLIA for determining the amount of total 25 OH vitamin D in serum in a quantitative manner. 25 OH During the initial incubation, vitamin D dissociates from its binding protein and binds to the specific antibody on the solid phase. After ten minutes, the tracer vitamin D attached to an isoluminol derivative is introduced. After a second incubation of 10 minutes, the loose material is removed using a wash cycle. The flash chemiluminescent process is then initiated by adding the beginning reagents. An inverse relationship exists between the amount of 25 OH vitamin D present in calibrators, controls, or samples and the light signal, which is measured by a photomultiplier as relative light unit (RLU).

REQUIREMENT OF MATERIALS:

- Magnetic particles (2. 4 mL)
- Conjugate (4. 5 mL)
- Assay Buffer (28. 0 mL)

REAGENT STORAGE AND STABILITY:

To aid in the resuspension of magnetic particles, the reagent component needs to be stored upright in the dark as soon as it is received. The reagents are stable at 2-8°C up to the expiration date when the reagent component is kept upright, light-protected, and unopened. Avoid freezing. The kit and reagent integral labels both state that the reagent integral should not be used after the specified expiration date. The reagent component should be returned to

storage at 2-8°C in the dark after use, sealed using the tape included with the kit. Stored properly, open use lasts four weeks. Avoid excessive exposure to light.

SPECIMEN COLLECTION AND PREPARATION:

It is possible to use fasting samples of SST tubes or human serum, EDTA plasma, lithium heparin plasma. Venipuncture should be used to draw blood aseptically, then the serum should be extracted as soon as the blood has had a chance to clot. It is not recommended to test materials that are hemolyzed, highly lipemic, include particulates, or clearly have microbial contamination. The samples should be kept at 2-8°C if the assay is run within 120 hours of sample collection; otherwise, they should be frozen (-20°C or lower) for storage. If samples were frozen during storage, thoroughly mix thawed samples before testing. Steers clear of frequent freeze-thaw cycles. Prior to assaying, look for and eliminate air bubbles. The initial test requires a minimum volume of 175 µL, and subsequent LIAISON® 25 OH Vitamin D TOTAL tests require an additional 25 µL.

ASSAY PROCEDURE:

Follow the LIAISON® Analyzer's operating instructions to the letter to ensure optimum test performance. Each test parameter is identified by a barcode on the reagent integral using the LIAISON® Analyzer. The integral cannot be applied if the analyzer is unable to read the barcode reader. Analysis tool LIAISON® XL: Reagent Integral Radio Frequency Identification transponder information is used to identify each test parameter.

RESULTS INTERPRETATION:

The LIAISON® Analyzer calculates the concentration automatically of 25 OH Vitamin D in the sample. This concentration 2. 5 = nmol/L. ×is expressed in ng/mL. To convert results to SI units: ng/mL

Assay range: 4. 0 to 150 ng/mL

On LIAISON® and LIAISON® XL, calibrators and controls may produce differing RLU or dosage results, but patient outcomes are comparable [234-239].

PARATHYROID HORMONE [PTH]

Chemiluminescence Method

PRINCIPLE:

Using direct chemiluminometric technology and antihuman PTH antibody concentrations that were constant in both the Lite and the Solid Phase Reagent, the ADVIA Centaur iPTH assay which is a two-site sandwich immunoassay was used. The first antibody is an acridinium ester-tagged polyclonal goat antihuman PTH (N-terminal 1-34) antibody. The second antibody is a polyclonal goat antihuman PTH (39-84 region) antibody that has been biotinylated. Streptavidin is covalently linked to paramagnetic latex particles in the solid state. The following tasks are carried out by the system automatically:

- 200 µl of the sample is poured into a cuvette.
- Dispenses 50 µL of Lite Reagent and then incubates it at 37° C for 6. 3 minutes.
- Dispenses 200 µL of Solid Phase which is then incubated at 37° C for 3. 0 minutes.
- Use Wash 1 to separate, aspirate, and wash the cuvettes.
- To start the chemiluminescent reaction, dispense 300 µL of each acid reagent (R1) and base reagent (R2) into the reaction chamber.

SPECIMEN COLLECTION AND HANDLING:

For this assay, EDTA plasma or serum is the suggested sample type.

The Clinical and Laboratory Standards Institute (formerly NCCLS) has provided the following guidelines for handling and storing blood samples:

- Collect all blood samples while adhering to general venipuncture safety measures.

- Centrifuge samples for 15 to 20 minutes at ≥ 1000 x g.
- Always keep tubes erect and stoppered.
- Only freeze samples once, then combine well after thawing.

Make sure that samples have the following qualities before placing them on the system:

- No fibrin or other debris was found in the samples. By centrifuging for 15 to 20 minutes at 1000 x g, remove particles.
- Samples are bubble free.

To maintain the integrity of the entire PTH molecule, patient samples must be handled properly. It has been shown that intact PTH is labile and prone to fragmentation. This instability is temperature and time dependent. The stability of patient samples is shown in the following table:

Sample stability was determined with the ADVIA Centaur iPTH assay.

REAGENTS:

- ADVIA Centaur iPTH Ready Pack primary reagent pack
- ADVIA Centaur Ready Pack ancillary reagent pack

ASSAY PROCEDURE:

The procedure is the same as system instructions or to the help system online.

EXPECTED VALUES:

Always consider the patient's medical history, clinical appearance, and other findings when interpreting the results.

The ACS:180® system was used to establish the reference range. 142 people appeared to be in good health and whose calcium levels ranged from 8. 0 to 10. 3 mg/dL provided EDTA

plasma samples. 95 percent of these people's intact PTH readings fell between 14 and 72 pg./mL (1. 48 and 7. 63 pmol/L), with a range of 11. 1 to 79. 5 pg/mL (1. 18 to 8. 43 pmol/L) overall. Each laboratory should choose its own reference range(s) for the diagnostic assessment of patient results, as is the case with all in vitro diagnostic assays [240-246].

CHAPTER-6

RESULTS

RESULTS

6. 1 Age wise distribution in Healthy controls, PreDiabetes and Diabetes patients

Table 1: Mean age wise distribution between healthy controls, prediabetes and diabetes

	Controls (n=75)	Prediabetic group (n=75)	Diabetes group (n=75)	p value
Mean Age in years	66.84±5.98	66.48±4.59	65.32±3.53	0.13

ANOVA test was applied.

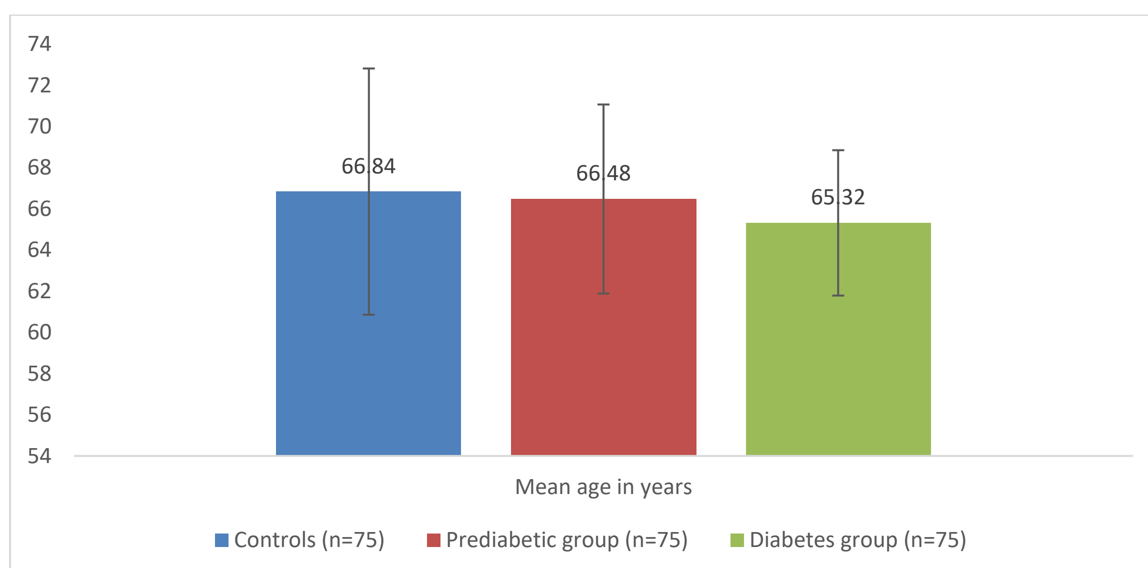


Figure 3: Graphical representation of mean age comparison between healthy controls, prediabetes and diabetes

Above table and figure represents that age of volunteers in control group was (66.84±5.98), Prediabetes group was (66.48±4.59) and diabetes group was (65.32±3.53). No significant relation was seen when assessed age in all the three groups of the study.

Most of patients with type 2 diabetes receive a diagnosis at ages 45–64. Sex, ethnicity, race and socioeconomic factors can also alter a person's risk of developing the condition.

6. 2 Gender wise distribution in controls, prediabetes and diabetes

Table 2: Gender wise distribution between healthy controls, prediabetes and diabetes

Gender	Controls (n=75)	Prediabetic group (n=75)	Diabetes group (n=75)	p value
Male	41 (54.7%)	48 (64%)	40 (53.3%)	0.35
Female	34 (45.3%)	27 (36%)	35 (46.7%)	

Chi square test applied

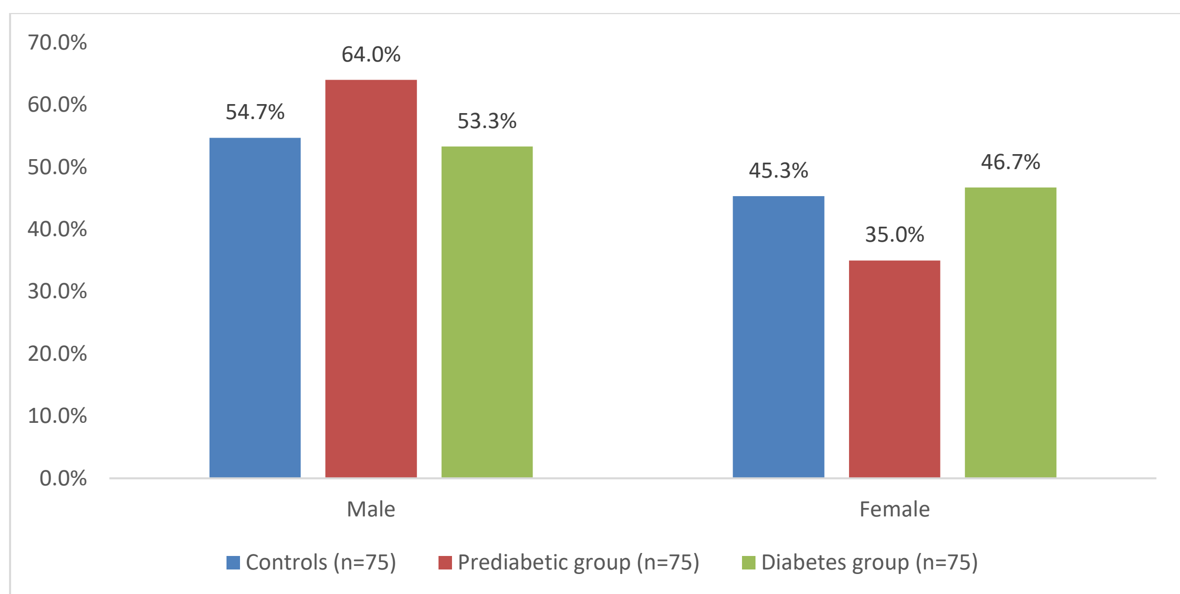


Figure 4: Graphical representation of Gender wise comparison between healthy controls, prediabetes and diabetes

Above table and figure represents that genders in control group was (Male 54.7% & Female 45.3%), Prediabetes group was (Male 64% & Female 36%), and diabetes group was (Male 53.3% & Female 46.7%), No significant relation was seen when assessed gender ratio in all the three groups of the study.

