A STUDY OF THE CORRELATION BETWEEN SERUM ADENOSINE DEAMINASE LEVELS AND FASTING SERUM INSULIN LEVELS IN TYPE 2 DIABETS MELLITUS

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LIST OF ABBREVIATION USED: -

ADA	Adenosine deaminase
cAMP	Cyclic adenosine monophosphate
HbA1C	Glycosylated hemoglobin
DM	Diabetes mellitus
AD	anno domini
HIV	Human immunodeficiency virus
AIDS	Acquired immunodeficiency syndrome
CVD	Cardiovascular disease
IDF	International diabetes federation
ATP	Adenosine triphosphate
AK	Adenosine kinase
FFA	Free fatty acid
DPP-4	Dipeptidyl peptidase-4
NPH	Neutral protamine Hagedorn
GLUT-2	Glucose transporter protein-2
HDL	High density lipoprotein
DNL	De novo lipogenesis
OGTT	Oral glucose tolerance test
QUICKI	Quantitative insulin sensitivity check index
HOMA-IR	Homeostasis model assessment insulin resistance
LDL	Low density lipoprotein
PKB	Protein kinase B
IL-6	Interleukin-6
TNF	Tissue necrosis factor

ABSTRACT

Introduction:

Diabetes mellitus (DM) represents a group of common metabolic diseases caused by genetic and environmental factors that result in inadequate insulin secretion and diminished sensitivity to endogenous or exogenous insulin. Adenosine replicates the effects of insulin on the metabolism of glucose and lipids in adipose tissue and the heart while blocking the effects of insulin on total hepatic glucose output, suggesting that adenosine leads to localised insulin resistance in the liver. Adenosine deaminase (ADA) is an enzyme involved in the purine metabolism that catalyses the hydrolytic cleavage of adenosine into inosine and ammonia, causing a drop in the levels of adenosine.

Aims and objectives: -

A study of insulin resistance with correlation between serum adenosine deaminase level and fasting serum insulin levels in type 2 diabetes mellitus.

Materials and methods: -

This is a cross sectional study in which 70 patients with newly diagnosed or known case of type 2 diabetes mellitus were taken based on inclusion and exclusion criteria who attended outpatient and admitted in BLDE (DEEMED TO BE UNIVERSITY) Shri B M Patil medical college hospital and research center, Vijayapura.

Result:

A total of 70 patients were included in the study. Serum ADA, mean fasting blood sugar, postprandial blood sugar, glycosylated haemoglobin, fasting serum insulin levels values were significantly elevated. Quantitative insulin sensitivity check index was measured for each patient. The mean and median value of serum ADA was 22.8109 and 20.5050; serum fasting insulin was 13.7349 and 11.8650; FBS was

174.31 and 165.50; PPBS was 219.86 and 207; HbA1C was 8.45 and 7.95. Mildly positive correlation was found between serum ADA with FBG, PPBG and HbA1C with r value of 0.085,0.193 and 0.157. Serum fasting insulin was mildly correlated with FBG and significant correlation with PPBS (p=0.027) and HbA1C (p = 0.022). Serum adenosine deaminase was positively correlated with QUICKI (r=0.126) and serum fasting insulin was strongly and significantly correlated with QUICKI (p=0.0001). Serum adenosine deaminase level and serum fasting insulin were mildly correlated with each other (r=0.201 and p=0.991).

Conclusion: -

In this study we correlated of Serum Adenosine Deaminase (ADA) levels and serum fasting insulin in patients of type 2 diabetes mellitus through a cross sectional study and we found: -

- Serum adenosine deaminase levels were found significantly higher in type 2 diabetes patients
- Mildly positive correlation was found between serum ADA with FBG, PPBG and HbA1C.
- There was a mild correlation between serum fasting insulin and FBG and a substantial correlation between PPBG andHbA1C.
- Serum adenosine deaminase level and serum fasting insulin were mildly correlated with each other.
- Serum adenosine deaminase was positively correlated with QUICKI
- Serum fasting insulin was strongly and significantly correlated with QUICKI

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INTRODUCTION

Diabetes mellitus (DM) represents a group of common metabolic diseases caused by genetic and environmental factors that result in inadequate insulin secretion and diminished sensitivity to endogenous or exogenous insulin. [(1)] It results in undesirable glucose levels and alterations to the metabolism of carbohydrates, fats, and proteins. Symptoms of hyperglycemia includes frequent urination, increased thirst and increased hunger.