6. 3 Duration of Diabetes in PreDiabetes and Diabetes patients

Table 3: Duration in months wise distribution between prediabetes and diabetes

	Prediabetic group (n=75)	**Diabetes group (n=75)**	**p value**
Duration in months	3.41±1.45	51.37±33.95	<0.01

Mann Whitney U test applied

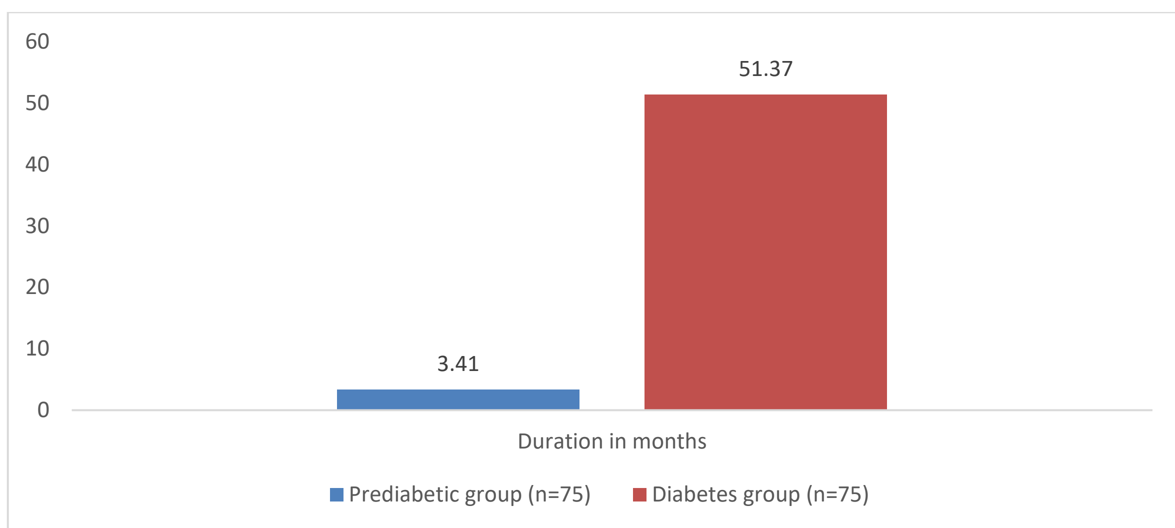


Figure 5: Graphical representation of Duration comparison in PreDiabetes and Diabetes patients

The above table and figure show that Duration of Prediabetes group was (3.41±1.45) and diabetes group was (51.37±33.95). Duration of diabetes is significantly higher in diabetes population (p<0. 01).

A longer diabetes and older age at diagnosis duration are proportionally to increase the risk of macro & micro vascular events, nephropathy, neuropathy & retinopathy**,** with the greatest risks observed in the longest duration of diabetes with the oldest age groups.

6. 4 BMI of Healthy controls, PreDiabetes and Diabetes patients

Table 4: Mean BMI distribution between healthy controls, prediabetes and diabetic patients

	Controls (n=75)	Prediabetic group (n=75)	Diabetes group (n=75)	p value
BMI (kg/m^2)	22.46±1.35	23.52±1.07	22.93±1.49	<0.001

ANOVA test was applied.

Table 5: Mean BMI Posthoc test p value comparison between between healthy controls, prediabetes and diabetes

	b/w Controls & prediabetes	b/w Controls & diabetes	b/w prediabetes & diabetes group (n=75)
BMI (kg/m^2)	<0.001	0.04	<0.01

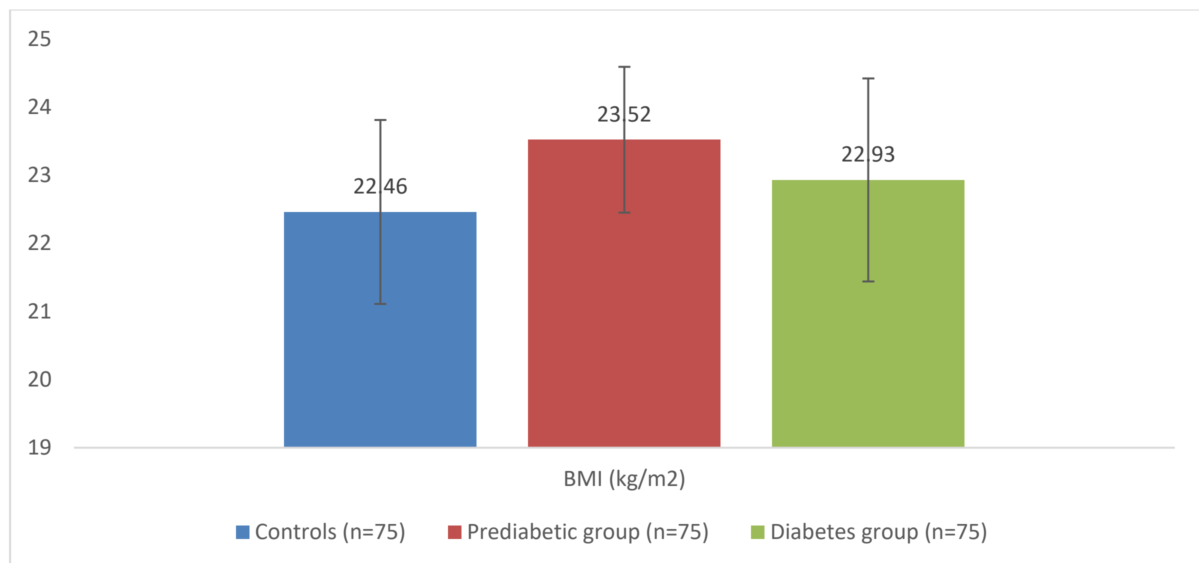


Figure 6: Graphical representation of BMI comparison among Healthy controls, PreDiabetes and Diabetes patients

Above table and figure represents that Mean BMI was 22.46±1.35 kg/m^2 in controls, 23.52±1.07 kg/m^2 in prediabetic group and 22.93±1.49 kg/m^2 in diabetic group. There was statistically significant difference between BMI of all three groups. BMI has calculated with

patient's weight of volunteers of the study and for control group it was (66. 55±4. 37), Prediabetes group was (70. 89±2. 66) and diabetes group was (68. 49±5. 56). Weight of prediabetes and diabetes volunteers is significantly higher in comparison to control population ($p<0.001$) and Height of volunteers of the study and for control group it was (172. 13±4. 45), Prediabetes group was (173. 65±4. 46) and diabetes group was (172. 79±4. 83). Height among the entire study group was found to be non-significant ($p>0.01$).

Overweight increses risk for prediabetes and type 2 diabetes, stroke and heart disease. Study indicated tall stature is associated with lower risk for developing type 2 diabetes.

6. 5 Blood Glucose & HbA1c level in Healthy controls, PreDiabetes and Diabetes patients

Table 6: Blood glucose & HbA1C levels between healthy controls, prediabetes and diabetes patients

	Controls (n=75)	**Prediabetic group (n=75)**	**Diabetes group (n=75)**	**p value**
Blood glucose fasting (mg/dl)	88.24±8.32	112.31±7.37	206.80±82.56	<0.001#
Blood glucose random (mg/dl)	104.01±17.52	160.87±13.71	240.51±110.77	<0.001#
HbA1C (%)	5.02±0.42	6.22±0.20	10.34±2.31	<0.001*

*ANOVA test was applied; Kruskal Wallis H test was applied#

Table 7: Blood glucose & HbA1C levels Posthoc test p value comparison between prediabetes and diabetes patients

	b/w Controls & prediabetes	**b/w Controls & diabetes**	**b/w prediabetes & diabetes group (n=75)**
Blood glucose fasting (mg/dl)	<0.001	<0.001	<0.001
Blood glucose random (mg/dl)	<0.001	<0.001	<0.001
HbA1C (%)	<0.001	<0.001	<0.001

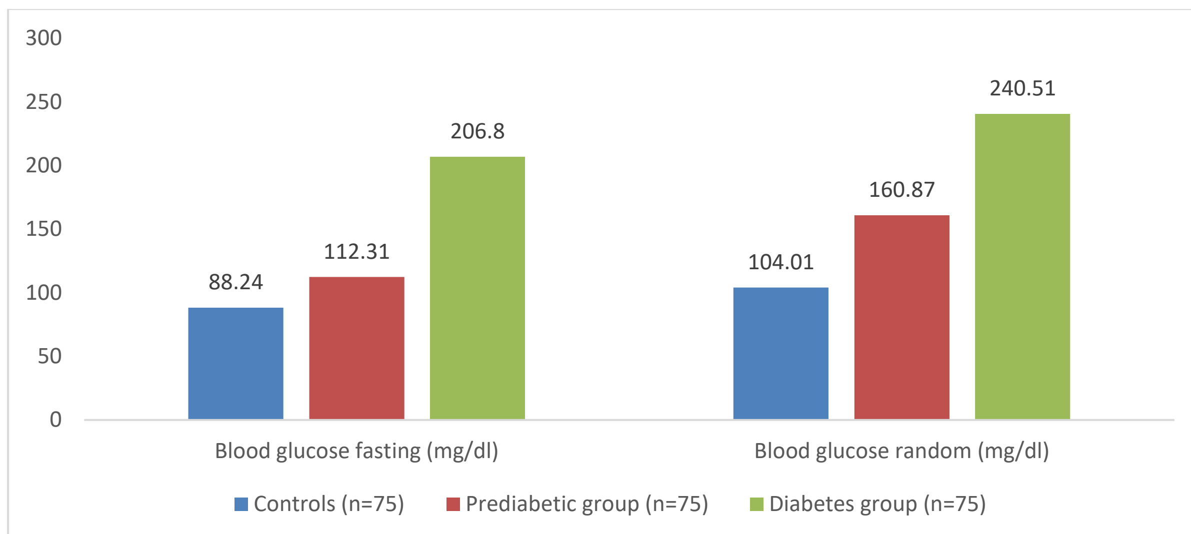


Figure 7: Graphical representation of Blood glucose fasting & blood glucose random comparison in Healthy controls, PreDiabetes and Diabetes

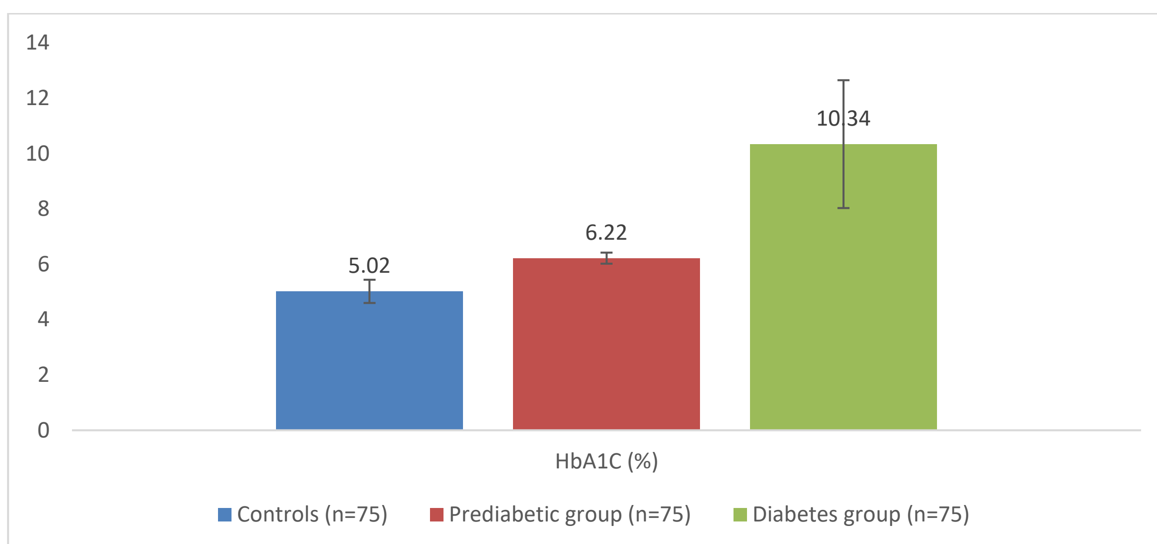


Figure 8: Graphical representation of HbA1c comparison between Healthy controls, PreDiabetes and Diabetes

The above table and figure show that the level of Fasting Blood Glucose in control group was (88. 24±8. 32), Prediabetes group was (112. 31±7. 37) and diabetes group was (206. 8±82. 5). Levels of Fasting Blood Glucose was markedly increased in diabetics and was found to be significant p<. 001. The level of Random Blood Glucose in control group was (104. 01±17.

52), Prediabetes group was (160. 87±13. 71) and diabetes group was (240. 51±110. 7). A level of Random Blood Glucose were markedly increased in diabetics and was found to be significant p<. 001. The level of HbA_{1c} in control group was (5. 02±0. 42), Prediabetes group was (6. 22±0. 20) and diabetes group was (10. 34±2. 31). Levels of $HbA_{1c}$was markedly increased in diabetics and was found to be significant p<. 001. A toxic effect is concequences of high blood glucose levels that directly affect osteoblast cell as a result of these modifications, bone mineralization and osteoblast development are inhibited.

6.6 **Liver Function Test (LFT) level in Healthy controls, PreDiabetes and Diabetes patients**

Table 8: LFT parameter levels between healthy controls, prediabetes and diabetes

	Controls (n=75)	**Prediabetic group (n=75)**	**Diabetes group (n=75)**	**p value**
SGOT (U/L)	22.97±8.76	24.80±10.11	31.44±18.50	<0.001#
SGPT (U/L)	25.33±9.53	29.42±18.82	29.53±19.20	0.79#
Albumin (g/dl)	4.17±0.51	4.70±0.56	4.66±0.63	<0.001*
Total protein (g/dl)	7.38±0.62	7.62±0.51	7.59±0.47	0.01*

*ANOVA test was applied; Kruskal Wallis H test was applied#

Table 9: LFT parameter Posthoc test p value comparison comparison between Healthy controls, PreDiabetes and Diabetic patients

	b/w Controls & prediabetes	**b/w Controls & diabetes**	**b/w prediabetes & diabetes group (n=75)**
SGOT (U/L)	0.14	<0.001	0.01
SGPT (U/L)	0.50	0.88	0.62

Albumin (g/dl)	<0.001	<0.001	0.64
Total protein (g/dl)	0.01	0.02	0.74

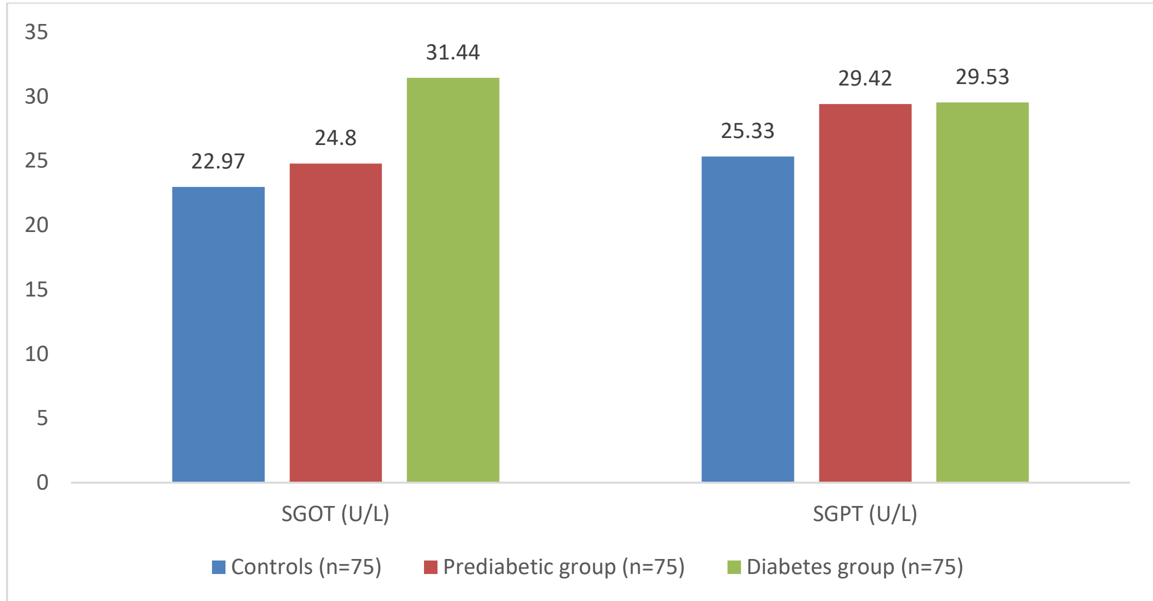


Figure 9: Graphical representation of SGOT & SGPT comparison comparison between Healthy controls, PreDiabetes and Diabetes patients

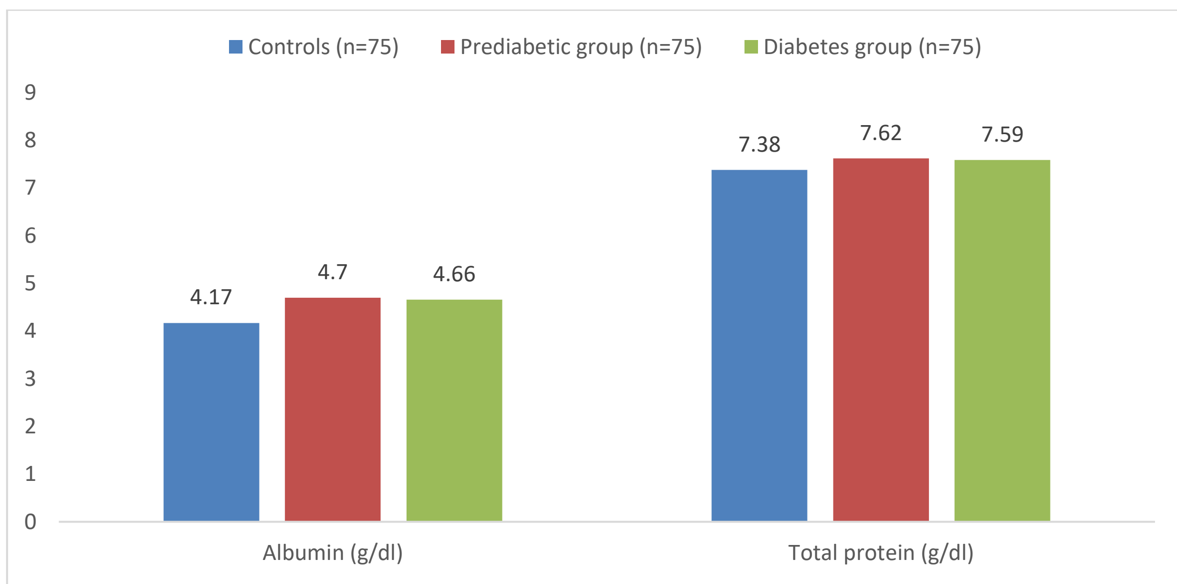


Figure 10: Graphical representation of Albumin & Total protein comparison between Healthy controls, PreDiabetes and Diabetes patients

The above table and figure that level of SGOT in control group was (22. 97±8. 76), Prediabetes group was (24. 8±10. 11) and diabetes group was (31. 44±18. 50). A level of SGOT were markedly increased in diabetics and was found to be significant p<. 001. The level of SGPT in control group was (25. 33±9. 5), Prediabetes group was (29.42±18.82) and diabetes group was (25.53±19.20). The concentration of serum SGPT was markedly increased in Prediabetics but decreased in diabetes and was found to be non-significant p>. 001. And the level of albumin in control group was (4. 17±0. 51), Prediabetes group was (4. 70±0. 56) and diabetes group was (4. 66±0. 63). Concentrations of serum albumin was markedly increased in prediabetics in comparison to diabetes and was found to be significant p<. 001. Level of Total protein in control group was (7. 38±0. 62), Prediabetes group was (7. 62±0. 51) and diabetes group was (7. 59±0. 47). Levels of Total protein were increased in diabetics and were found to be non-significant p>. 001.

Albuminaria is a marker of metabolic syndrome, CVD and nephropathy. SGOT elevated in pre diabetes & diabetes cause of liver tissue strain due to hyperlipidemia and progression to overt diabetes.

6. 7 Renal FunctionTest (RFT) levels between controls, prediabetes and diabetes

Table 10: RFT parameter levels between Healthy controls, PreDiabetes and Diabetes

	Controls (n=75)	**Prediabetic group (n=75)**	**Diabetes group (n=75)**	**p value**
Urea (mg/dl)	28.19±8.17	30.34±9.47	29.96±10.27	0.42#
Creatinine (mg/dl)	0.93±0.19	0.98±0.15	0.99±0.15	0.06*

*ANOVA test was applied; Kruskal Wallis H test was applied#

Table 11: RFT parameter Posthoc test p value comparison between Healthy controls, PreDiabetes and Diabetes patients

	b/w Controls & prediabetes	b/w Controls & diabetes	b/w prediabetes & diabetes group (n=75)
Urea (mg/dl)	0.16	0.52	0.14
Creatinine (mg/dl)	0.07	0.03	0.69

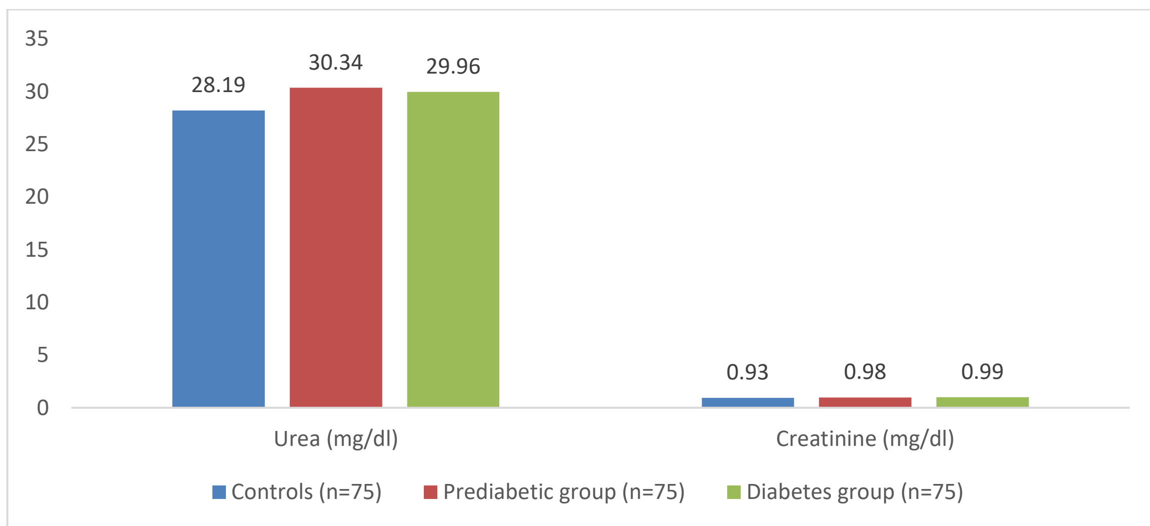


Figure 11: Graphical representation of Urea & Creatinine comparison between Healthy controls, PreDiabetes and Diabetes patients

The above table and figure represent that level of Urea in control group was (28.19±8. 17), Prediabetes group was (30.34±9. 47) and diabetes group was (29. 96±10. 27). Concentrations of Serum Urea was seen to be more increased in Prediabetics in comparison to diabetes and was found to be non-significant p>. 001. And the level of Creatinine in control group was (0. 93±0.19), Prediabetes group was (0.98±0.15) and diabetes group was (0.99±0.15). Concentrations of Serum Creatinine increased in Prediabetics and in diabetes patients and were found to be non-significant p>. 001. High creatinine level may improve glucose metabolism and stimulate insulin-resistance and in diabetic individuals and urea suppresses

sensitivity & insulin secretion. We can conclude that increase levels of urea are associated with higher risk of incident in pre diabetes mellitus.

6. 8 Lipid profile levels in Healthy controls, PreDiabetes and Diabetes patients

Table 12: Lipid profile levels between Healthy controls, PreDiabetes and Diabetes patients

	Controls (n=75)	**Prediabetic group (n=75)**	**Diabetes group (n=75)**	**p value**
TG (mg/dl)	106.30±25.66	137.44±51.79	148.90±68.84	<0.001
T cholesterol (mg/dl)	124.86±33.72	179.13±38.71	169.92±50.16	<0.001
HDL (mg/dl)	59.58±12.46	56.63±10.67	53.66±10.91	<0.01
LDL (mg/dl)	77.31±18.32	90.34±27.68	78.55±30.07	<0.01

Kruskal Wallis H test was applied

Table 13: Lipid parameter levels Posthoc test p value comparison between Healthy controls, PreDiabetes and Diabetes patients

	b/w Controls & prediabetes	**b/w Controls & diabetes**	**b/w prediabetes & diabetes group (n=75)**
TG (mg/dl)	0.001	<0.001	0.51
T cholesterol (mg/dl)	<0.001	<0.001	0.16
HDL (mg/dl)	0.08	<0.01	0.22
LDL (mg/dl)	0.02	0.40	<0.01

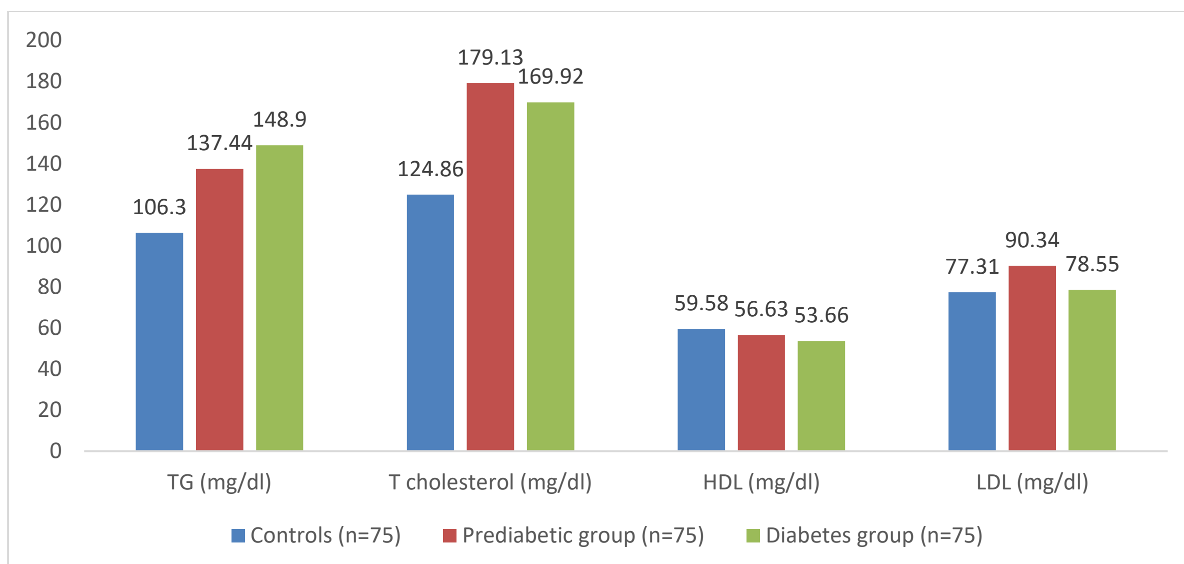


Figure 12: Graphical representation of Lipid profiles comparison between Healthy controls, PreDiabetes and Diabetes patients

Triglycerides in the control group were (106. 30±25. 66), Prediabetes group was (137. 44±51. 79) and diabetes group was (148. 90±68. 84). A level of Triglycerides was markedly increased in diabetics and was found to be significant p<. 001. In sequence the level of Cholesterol in the control group was (124. 86±33. 72), Prediabetes group was (179. 08±38. 75) and diabetes group was (169. 92±50. 16). A level of Cholesterol was markedly increased in diabetics and was found to be significant p<. 001. And the level of HDL in control group was (59. 58±12. 46), Prediabetes group was (56. 68±10. 64) and diabetes group was (53. 66±10. 91). Levels of markedly decreased in diabetics and was found to be non- significant p>. 001. The level of LDL in control group was (77. 31±18. 32), Prediabetes group was (90. 37±27. 67) and diabetes group was (78. 55±30. 07). Levels of Lulwa's were found to be non-significant p>. 001.