Type-2 diabetes mellitus, which earlier known as "noninsulin-dependent diabetes" or "adult-onset diabetes," represents 90–95 percent of all diabetes. Numerous things can lead to type 2 diabetes. Beta-cells are not destroyed by the immune system, despite the fact that the precise causes are unknown, and patients do not have any other known diabetes-causing factors. Despite the appearance of normal or raised insulin levels in type 2 diabetes mellitus patients, the failure to normalise blood glucose is caused by a relative deficiency in glucose-stimulated insulin production. As a result, insulin secretion in these patients is impaired and insufficient to compensate for insulin resistance. [(2)]

Adenosine deaminase (ADA), also known as adenosine amidohydrolase (EC 3.5.4.4), is an enzyme involved in the purine metabolism that catalyses the hydrolytic cleavage of adenosine into inosine and ammonia, causing a drop in the adenosine concentrations. Adenosine replicates the effects of insulin on the metabolism of glucose and lipids in adipose tissue and the heart while blocking the effects of insulin's impact on liver glucose production overall, suggesting that adenosine leads to localised insulin resistance in the liver.[(3)] Adenosine primarily inhibits cyclic AMP accumulation, whereas insulin inhibits lipolysis through a noncyclic AMP-dependent mechanism. According to John N. Fain et al., unless adenosine keeps cyclic AMP accumulation at low levels, insulin cannot suppress lipolysis since there are significant quantities of lipolytic chemicals. [(4)] Because ADA's main biological activity is identified in T lymphocyte function, it was regarded to be an excellent marker of cell-mediated immunity and played an important

role in lymphocyte proliferation and differentiation. [5]

Impairment of lymphocyte function and increased susceptibility to infections are typically linked to human diabetes. [6] Studies on type 2 diabetes have found increased ADA activity [3,7], and concluded that the ADA measures oxidative stress and peroxidation of lipid in diabetes. While Anju Gillet et al. observed that type 2 diabetes mellitus increases HbA1c levels along with a rise in serum Insulin levels [6].

Several proinflammatory oxidative stress mediators, including proinflammatory cytokines like tumour necrosis-alpha, IL-1 beta, and IL -6, numerous chemokines and adipocytokines, epigenetic factors, and other metabolic and transcriptional pathways, are associated with low-grade tissue-specific inflammatory responses causing insulin resistance.

In addition, proinflammatory mediators enhances the stimulation of cytokine signalling proteins, this in turn stops the beta cells in pancreatic islets from activating the insulin signalling receptor. [8]

REVIEW OF LITERATURE

Although diabetes mellitus was first recognised in antiquity, its history has already been marked by multiple cycles of discovery, oblivion, and rediscovery. The first clinical descriptions of diabetes and its complications date back to antiquity, but it was not until the 16th to 18th centuries that DM was recognised as a distinct medical entity. In the mid-to-late 19th century, which might be termed the initial experimental period, the glucoregulatory activity of the pancreas became evident and the biochemical irregularities of diabetes were first discovered. [(9)]

Eventually, the 20th century has witnessed a significant rise in diabetes understanding. Significant scientific, clinical, and social effects have resulted from the discovery of insulin in 1921–1922. Some key developments in the scientific and clinical understanding of diabetes may be summarised as follows :-[(10)]

- As early as 1550 BC, in an ancient Egyptian papyrus that George Ebers uncovered, polyuric states that clinically resembled DM were recorded.
- Sushruta and Charaka, two Indian doctors, first described the sweet flavor of urine in diabetes in the fifth and sixth centuries AD. Thomas Willis later described it in 17th century. In order to distinguish diabetes mellitus from different polyuric conditions in which urine was bland, John Rollo and others originally adopted the word in the late 18th century which makes reference to the honeyed taste of urine.
- Matthew Dobson observed in 1776 that diabetic urine and serum both contained sugar and came to the conclusion that diabetes was a systemic ailment rather than a kidney disease.
- Between the middle and end of the 19th century, Claude Bernard produced a number of discoveries on metabolism and diabetes. He described how glucose is stored in the liver as

glycogen and how experimental animals experience hyperglycemia.

- Edvard Laguesse proposed that the pancreatic islets produced a chemical that may lower blood sugar levels in 1893 and called them after Paul Langerhans, who had first characterised them in 1869. In1909, more than ten years before it was ever discovered, Jean de Meyer gave this putative hormone the name insulin.
- During the first two decades of the 20th century, a number of researchers, including George Zuelzer (Germany) and Nicolas Paulesco (Romania), extracted active but enhanced hypoglycemic extracts from the pancreas; nonetheless, hazardous side effects prevented their official testing in diabetic patients.
- At the University of Toronto, Frederick G. Banting, Charles H. Best, James B. Collip, and J.J.R. Macleod collaborated on the creation of insulin in 1921. Insulin was first isolated from a human diabetic (Leonard Thompson) in January1922, and it was discovered that the isolates lower blood glucose levels in pancreatectomized dogs.

Classification: -

It can be broadly categorised into the following groups: -

1. Type 1 diabetes mellitus (owing to autoimmune cell damage, which leads to insulin deficit)

2. Type 2 diabetes mellitus (owing to substantial loss of adequate b-cell insulin secretion with insulin resistance)

3. Gestational diabetes mellitus (diabetes detected in the 2^{nd} or 3^{rd} trimester of pregnancy that was not present before gestation)

4. Particular type of diabetes owing to other causes such as, monogenic diabetes syndromes (which includes neonatal diabetes and maturity-onset diabetes of the young), drug- or chemicals causing diabetes (as when using glucocorticoids or in the treatment of HIV/AIDS, or after organ transplantation) and diseases of the exocrine pancreas (such as pancreatitis and cystic fibrosis) [11,12]

Type-2 Diabetes Mellitus diagnostic criteria: - [13]

Fasting blood sugar levels more than or equivalent to 126 mg/dl (7.0 mmol/l)

OR

Increased frequency of urination, extreme thirst, unanticipated weight loss, and other symptoms, along with a random blood sugar measurement of 200 mg/dl (11.1 mmol/l) or higher

OR

Blood glucose level ≥200 mg/dl (11.1 mmol/l), measured two hours after a 75-gram glucose load

OR

HbA1c ≥ 6.5%.

PATHOPHYSIOLOGY [14]: -

A pro-inflammatory condition, as well as the interaction of hereditary and environmental variables, contributing to type 2 diabetes mellitus**.** (FIG-1) [15]

1. First-degree relatives are at a five-to ten-fold higher risk due to genetic factors.

2. Environmental Factors like sedentary lifestyle and obesity

3. Insulin resistance which causes hyperglycemia due to: -

i) reduce glucose utilisation causing hyperglycaemia.

ii) elevated glucose production in the liver.

iii) High amounts of free fatty acids and cytokines in obesity cause hyperglycemia.