6.9 Metabolic markers levels between controls, prediabetes and diabetes

Table 14: Metabolic marker levels between Healthy controls, PreDiabetes and Diabetes patients

	Controls (n=75)	**Prediabetic group (n=75)**	**Diabetes group (n=75)**	**p value**
Calcium (mg/dl)	9.37±0.59	9.18±0.57	9.16±0.91	0.14*
Phosphorus (mg/dl)	3.50±0.53	3.49±0.49	3.41±0.54	0.56*
ALP (U/L)	72.58±16.74	77.72±22.94	87.23±21.31	<0.001#
Vitamin D (ng/ml)	43.86±20.70	32.04±18.05	31.63±16.32	<0.001#
PTH (pg/ml)	45.35±19.44	46.7±23.99	50.47±18.68	0.06#
Osteocalcin (ng/ml)	20.04±7.96	14.11±5.10	14.71±5.20	<0.001#

*ANOVA test was applied; Kruskal Wallis H test was applied#

Table 15: Metabolic marker levels Posthoc test p value comparison between Healthy controls, PreDiabetes and Diabetes patients

	b/w Controls & prediabetes	**b/w Controls & diabetes**	**b/w prediabetes & diabetes group (n=75)**
Calcium (mg/dl)	0.05	0.09	0.85
Phosphorus (mg/dl)	0.88	0.33	0.39
ALP (U/L)	0.35	<0.001	<0.01
Vitamin D (ng/ml)	<0.001	<0.001	0.69
PTH (pg/ml)	0.79	0.03	0.05
Osteocalcin (ng/ml)	<0.001	<0.001	0.56

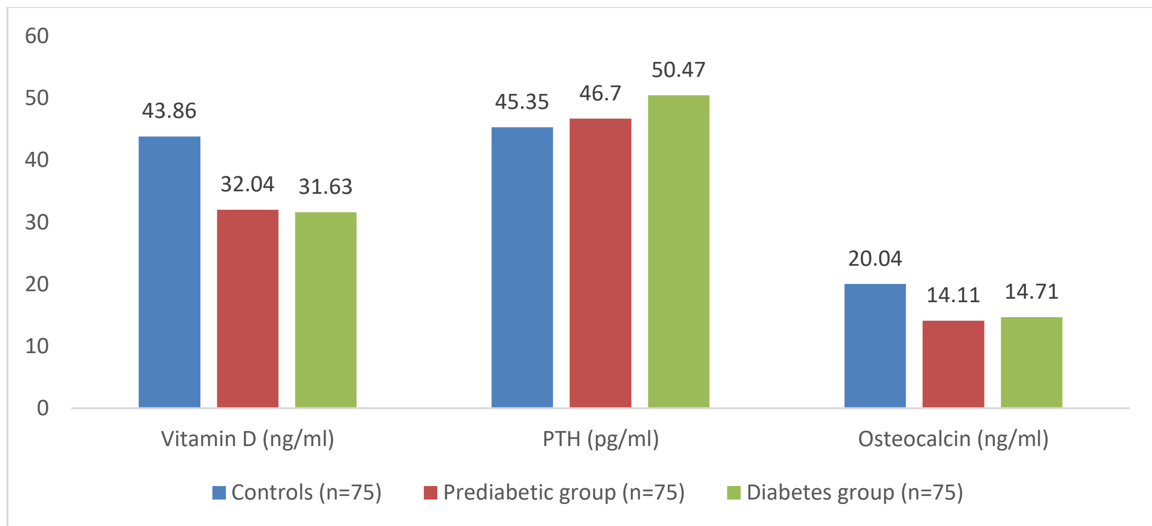


Figure 13: Graphical representation of Vitamin D, Parathyroid Hormone & Osteocalcin comparison between Healthy controls, PreDiabetes and Diabetes patients

The above table and figure represents that level of Calcium in control group was (9.37±0. 59), Prediabetes group was (9.18±0. 57) and diabetes group was (9.16±0. 91). Levels of Calcium were markedly decreased in diabetics and were found to be significant p<. 014. The level of Phosphorus in the control group was (3. 50±0. 53), Prediabetes group was (3. 49±0. 49) and diabetes group was (3. 41±0.549). A level of Phosphorus was markedly decreased in diabetics and was found to be significant p<. 0.56* in sequence the level of ALP in control group was (72. 58±16. 74), Prediabetes group was (77. 72±22. 94) and diabetes group was (87. 23±21. 31). Levels of ALP was markedly increased in diabetics patients and was found to be significant p<0. 001. And the level of Vitamin D in control group was (43. 86±20.70.), Prediabetes group was (32.04±18.05) and diabetes group was (31.63±16. 32). A level of Vitamin D was markedly increased in pre-diabetics while slight decrease in comparison to pre-diabetics in diabetes patients and was found to be significant p<0. 001.

The level of Parathyroid Hormone in control group was (45. 35±19. 4), Prediabetes group was (46. 7±23. 9) and diabetes group was (50. 47±18. 6). Levels of Parathyroid Hormone was markedly increased in diabetics and was found to be significant p<0. 06. And the level of

Osteocalcin in control group was (20. 04±7. 9), Prediabetes group was (14. 11±5.1) and diabetes group was (14.71±5.2). Levels of Osteocalcin was markedly decreased in diabetics and was found to be significant p<0. 001.

6. 10 FGF-23 & IGF level in Healthy controls, PreDiabetes and Diabetes patients

Table 16: FGF-23 & IGF 1 levels between Healthy controls, PreDiabetes and Diabetes patients

	Controls (n=75)	Prediabetic group (n=75)	Diabetes group (n=75)	p value
FGF23 (pg/ml)	23.92±8.56	26.72±11.18	31.30±8.23	<0.001
IGF1 (ng/ml)	80.68±44.59	67.01±39.89	65.30±36.62	0.06

Kruskal Wallis H test was applied

Table 17: FGF 23 & IGF1 Posthoc test p value comparison between healty controls, prediabetes & diabetes

	b/w Controls & prediabetes	b/w Controls & diabetes	b/w prediabetes & diabetes group (n=75)
FGF23 (pg/ml)	0.24	<0.001	<0.001
IGF1 (ng/ml)	0.06	0.03	0.81

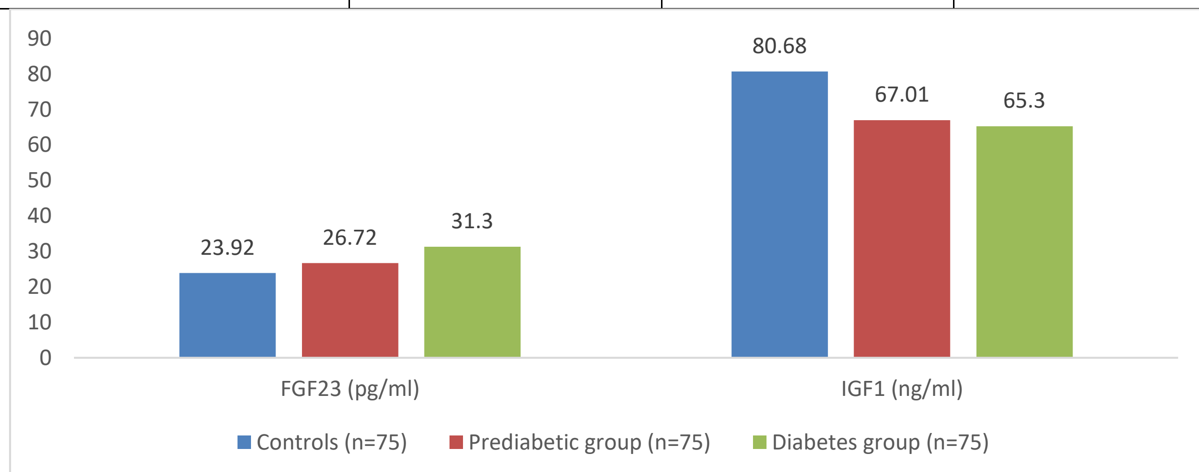


Figure 14: Graphical representation of FGF-23 & IGF 1 comparison between Healthy controls, PreDiabetes and Diabetes patient

The above table and figure represents that the level of FGF-23 in control group was (23. 92±8. 56), Prediabetes group was (26.72±11. 18) and diabetes group was (31. 30±8. 23). **Levels of FGF-23 were markedly increased in diabetics and was found to be significant p<. 001.**

The level of IGF-1 in control group was (80. 68±44. 59), Prediabetes group was (67. 01±39. 89) and diabetes group was (65.30±36.62). **A level of IGF-1was markedly decreased in diabetics and was found to be significant p<0. 06**.

Table 18 : Correlation of FGF23 and IGF-1 with different metabolic markers in controls

		FGF23 (pg/ml)	IGF1 (ng/ml)
Calcium (mg/dl)	r value	-.133	-.005
	p value	.257	.969
	N	75	75
Phosphorus (mg/dl)	r value	.021	.183
	p value	.855	.116
	N	75	75
ALP (U/L)	r value	.091	-.032
	p value	.436	.787
	N	75	75
Vitamin D (ng/mL)	r value	.075	-.035
	p value	.523	.767
	N	75	75
PTH (pg/ml)	r value	.193	-.049
	p value	.097	.677
	N	75	75
Osteocalcin (ng/ml)	r value	.121	.029
	p value	.299	.804
	N	75	75

Spearman correlation coefficient were used

The “Spearman coefficient of correlation” for all the bone metabolic markers such as Phosphorus and FGF 23 level (r value = .021; p value = 0.85) , between Phosphorus and IGF 1 level (r value = .18; p value = 0.12) , ALP and FGF23 level (r value = .091; p value =

0.44), Vit D and FGF23 level (r value = .075; p value = 0.52), PTH and FGF23 level (r value = .19; p value = .098), Osteocalcin and FGF23 level (r value = .12 ; p value = .29) Osteocalcin and IGF1 level (r value = .029; p value = 0.80) showing very weak correlation which was statistically significant.