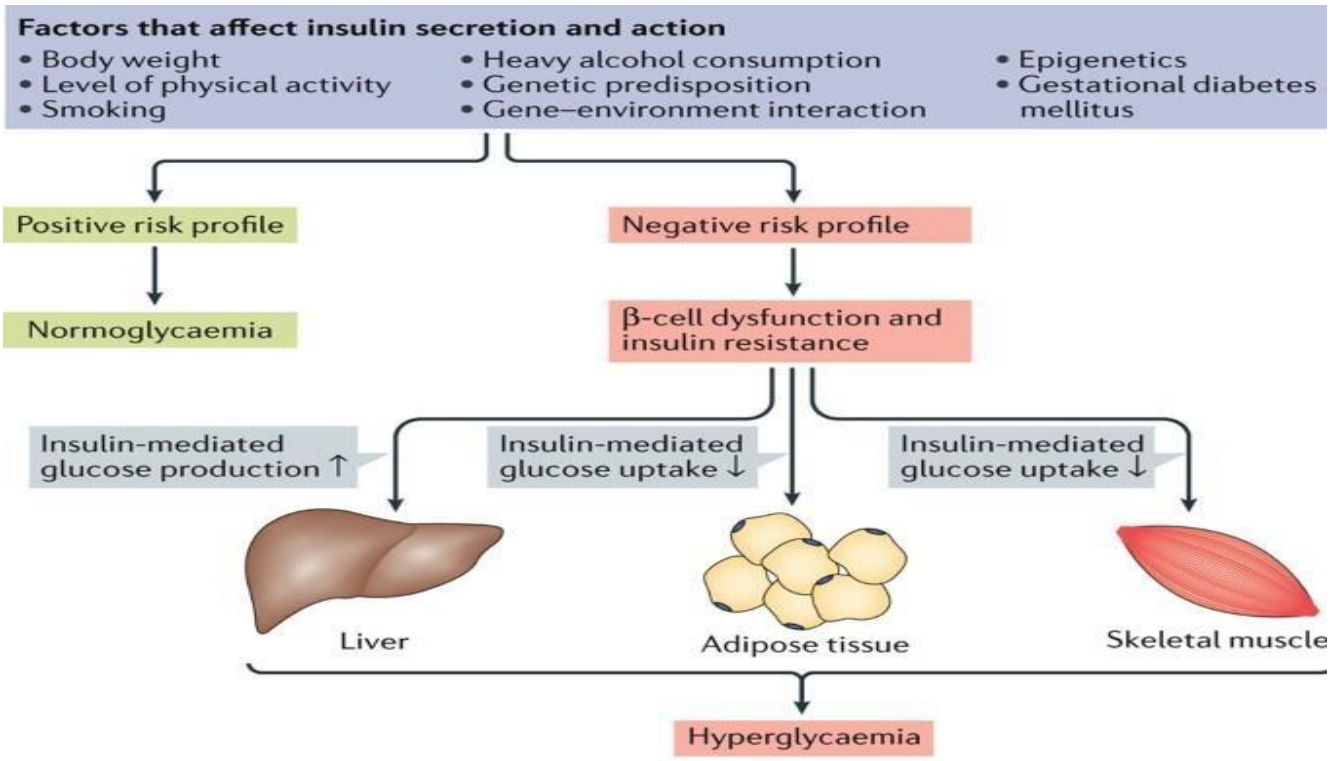

FIG.-1

EPIDEMIOLOGY: -

In addition to cancer, cardiovascular disease (CVD), and respiratory diseases as three causes of death, diabetes is one of the most serious health emergencies worldwide of this century. The International Diabetes Federation (IDF) estimated approximately 537 million persons in the 20-to 79-year-old age in year 2021, with that number expected to climb to 643 million by 2030 and 783 million by the year 2045. [(16)]

In India, diabetes has grown to be a serious public health issue. The World Health Organization estimated that there were 31.7 million diabetics in our nation in 2000, and that number will increase to

71.4 million by 2030. India has emerged as the world's diabetes capital, with the largest population of people with diabetes.

The current rate of DM among Indians living in urban areas is 12.1 percent. According to data, type 2 diabetes strikes decade earlier in Indians than it does in the West. Because of early-onset, late diagnosis, and poor management, have resulted in a decline in productivity and higher rate of morbidity and mortality. [17]

RISK FACTORS: -

Type-2 diabetes mellitus risk factors include advancing age, obesity, and inactivity. Diabetes frequently co-occurs with other risk factors, such as hypertension, dyslipidemia, insulin resistance, and abdominal obesity, collectively known as the "metabolic syndrome," through which cardiovascular disease risk is raised. [18] It frequently has a significant hereditary propensity or a history among first-degree relatives. In this era of precision medicine, the genetic epidemiology of type 2 diabetes is deeply studied but yet poorly understood. [19]

TREATMENT RECOMMODATIONS: - [20,21]

1. The first drug of preference for treating type-2 diabetes is metformin.
2. Metformin should be continued once it has been started if no side effects appear and if not contraindicated; additional pharmaceuticals, such as insulin, should be used together with metformin.
3. In some patients, early combination therapy is advantageous at the beginning of treatment to delay the onset of treatment failure.
4. When there are signs of continuous catabolism, such as weight loss, hyperglycemia symptoms, or when blood sugar levels are extremely high (300 mg/dL or 16.7 mmol/L), or HBA1C, 10% (86 mmol/mol), it is advantageous to start insulin therapy.
5. Pharmacologic medicines should be started with a patient-centered strategy. Cardiovascular comorbidities, the danger of hypoglycemia, the influence on weight, the cost, the possibility of side

effects, and patient preferences should all be taken into account.

6. Sodium-glucose cotransporter-2 inhibitors or glucagon-like peptide-1 receptor agonists demonstrated cardiovascular disease benefit are advised for patients with type 2 diabetes mellitus who have cardiovascular disease or established kidney disease.
7. Oral medications can be used to treat type 2 diabetic patients who need more considerable glucose lowering; where possible, Insulin is effective for glucagon-like peptide-1 receptor agonists.
8. Treatment should be escalated in patients not achieving treatment goals in type 2 diabetes mellitus patients.

ADENOSINE

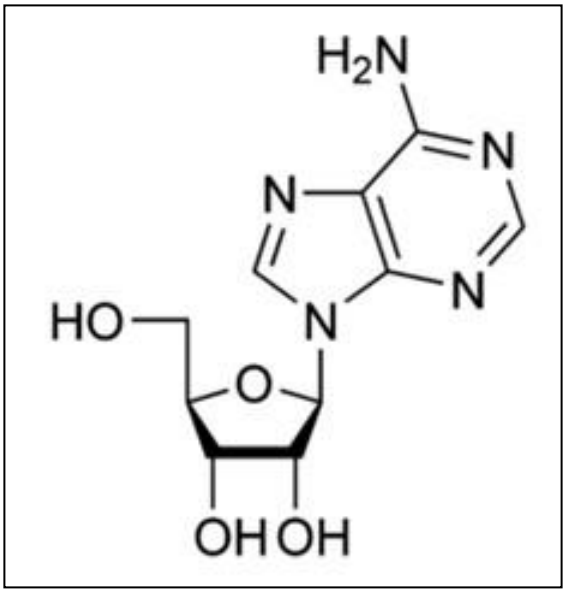

FIG.-2

An endogenous nucleoside called adenosine (FIG. 2) is made up of adenine joined to a ribose. In various mammalian tissues, it is a necessary chemical for life. [22]

Despite the fact that adenosine has a variety of effects, our understanding of the substance is mostly based on how it affects the cardiovascular system. Drury and Szent-ground-breaking Gyorgyi's ([23] study from a century ago revealed that an adenine compound's intravenous injection might alter heart rate. This adenine substance was probably adenosine. Adenosine wasn't employed in clinical settings to treat patients with supraventricular tachycardia until almost 60 years later [24]. In 1970 Sattin and Rall demonstrated how adenosine controls cell function by interacting with particular cell surface receptors. Adenosine can either be produced by the enzymatic cleavage of extracellular ATP or exocytosed out of the intracellular space. Injured neurons and glial cells may potentially release ATP, avoiding the

compromised plasma membrane. [24,25]

Adenosine synthesis and metabolism: -

Adenosine, an endogenous purine nucleoside, is generally present at low concentrations in the extracellular space but rises sharply in physiologically stressful settings. [26]

AMP and S-adenosylhomocysteine, which are carried across cell membranes by nucleoside transporters, are two separate substrates that are used in two different routes to generate adenosine at intracellular and extracellular locations [27,28]. Adenosine is quickly phosphorylated into AMP by adenosine kinase following intracellular reuptake, or it is rapidly deaminated into inosine by adenosine deaminase. These pathways employ strong enzymatic regulation to maintain the level of intracellular adenosine concentrations. The suppression of the enzymes involved in adenosine's metabolic transition is one method for raising local concentrations of the substance. In an effort to raise amounts of endogenous adenosine, inhibition of adenosine deaminase (ADA) and adenosine kinase (AK) have drawn a lot of attention. [29]