In sequence Calcium and FGF23 level (r value = -.13; p value = 0.26) , Calcium and IGF 1 level (r value = -.005; p value = 0.97), between ALP and IGF 1 level (r value = -.032; p value = 0.79), between Vit D and IGF 1 level (r value = -.035; p value = 0.77) , PTH and IGF1 level (r value = -.049; p value = 0.68) showing very weak negative correlation between parameters which was statistically significant. Parameters were found to be less that 0.5 in the case of control group respectively (Table18). These correlations coefficient between the calcium and FGF-23 & IGF-1 were found to be and concluded as negatively correlated. The ALP and IGF-1 is significantly negatively correlated when compared to calcium and IGF-1. The correlation coefficients of Osteocalcin with FGF-23 were found to be 0.12 and that with IGF-1 was 0.29. The positive correlation between phosphorous and FGF-23 is stronger than between IGF-1. (Source: Vargas, 2016)

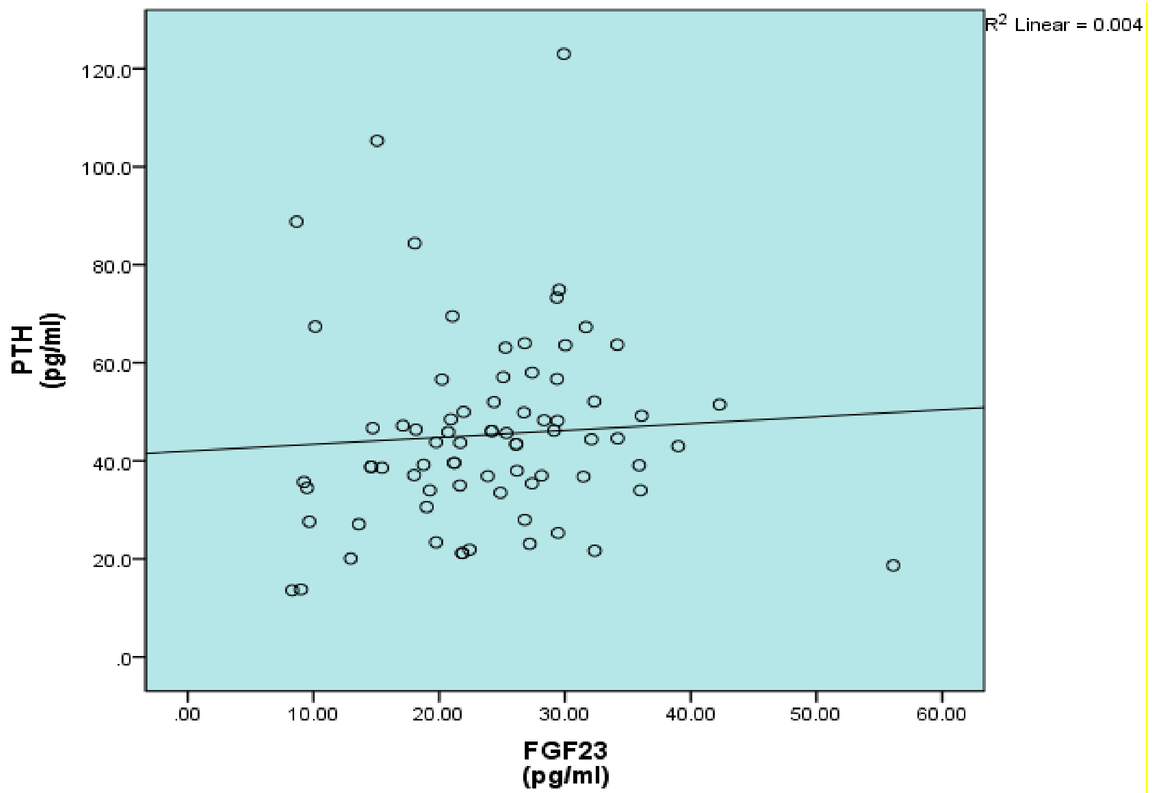


Figure 15: Scatterplot showing correlation of FGF23 with PTH in controls

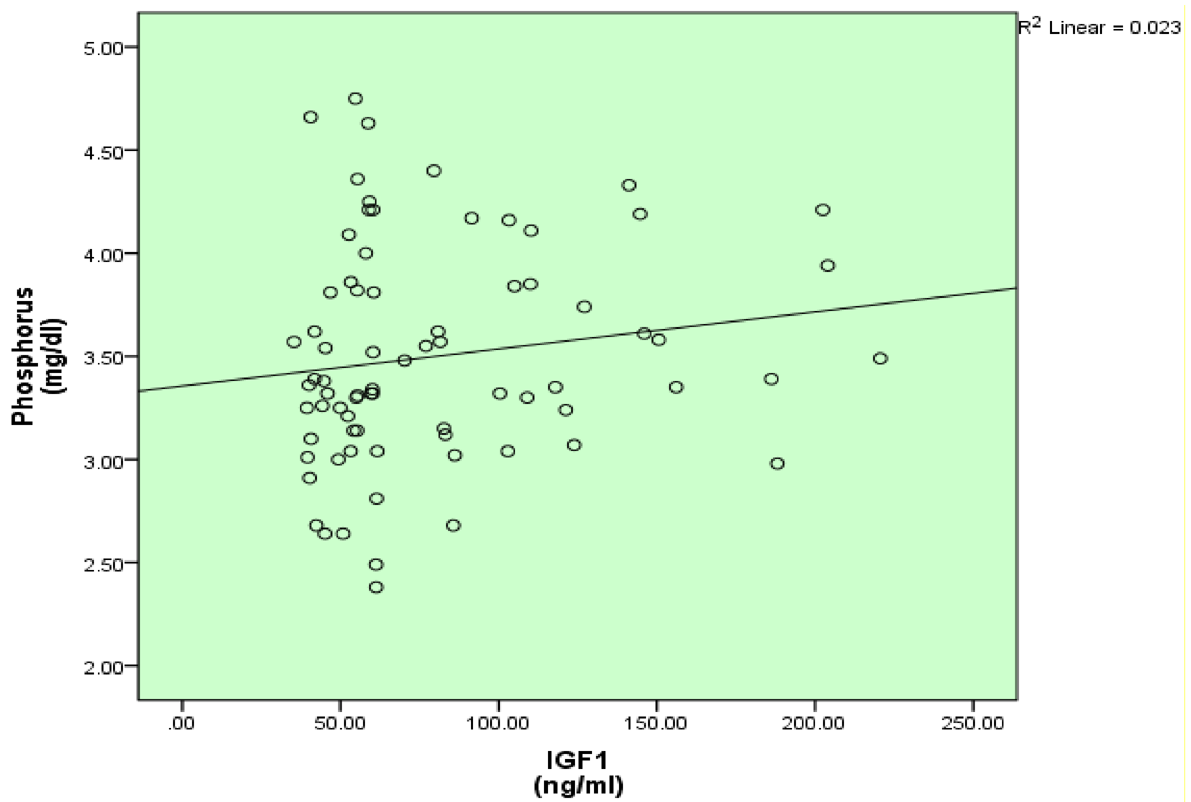


Figure 16 : Scatterplot showing correlation of IGF-1 with Phosphorus level in controls

Table 19 : Correlation of FGF23 and IGF-1 with different metabolic markers in pre-diabetics

		FGF23 (pg/ml)	IGF1 (ng/ml)
Calcium (mg/dl)	r value	.035	.076
	p value	.764	.515
	N	75	75
Phosphorus (mg/dl)	r value	.141	.075
	p value	.227	.524
	N	75	75
ALP (U/L)	r value	-.226	.072
	p value	.051	.539
	N	75	75
Vitamin D (ng/mL)	r value	.105	**-.253**
	p value	.372	**.028**
	N	75	**75**
PTH (pg/ml)	r value	.186	.173
	p value	.111	.138
	N	75	75
Osteocalcin (ng/ml)	r value	.087	-.084
	p value	.457	.472
	N	75	75

Spearman correlation coefficient were used

The "Spearman coefficient of correlation" for all the bone metabolic markers in prediabetes such as Calcium and FGF23 level (r value = .035; p value = 0.76) , Calcium and IGF 1 level (r value =-.076; p value = 0.51), Phosphorus and FGF 23 level (r value = .14; p value = 0.22) , between Phosphorus and IGF 1 level (r value = .07; p value = 0.52) , ALP and IGF 1 level (r value = .072; p value = 0.54), Vit D and FGF23 level (r value = .11; p value = 0.37), PTH and FGF23 level (r value = .19; p value = .11), PTH and IGF1 level (r value = -.17; p value

= 0.14) Osteocalcin and FGF23 level (r value = .09 ; p value = .46) showing positive weak correlation which was statistically significant.

In sequence between ALP and FGF23 level (r value = -.23; p value = 0.51), Vit D and IGF 1 level (r value = -.25; p value = 0.028), Osteocalcin and IGF1 level (r value = -.08; p value = 0.47) showing weak negative correlation between parameters which was statistically significant. Parameters were found to be less that 0.5 in the case of control group respectively (Table19). These correlations coefficient between the ALP and FGF-23 were found to be and concluded as negatively correlated. The correlation coefficients of Osteocalcin with FGF-23 were found to be positively correlated and negatively correlated with the IGF-1. The Vitamin D and IGF-1 is significantly negatively correlated when compared to Vitamin D and FGF-23.

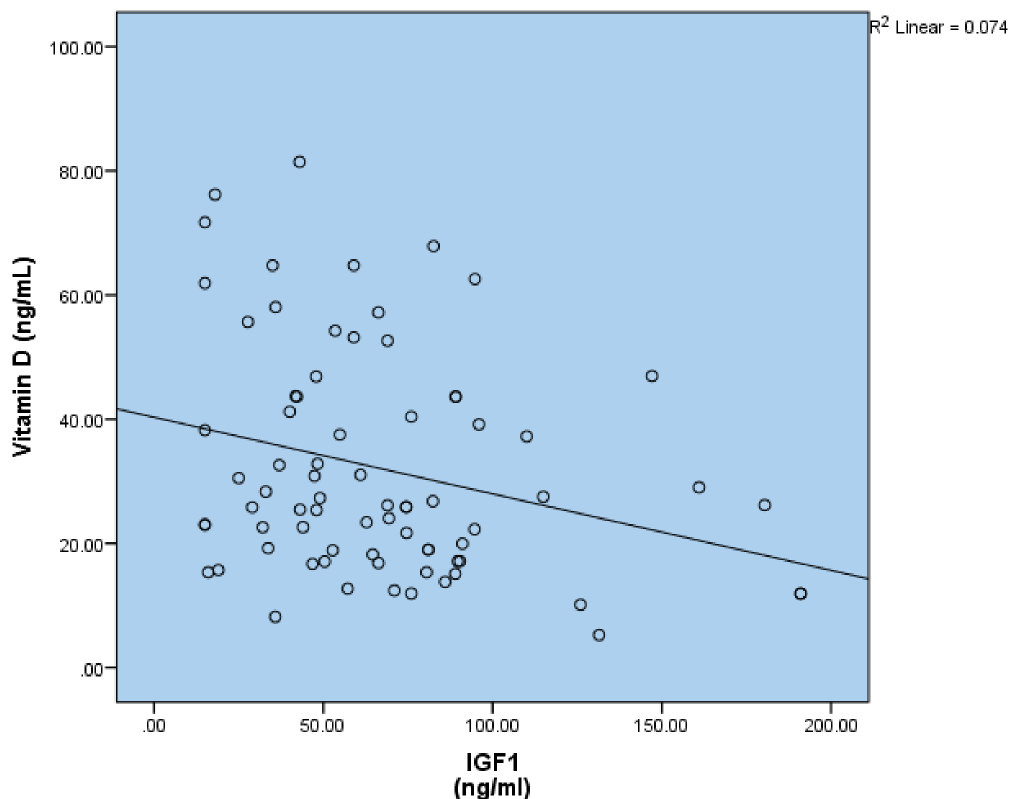


Figure 17: Scatterplot showing correlation of IGF-1 with Vitamin D level in pre-diabetics

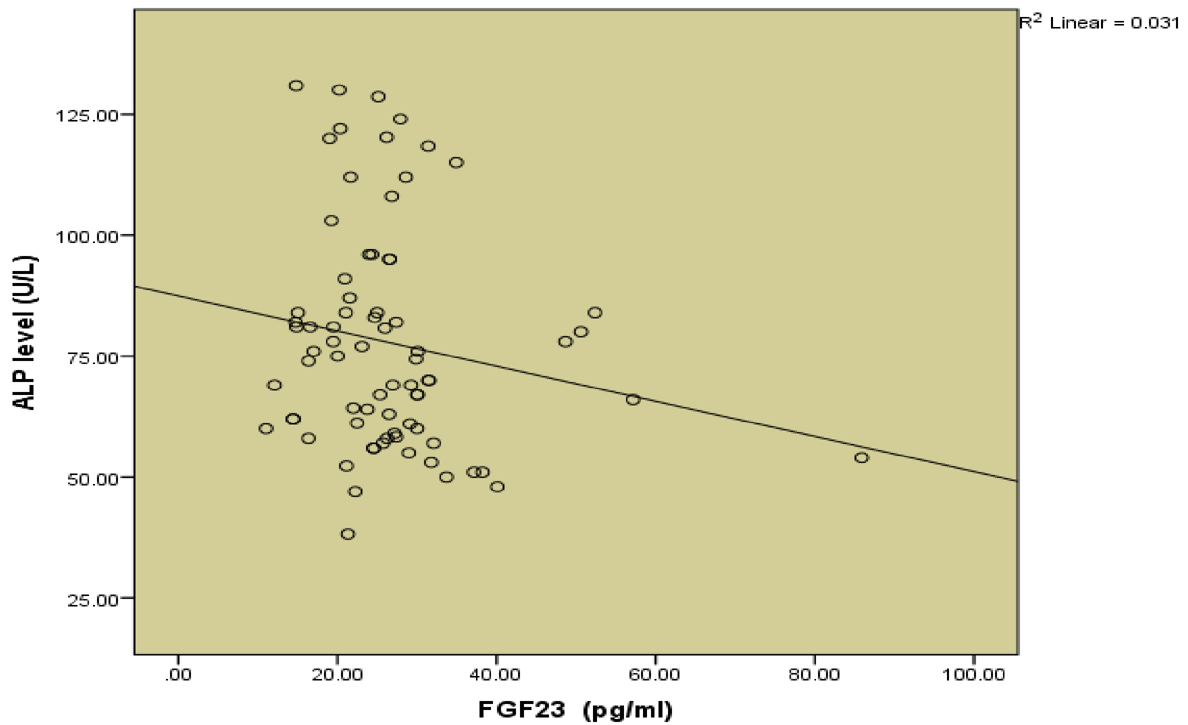


Figure 18: Scatterplot showing correlation of FGF23 with ALP level in pre-diabetics

Table 20: Correlation of FGF23 and IGF-1 with different metabolic markers in diabetics

		FGF23 (pg/ml)	IGF1 (ng/ml)
Calcium (mg/dl)	r value	-.005	.026
	p value	.964	.825
	N	75	75
Phosphorus (mg/dl)	r value	.036	.003
	p value	.760	.980
	N	75	75
ALP (U/L)	r value	-.124	-.215
	p value	.288	.065
	N	75	75
Vitamin D (ng/mL)	r value	.052	-.052
	p value	.659	.659
	N	75	75
PTH (pg/ml)	r value	-.064	.086
	p value	.584	.465
	N	75	75
Osteocalcin (ng/ml)	r value	-.079	.057
	p value	.498	.625
	N	75	75

Spearman correlation coefficient were used

The "Spearman coefficient of correlation" for all the bone metabolic markers in diabetes such as Calcium and IGF 1 level (r value =-.026; p value = 0.83), Phosphorus and FGF 23 level (r value = .036; p value = 0.76) , between Phosphorus and IGF 1 level (r value = .003; p value = 0.98) , Vit D and FGF23 level (r value = .05; p value = 0.66), PTH and IGF1 level (r value =.08; p value = 0.46), Osteocalcin and IGF1 level (r value = .05; p value = 0.62) showing positive weak correlation which was statistically significant.