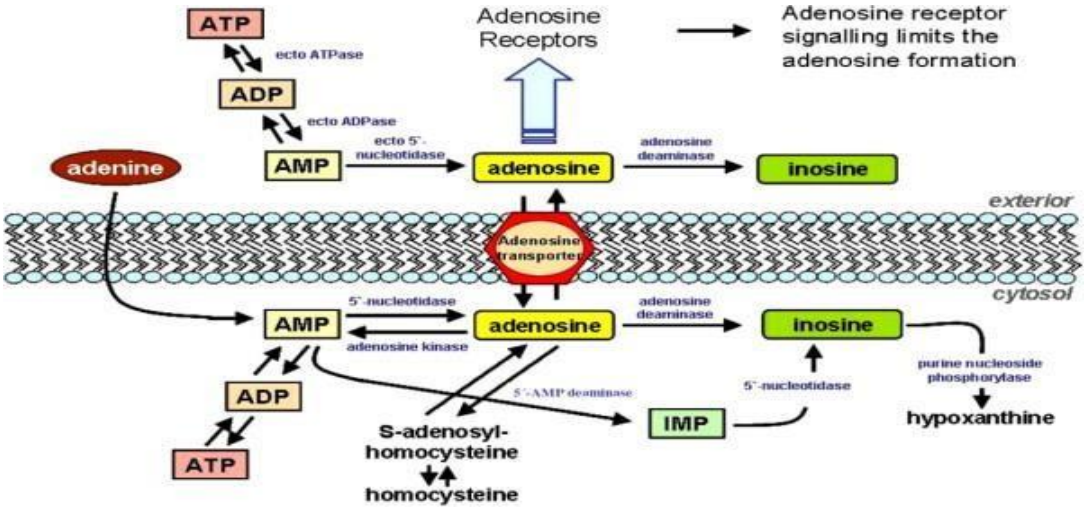

FIG.-4

ADENOSINE RECEPTORS: -

The two primary kinds of purinergic receptors are P1 receptors (also known as adenosine receptors) and P2 receptors (also known as nucleotide receptors) (Fredholm et al., 1994).

There are four subtypes of adenosine receptors—A1, A2A, A2B, and A3—based on their molecular, biochemical, and pharmacological characteristics.

The amino acid sequences of every cloned adenosine receptor are in agreement with the three-dimensional structure anticipated for G-protein coupled receptors. According to this theory, the N- and C-terminal of the protein are located on the intracellular and extracellular sides of the plasma membrane, respectively, and these receptors are made up of seven alpha-helical membrane-spanning domains connected by three extra and three intracellular loops. The transmembrane regions have the most homology between subtypes. These areas are thought to be important in ligand binding and activation along with the 2nd extracellular loop, while the 3rd intracellular loop is thought to be where the G-protein interaction occurs. **(30)**

PHYSIOLOGICAL RESPONSE TO ADENOSINE RECEPTORS:-[30,31]

Subtype of physiological response	Receptor
1. Central nervous system	
• Anticonvulsant action	A1
• Reduction in locomotor activity	A2A
• Sedation	A1
2. **Peripheral nervous system**	
• Inhibition of parasympathetic nerves	A1
• Inhibition of sympathetic nerves	A1
• Stimulation of chemoreceptor	A2A/A2B
• Parasympathetic nerve stimulation	A2A
• Sensory nerve modulation	A1/ A2A/A2B/A3
3. **Cardiovascular system**	
• Vasodilatation (vascular smooth muscle)	A2A/A2B/A3
• Vasoconstriction	A1
• Preconditioning	A1/A3
• Platelet aggregation inhibition	A2A
4. **Respiratory system**	

- Bronchodilatation A2A/A2B
- Broncho-constriction A1

5. Renal system

- Antidiuresis (tubular sodium reabsorption) A1
- Afferent arteriole constriction A1
- Efferent arteriole dilation A2AA1
- Mesangial cell contraction A1
- Tubuloglomerular feedback response
- Renin activation A1/A2A

6. Gastrointestinal system

- Gastric acid inhibition A1
- Stimulation of intestinal secretion A2A

7. Hormonal and exocrine system

- Inhibition of lipolysis A1
- Increase of insulin sensitivity A1
- Pancreatic insulin secretion inhibition A1
- Pancreatic glucagon stimulation A2A/A2B
- Stimulation of gluconeogenesis A2A/A2B

8. Immune system

• Neutrophil superoxide inhibition	A2A
• Inhibition of expression of cell adhesion molecules	A2A
• Cytokine synthesis inhibition	A2A/A2B/A3
• Degranulation of mast cell	A2A/A2B