In sequence between Calcium and FGF23 level (r value = -.005; p value = 0.96) ALP and FGF23 level (r value = -.12; p value = 0.29), ALP and IGF 1 level (r value = -.22; p value = 0.07)Vit D and IGF 1 level (r value = -.05; p value = 0.66) , PTH and FGF23 level (r value = -.06; p value = .58), Osteocalcin and FGF23 level (r value = -.08; p value = .49) showing weak negative correlation between parameters which was statistically significant. Parameters were found to be less that 0.5 in the case of control group respectively (Table 20). These correlations coefficient between the ALP with IGF 1 and FGF 23 were found to be and concluded as negatively correlated.

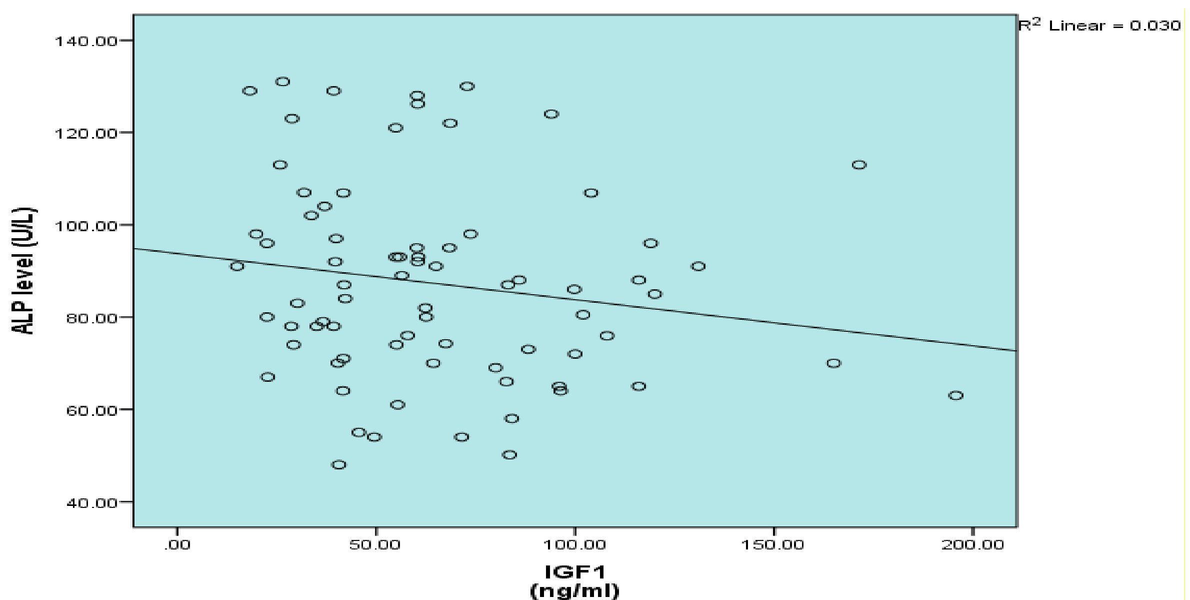


Figure 19: Scatterplot showing correlation of IGF-1 with ALP level in diabetics

Table 21: Correlation of FGF23 and IGF-1 with different metabolic markers in all study subjects

		FGF23 (pg/ml)	IGF1 (ng/ml)
Calcium (mg/dl)	r value	-.064	.061
	p value	.336	.364
	N	225	225
Phosphorus (mg/dl)	r value	.045	.095
	p value	.506	.155
	N	225	225
ALP (U/L)	r value	.005	-.084
	p value	.941	.211
	N	225	225
Vitamin D (ng/mL)	r value	.019	-.067
	p value	.774	.315
	N	225	225
PTH (pg/ml)	r value	**.153**	.055
	p value	**.021**	.408
	N	**225**	225
Osteocalcin (ng/ml)	r value	-.030	.060
	p value	.653	.371
	N	225	225

Spearman correlation coefficient were used

The "Spearman coefficient of correlation" for all the bone metabolic markers in controls, prediabetic & diabetics such as Calcium and IGF 1 level (r value =.061; p value = 0.35), Phosphorus and FGF 23 level (r value = .05; p value = 0.51) between Phosphorus and IGF 1 level (r value = .09; p value = 0.16) , Vit D and FGF23 level (r value = .02; p value = 0.77), PTH and FGF23 level (r value = .15; p value = .02), ALP and FGF23 level (r value = .005; p value = 0.94), PTH and IGF1 level (r value = .05; p value = 0.41) Osteocalcin and IGF1 level (r value = .06; p value = 0.37) showing positive weak correlation which was statistically significant.

In sequence between Calcium and FGF23 level (r value = -.06; p value = 0.34) ALP and IGF 1 level (r value = -.08; p value = 0.21), Vit D and IGF 1 level (r value = -.07; p value =

0.32) , Osteocalcin and FGF23 level (r value = -.03 ; p value = .65) showing weak negative correlation between parameters which was statistically significant. Parameters were found to be less that 0.5 in the case of control group respectively (Table 21). These correlations coefficient between the PTH and FGF-23 were found to be and concluded as positively significantly correlated.

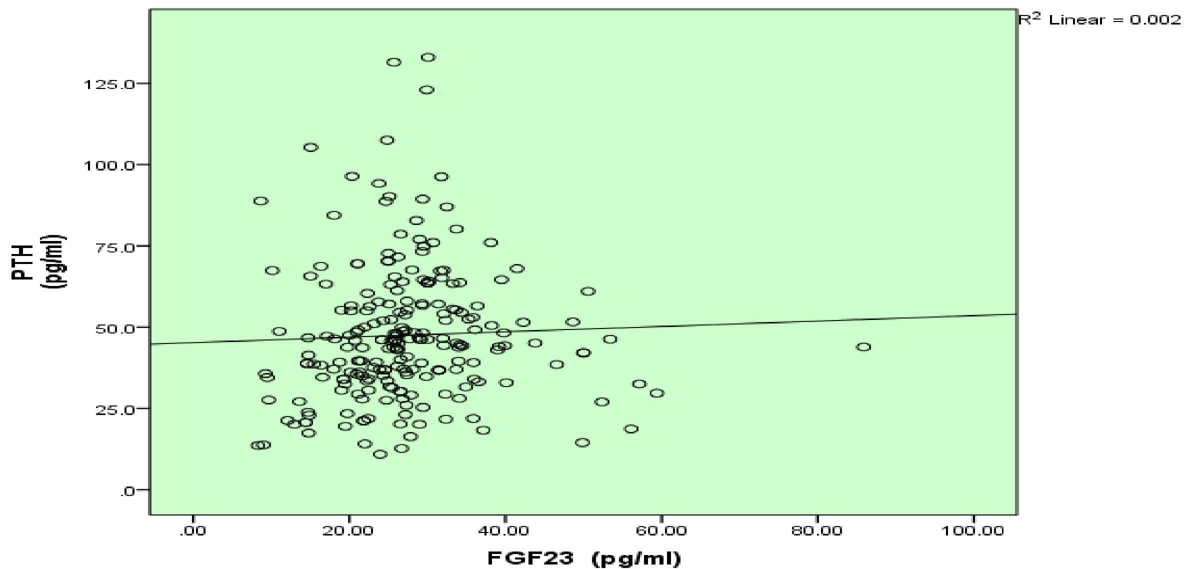


Figure 20: Scatterplot showing correlation of FGF23 with PTH level in all study subjects

Table 22: Correlation of Blood glucose and HbA1C with different metabolic markers in all study subjects

		Blood Glocose F (In Gm%)	Blood Glocose R (mg/dl)	HbA1c (%)
FGF23 (pg/ml)	r value	**.325**	.064	**.301**
	p value	**.000**	.568	**.000**
	N	144	81	225
IGF1 (ng/ml)	r value	**-.176**	-.072	-.100
	p value	**.035**	.525	.134
	N	144	81	225
Calcium (mg/dl)	r value	**-.174**	-.015	-.098
	p value	**.037**	.894	.144
	N	144	81	225

Phosphorus (mg/dl)	r value	-.078	.036	-.057
	p value	.355	.748	.398
	N	144	81	225
ALP (U/L)	r value	**.335**	**.307**	**.288**
	p value	**.000**	**.005**	**.000**
	N	144	81	225
Vitamin D (ng/mL)	r value	**-.259**	**-.286**	**-.257**
	p value	**.002**	**.010**	**.000**
	N	144	81	225
PTH (pg/ml)	r value	.044	.090	.107
	p value	.601	.423	.110
	N	144	81	225
Osteocalcin level (ng/ml)	r value	**-.249**	-.160	**-.286**
	p value	**.003**	.153	**.000**
	N	144	81	225

Spearman correlation coefficient were used

The "Spearman coefficient of correlation" for all the blood glucode and Hba1c in controls, prediabetes and diabetes such as FGF23 with fasting blood glucose level (r value = .35; p value = 0.33) and HbA1c level (r value = .30; p value = 0.0) were found positively significantly correlated when compared to FGF23 with random blood glucose level (r value = .06; p value = 0.57), and Phosphorus with fasting blood glucose level (r value = -.04; p value = .75),ALP were found positively correlated with fasting blood glucose level (r value = .34; p value = 0.0) and with random blood glucose level (r value = .30; p value = 0.05), ALP with HbA1c (r value = .29; p value = 0.0) level also were found positively significantly correlated compared to PTH with fasting blood glucose level (r value = .04; p value = 0.06) and PTH with random blood glucose level (r value = .09; p value = 0.42), PTH with HbA1c level (r value = .10; p value = 0.11) all were showing positive weak correlation which was statistically significant.

In sequence between Vitamin D with fasting blood glucose level (r value = -.26; p value = .2), Vitamin D with random blood glucose level (r value = -.29; p value = .10), Vitamin D with HbA1c level (r value = -.26; p value = 0.0),IGF 1 with fasting blood glucose level (r

value = -.18; p value = .04) , IGF 1 with random blood glucose level (r value = -.07; p value = 0.53), IGF 1 with HbA1c level (r value = -.10; p value = 0.13), Calcium with fasting blood glucose level (r value = -.17; p value = .04) , Calcium with random blood glucose level (r value = -.02; p value = 0.9), Calcium with HbA1c level (r value = -.09; p value = 0.14) Phosphorus with fasting blood glucose level (r value = -.08; p value = .35), Phosphorus with HbA1c level (r value = -.06; p value = .39) Osteocalcin with fasting blood glucose level (r value = -.25; p value = .03), Osteocalcin with random blood glucose level (r value = -.16; p value = .15), Phosphorus with HbA1c level (r value = -.29; p value = 0.0) showing weak negative correlation between parameters which was statistically significant. Parameters were found to be less that 0.5 in the case of control group respectively (Table 22). The correlations coefficient between the Vitamin D with fasting, random & Hba1c were found to be and concluded as negatively significantly correlated. The correlation coefficients of Osteocalcin with fasting, random & Hba1c were found to be and concluded as negatively significantly correlated FGF-23 were found to be positively correlated.

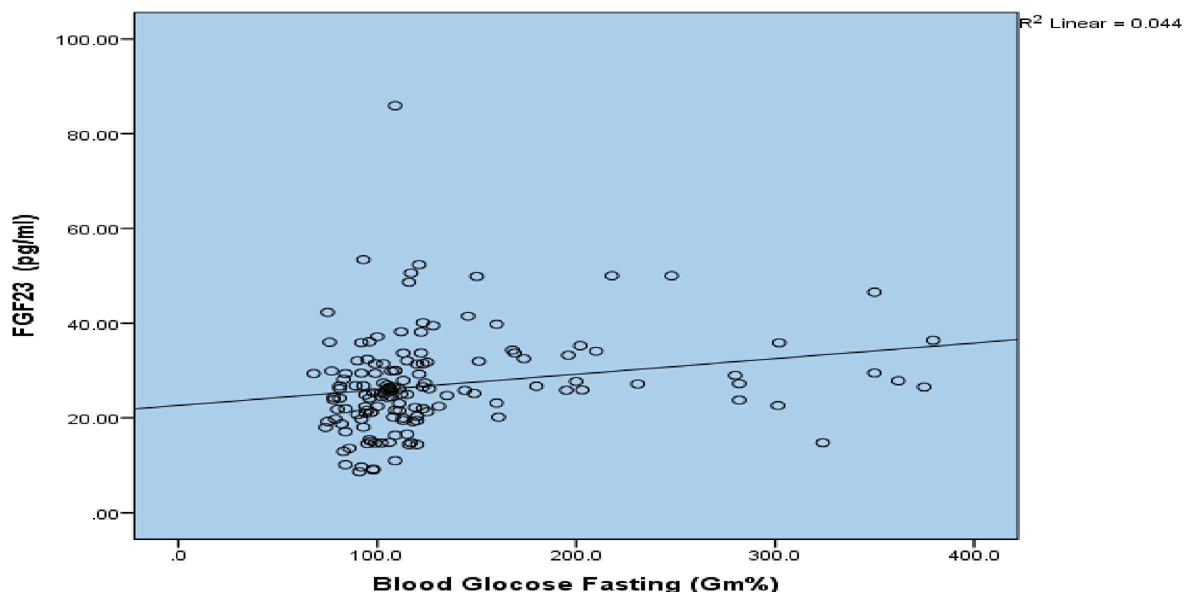


Figure 21: Scatterplot showing correlation of FGF23 with Fasting blood glucose in all study subjects

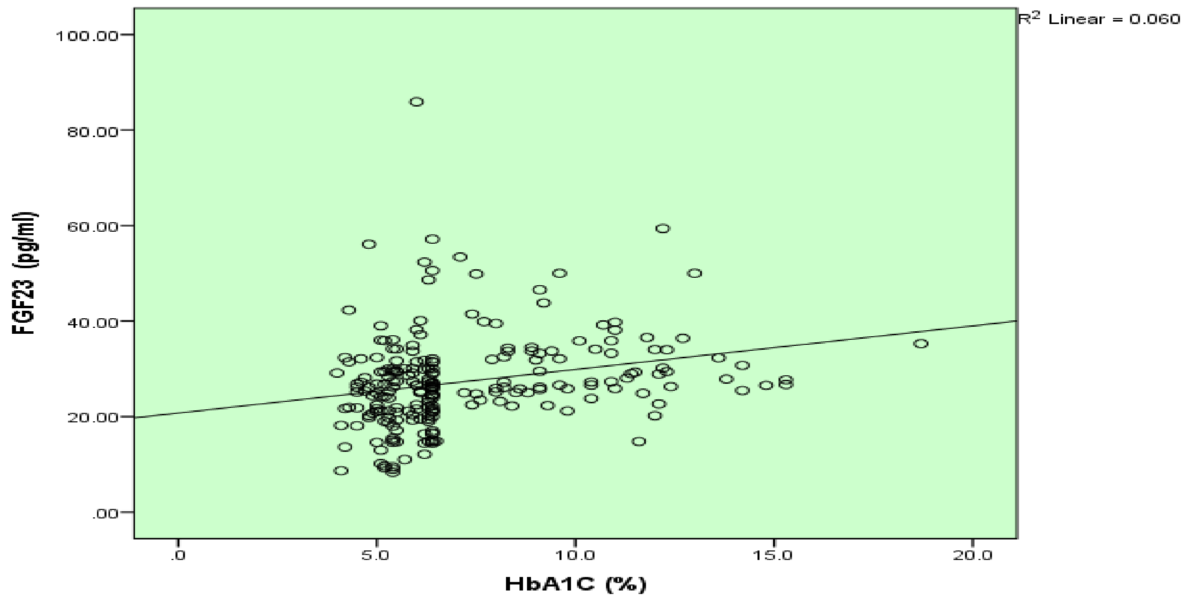


Figure 22: Scatterplot showing correlation of FGF23 with HbA1C level in all study subjects

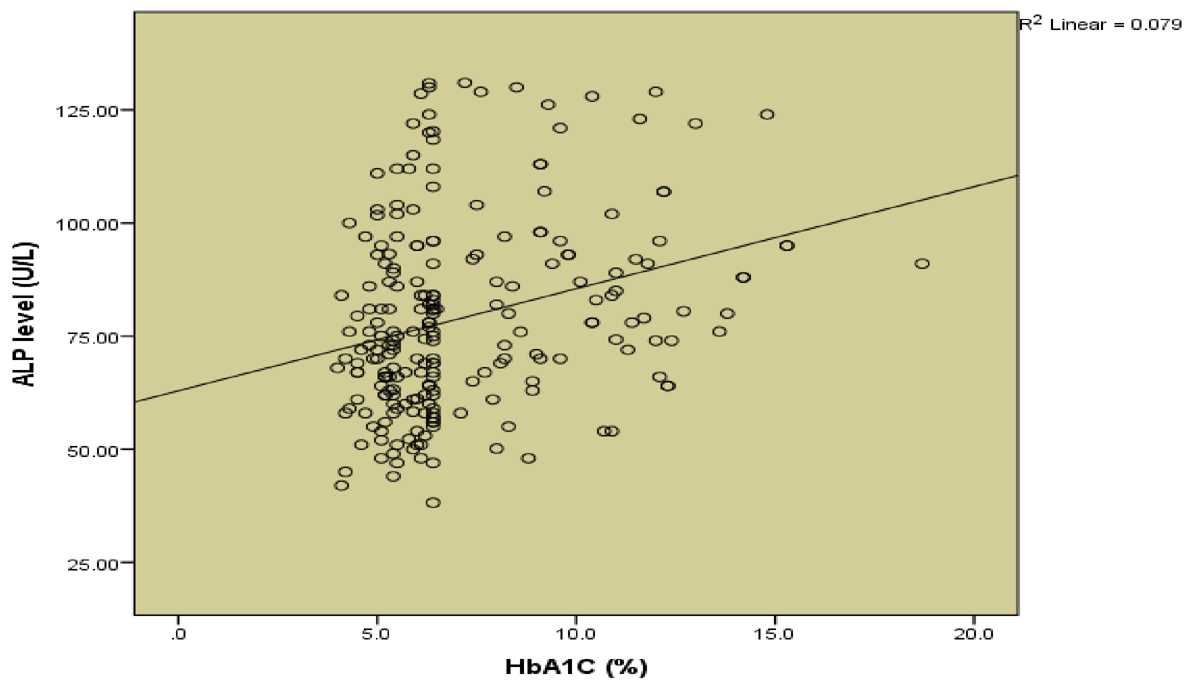


Figure 23 : Scatterplot showing correlation of ALP with HbA1C level in all study subjects

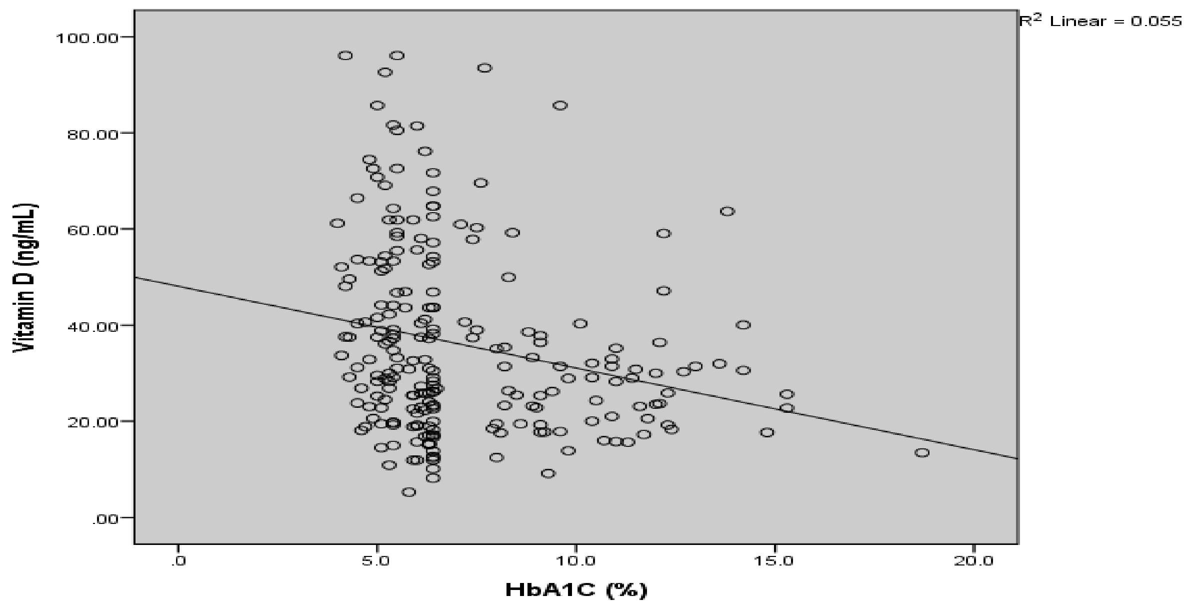


Figure 24 : Scatterplot showing correlation of HbA1C with Vitamin D level in all study subjects

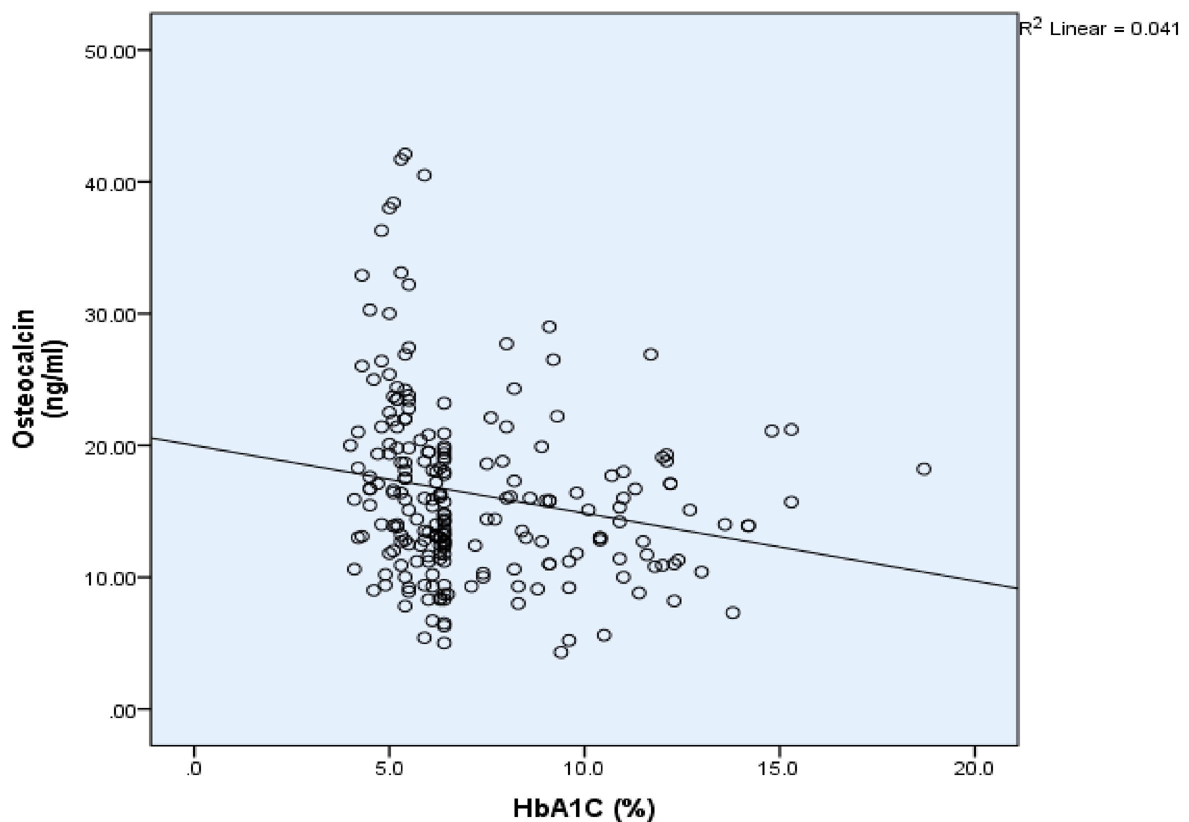


Figure 25 : Scatterplot showing correlation of HbA1C with osteocalcin level in all study subjects

CHAPTER-7

DISCUSSION

DISCUSSION

The current theory is supported by case-control hospital research that makes use of a range of measuring variables related to poor bone metabolism and diabetes. In persons with T2DM and prediabetes, the effects of FGF-23 and insulin-like growth factor-1 on altered bone metabolism were the main focus of this investigation. The study's subjects, on average, were over 60 years old and had been diagnosed with a number of cofounding diabetes-related conditions, some of which could worsen the condition if changed. In this study the parameters which shows significant results are Duration, Weight, BMI, Random and Fasting Blood Glucose, SGOT, Albumin, Calcium, Phosphorus, ALP, Vitamin D, HbA1c%, FGF-23, Parathyroid hormone, IGF-1, Osteocalcin, Triglycerides and Cholesterol which is a positive indication for this case-control study that this altered parameters can lead to alteration in bone metabolism which in turn leads to diagnosis of parameters for diabetes.