ADENOSINE AND TYPE 2 DIABTES MELLITUS: -

Type-2 DM is a pathophysiological disorder marked by an insufficient β cell response to the accumulating insulin resistance that occurs with ageing, inactivity, and fat.[32] Obesity-induced insulin resistance, metabolic syndrome, and type 2 diabetes mellitus are all primarily caused by chronic low-grade inflammation.[33] Indeed, immune cells such as macrophages invade the liver, muscle, pancreas, and adipose tissue, and various cell populations' characteristics switch from being anti-inflammatory to being pro-inflammatory.[33]

ADENOSINE IN ADIPOSE TISSUE: - (FIG.-4)

Obesity-related adipose tissue dysfunctionality is characterised by reduce storage of triglyceride and increased lipolysis [34], which result in elevated levels of free fatty acids (FFAs) in the bloodstream [35] and a corresponding excess of FFAs in liver and the skeletal muscle.[34] Adenosine plays a role in controlling adipocyte function by being secreted from fat cells in adipose tissue.[36,37,38]) Adenosine primarily stimulates the A1 adenosine receptor to prevent lipolysis and increase lipogenesis.[39,40] According to pharmacological research, the A1R mediates the antilipolytic activity of adenosine. It has also been demonstrated that endogenous adenosine at low levels is adequate to prevent lipolysis. Adenyl cyclase is inhibited by activation of inhibitory Gi protein-coupled receptors, which also results in lower levels of cAMP and lipolysis. In adipose tissue, adenosine also exerts other effects, such as inducing leptin release. A2B adenosine receptors, however, reduce maturation of adipocyte and inflammation in adipose tissue.

ADENOSINE IN SKELETAL MUSCLE: - (FIG-4)

As a result of lipid accumulation and anomalies in fatty acid metabolism, such as alterations in fatty acid consumption in muscle, fatty acid oxidation, triglyceride synthesis, and lipolysis, skeletal muscle develops insulin resistance. [41] A1 adenosine receptors were the main pathway through which adenosine inhibited the transport of glucose into skeletal muscle. [42]

ADENOSINE IN PANCREAS: - [43]

Adenosine and its precursors have been shown to inhibit the secretion of insulin. It has been suggested that the adenosine receptor responsible for this suppression of release is the A1R. A2BR antagonists were able to overcome the inhibition effect of an unspecific adenosine agonist on insulin release. However, agonists of the A1R, A2AR, and A3R inhibited the release of insulin produced by glucose. These findings suggest that additional adenosine receptors may also be involved in the production of insulin. The mechanism underlying these discoveries is currently unknown. Adenosine has also been shown to stimulate glucagon release, which is thought to be mediated by A2 receptors.

ADENOSINE IN LIVER: - (FIG-4)[44]

Adenosine promotes the synthesis of cyclic AMP and regulates glycogen inhibition and gluconeogenesis most likely through the A2B receptor of adenosine in hepatocytes.