Diabetes-related metabolic changes have a significant impact on bone metabolism, increasing the fracture risks in people having diabetes type 1 and type 2 by six and two times, respectively [247]. Although numerous research has demonstrated the detrimental diabetes effects on bone strength and quality, the issue is still up for debate [248, 249].

Hyperglycemia - Hyperglycemia can reduce bone density in a number of ways. High glucose levels can have toxic effects that directly affect osteoblast function and quantity [250]. Through osmotic and non-osmotic mechanisms, high glucose levels can independently alter the amounts of osteoblast gene expression [251]. As a result of these modifications, bone mineralization and osteoblast development are inhibited. Osteoblast growth is hindered by high glucose levels, which reduces the osteoblast's capacity to react to 1, 25(OH)-vitamin D3. The vitamin D receptors are indirectly regulated as a result. Glycation end product production: A variety of proteins can be glycosylated by high glucose levels through non-

enzymatic processes, resulting in substances known as advanced glycosylation end-products (AGEs).

These biochemicals are believed to contribute to the genesis of diabetes since they are found in a variety of diabetic tissues in humans [252]. It reveals that AGEs and hyperglycemia play a significant part in the bone's fragility in types of diabetes [253]. The buildup of AGEs in cortical bone leads to an increased creation for cross-links between collagens. As this process makes collagen more rigid and durable, it has no impact on the mineralization of bones. The increased fragility of bone and fracture in people affected with diabetes may be explained by the negative relationship for AGEs and human trabecular bone size and fragility [254]. An accumulation of AGEs directly limits bone cell proliferation and differentiation in addition to the direct consequences of too much glucose. ROS and cellular death can result from the production and accumulation of AGEs, which is referred to as oxidative stress. [255]. High glucose levels enable bone marrow mesenchymal cells to differentiate more quickly into adipocytes, which could increase adipogenesis and reduce osteogenesis [256].

The following activities of IGF-1, insulin, and other growth factors:

In addition to IGF-1, some osteogenic effects are shown by insulin and stimulate the growth and differentiation of osteoblasts in both direct and indirect manner [257]. The insulin and IGF-1 receptors on the osteoblast are the direct mediators of the activity [258-260]. For instance, it has been demonstrated that exogenous insulin causes cultured osteoblast cells to produce more collagen [261]. The modulation of blood glucose levels, PTH, IGF-1, and vitamin D impacts are the indirect effects of insulin [262-264]. In T1DM animal models, claims of decreased bone density and osteoporosis have surfaced [265,266]. Additionally, it has been demonstrated in clinical research that T1DM people had lower BMD for neck of femur than diabetes type 2 subjects [267]. This discovery is explained by the absence of

insulin in patients with T1DM, which may also explain why juvenile T1DMs have increased risk for osteopenia and osteoporosis [268]. However, higher BMD and obesity levels in T2DM patients may be to blame for the higher BMD levels observed in T2DM patients compared to T1DM patients [269].

IGF-1, an insulin mimic, may have an impact on metabolism of bone. In fact, because it encourages osteoblast recruitment and matrix bone production while preventing bone loss and collagen breakdown, IGF-1 was assessed as an important regulator of metabolism of bones [270]. The number of osteoblasts is normal in spontaneously diabetic BB rats, but there is a deficit in bone mineralization that resembles osteomalacia (osteomalacia-like mineralization defect) [271]. Additionally, using IFG-1 with a controlled release as a pharmacological model showed promising outcomes for bone abnormalities that do not repair under standard therapy circumstances [272]. In postmenopausal women with T2D, clinical studies have shown a link between vertebral fractures and reduced levels of IGF-1 [273]. 17-estradiol and IGF-1 are recognized as strong hormonal predictors for hip and femoral neck BMD in young men; however, in men over 60, the connection for IGF-1 is no longer significant. [274]. IGF-1 levels, on the other side, are decreased in T1DM patients than in T2DM patients [275]. Last but not least, IGF-1 may be taken into consideration for predicting fracture risk in diabetes [276], according to cross-sectional studies that looked at the relationship between fracture and bone turnover markers (BTMs). More research should definitely be done to completely comprehend associated pathways.

The metabolism of calcium and its effects on bones are crucial. Therefore, any change that upsets the equilibrium between systemic factors that regulate calcium levels may lead to bone loss [277]. Together, type 1 or type 2 diabetes and vitamin D deficiency significantly raise the risk of osteopenia and osteoporosis [278,279]. A quantitative imbalance in the

parathyroid hormone may promote bone loss and an elevated risk of fracture since it also has some moderating effects on osteoblasts [280, 281]. Numerous processes, the majority of which are linked to calcium loss through the kidney, can affect calcium metabolism as a result of diabetes. Diabetes causes diabetic rats to release more calcium from their kidneys than non-diabetic rats do. Additionally, the levels of 1,25(OH)2-D3 and vitamin D binding proteins are almost ten times lower in the experimental group than in the control group. In diabetic mice, there is a correlation between lower bone density and the amount of calcium secreted in urine. The kidney was subjected to a Quantified-PCR analysis, which identified changes in the levels of the mRNAs for 25-hydroxyvitamin D24-hydroxylase (down regulation) and 25-hydroxyvitamin D-1-hydroxylase (up regulation), as well as the down regulation of the genes for plasma membrane Ca-ATPase, vitamin D receptors, and calcium transferring receptors (VDR) [282].

Lack of vitamin D during the first two years of life is the primary cause of rickets. Adults with vitamin D deficiency may develop osteoporosis, osteomalacia, or both [283]. It has been determined that it has a role in the development of both forms of diabetes [284]. Vitamin D appears to affect the immune system in T1DM, but its ameliorating effects on B cell activity and enhanced insulin sensitivity are more apparent in type 2 diabetes (T2DM). T1DM may also be correlated with some genetic variations linked to low vitamin D levels.

Regarding incidence, complications, management, and death, there is a link between insufficiency of vitamin D and diabetes. [285-289]. It is controversial whether vitamin D deficiency is a complication of diabetes or a risk factor for both types of diabetes, despite the fact that numerous research has suggested it may be [290]. The impact of vitamin D supplementation on the prevalence of diabetes has not been studied in any randomized clinical studies [291].

PTH: Increased PTH levels have been linked to insulin resistance, according to research [295]. Furthermore, there is a correlation between reduced PTH levels and vertebral fracture in people with T2 diabetes [296], which could be explained by PTH's diminished anabolic effects on bones. Young patients with insulin-dependent diabetes had PTH, calcium, and phosphate levels that were within the normal range, but the serum levels of vitamin D3 were lower, possibly as a result of their lower cortical bone density. It is generally known that PTH and teriparatide (PTH 1-34, TPTD), a synthetic peptide fragment of PTH, have anabolic effects on bone when given occasionally. The anabolic mechanism of action of PTH therapy may be indicated by elevated serum levels of osteocalcin [297].

Trials have shown that PTH has a bone-healing effect, and a few case reports have suggested that TPTD may help patients with diabetes recover stress and non union fractures more quickly. The beneficial effects of PTH on osteoporosis brought on by thiazolidinediones (TZDs) are also an exciting area for research [298-300].

FGF-23 is assessed as the possible biomarker in detection of diabetes mellitus as its concentration greatly increased in diabetes when compared to controls. Levels of FGF-23 affects several other parameters especially glucose levels in random and fasting blood that was markedly increased in diabetes mellitus patients and was found to regulate gene expression and formation of bone in osteoblast cells which synthesize FGF-23 production. FGF-23 levels and glucose levels did not significantly correlate in the current findings. In diabetes patients, there is a substantial correlation between HbA1c% and blood glucose levels [301].

The bone derived FGF23 prevents the kidney from producing vitamin D hormone and reabsorbing phosphate. FGF23's endocrine activities in the kidney are Klotho dependent because co-receptor Klotho is necessary for high-affinity binding of the hormone to FGF

receptors to take place at physiological levels of the hormone. It is widely known that people with adequate renal function experience phosphate squandering when their blood contains high levels of intact FGF23. A significant amount of study has concentrated on the pathophysiological effects of FGF23 excess due to the significance of diseases like chronic kidney disease and phosphate-wasting diseases that are linked to gains in FGF23 function. Less attention has been placed on FGF23's function in healthy physiology. However, in addition to its crucial physiological role in regulating the sodium-phosphate co-transporters and 1-hydroxylase expression in proximal renal tubules, recent studies utilizing loss-of-function models have shown that FGF23 also plays a crucial physiological role in stimulating calcium and sodium reabsorption in distal renal tubules. FGF23 is also beginning to have a role in the autocrine/paracrine control of bone mineralization and alkaline phosphatase expression. FGF23 inhibits alkaline phosphatase in bone independently of Klotho, in contrast to its effect on the kidney. In the bone microenvironment, FGF23 may also physiologically limit the development of hematopoietic stem cells along the erythroid lineage. FGF23's physiological function in organs other than the kidney and bone is not fully understood [302]. The mini-goal of the study is to highlight what is currently known about FGF23's and IGF-1 intricate physiological functions on altered bone metabolism. Finally The correlations coefficient between the FGF23 & Vitamin D with fasting, random & Hba1c were found negatively significantly correlated. And the correlation coefficients of Osteocalcin with fasting, random & Hba1c were also found to concluded as negatively significantly correlated

Similarily, the correlations coefficient between the ALP and FGF-23 were found to be and concluded as negatively correlated. The correlation coefficients of Osteocalcin with FGF-23 were found to be positively correlated and negatively correlated with the IGF-1. The Vitamin D and IGF-1 is significantly negatively correlated as to Vitamin D and FGF-23.

CHAPTER-8

CONCLUSION

CONCLUSION

There is a lot of data to support the notion that both forms of diabetes increase the risk of fracture and have a detrimental impact on bone strength. We could argue that, of the various pathways discussed in this work, hyperglycemia and AGEs play a more significant impact. However, research on additional mechanisms by which diabetes affects bone fragility is highly encouraged. We are more likely to discover workable therapeutic strategies in this way for the appropriate and prompt management of osteoporosis. It may also be beneficial to examine how diabetic sequelae such microangiopathy and macroangiopathy, neuropathy, and nephropathy affect bone health. Although bone can influence the body's metabolism of glucose and the chance of getting diabetes, the exact method by which it does so is yet unknown. In this sense, it's also critical to emphasize the significance of studying hormones derived from bone in scientific studies. All of the discussions we've just discussed have the ability to present a fresh, intriguing idea. The bone is an endocrine organ that secretes substances like adiponectin, osteocalcin, and vastatin that can alter the body's metabolism rather than being a fundamental organ that just receives input from other organs.

Since its discovery, research into the regulation and function of FGF23, a strong phosphaturic hormone, has attracted considerable attention. Studies have shown that FGF23 is tightly controlled at the transcriptional and posttranscriptional levels by both systemic and local stimuli, with the kidney being its primary target organ. An imbalanced phosphate homeostasis and vitamin D metabolism would result from an excess or deficiency of FGF23, which would be caused by a disruption in its regulation and would significantly contribute to the development of skeletal abnormalities. However, direct effects of abnormal FGF23 activity on bone have also been postulated. Therefore, both direct and indirect processes may be used by FGF23 to regulate bone production and modeling/remodeling. Aside from these

extra physiological functions, FGF23 also enhances calcium and salt retention in the kidney and inhibits renal phosphate reabsorption. It also supports a normal response for the kidney to PTH. There is currently no evidence that FGF23 contributes to the normal physiology of organs other than the kidney and bone, despite the fact that excessive FGF23 may target a number of non-canonical tissues. Finally the correlation coefficients of Osteocalcin with FGF-23 were found to be positively correlated and negatively correlated with the IGF-1. The Vitamin D and IGF-1 is significantly negatively correlated as to Vitamin D and FGF-23.

The correlations coefficient between the FGF23 & Vitamin D with fasting, random & Hba1c were found negatively significantly correlated. And the correlation coefficients of Osteocalcin with fasting, random & Hba1c were also found to concluded as negatively significantly correlated

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CHAPTER-9

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