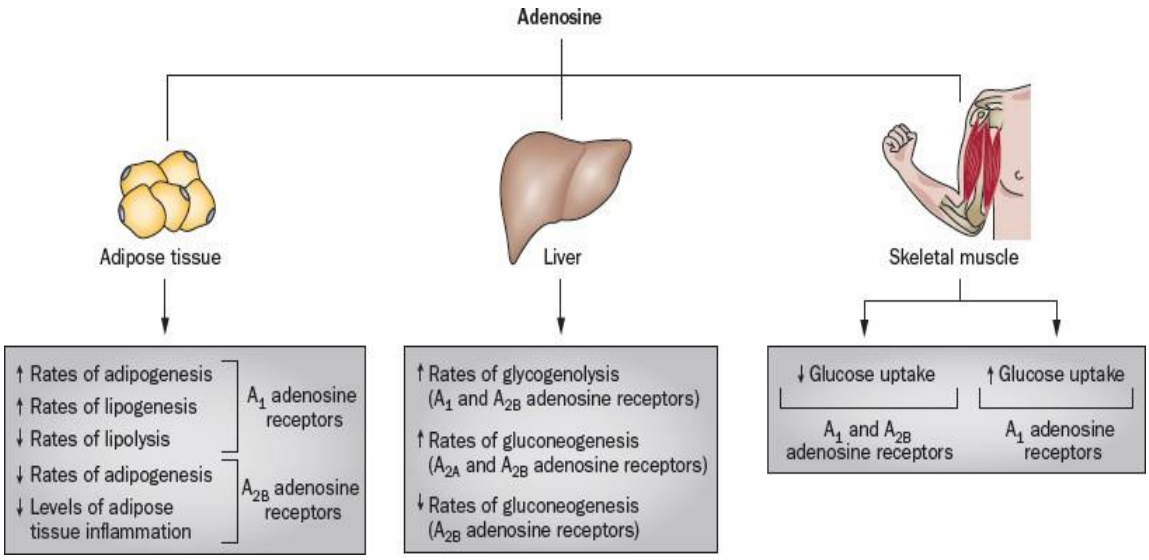

FIG.-4

ADENOSINE DEAMINASE: -

Adenosine deaminase (Adenosine aminohydrolase EC 3.5.4.4), an enzyme which is involved in the metabolism of purine nucleosides, by catalysing the irreversible hydrolytic deamination of adenosine (Ado) and 2´-deoxyadenosine (2´-dAdo) to inosine and 2´-deoxyinosine, respectively. Further metabolism of these deaminated nucleosides leads to hypoxanthine, which can be either transformed into uric acid by xanthine oxidase or salvaged into mononucleotides by the action of hypoxanthine-guanine phosphoribosyl- transferase. **(45)**

The enzyme is abuduntly distributed in vertebrate tissues and plays a critical role in anumber of physiological systems. (45)

ISOFORMS: -

There are two ADA isoforms.: -

- ADA1 is found in the cytoplasm, nucleus, and cell membrane of the majority of cells, including lymphocytes and macrophages bound to dipeptidyl peptidase-4 (aka, CD26). ADA1 occurs in both a small form -monomer and a large form - dimer and is largely involved in intracellular action.[46] A "conversion factor in the lung" controls the transition of small to large forms.[47]

- In the human spleen, ADA2 was originally discovered. [48] Later, it was discovered to coexist with the ADA1 isoform in other cells, including macrophages. The action of killing parasites is increased by the two isoforms, which govern the ratio of adenosine to deoxyadenosine. ADA2 only occurs as a homodimer and is ubiquitously present in human plasma and serum. [49]

ADENOSINE DEAMINASE AND DIPEPTIDYL PEPTIDASE -4: -

A prolyl oligopeptides family ectopeptidase found in cellular membranes, DPP-4 is also called as adenosine deaminase (ADA) binding protein or CD26. [50,51]. DPP-4 is typically expressed by endothelial and epithelial cells in mammals, especially in the bone marrow, gut, liver, and kidney. This molecule's enzymatic activity participates in the control of cellular processes by interacting with a variety of external substrates. [50,51] DPP-4 is also present in immune system cells, particularly T cells, where it interrelates with additional signalling pathways (CD3) and functions as a T cell stimulator (especially CD4+ T cells); this encourages T-cell response to foreign antigens, early signal transduction, boosted cytokine release, aided cell division, elevated expression of markers for activated T-cells (CD25, CD71, and CD69), and aided trigger cell differentiation. [52]

DPP-4 can bind to ADA as well. The cell membrane's ADA activity is the cause, which stops T-cell proliferation by breaking down adenosine, the action of DPP-4 with ADA and the reorganisation of ADA on the cell membrane can lead to an increase in T-cell proliferation and cytokine production. [53,54]

Increased ADA activity has been observed in type 2 diabetic patients (T2DM). [55,56]. Even if the exact process causing a rise in serum and tissue ADA activity isn't really understood, increased ADA activity in insulin-sensitive tissues would result in lower levels of adenosine, which encourages glucose absorption inside cells. Inflammation, T-cell activity, and cellular proliferation are all linked to the pathophysiology of insulin resistance; therefore, if ADA action is diminished, insulin sensitivity may be enhanced. [57]

ADENOSINE DEAMINASE AND INSULIN RESISTANCE: -

Adenosine mirrors insulin's role in lipid and glucose metabolism in adipose tissue and the heart while blocking insulin's impact demonstrating that it results in selective insulin resistance in the liver based on total hepatic glucose production. [3] Adenosine is a substance that largely reduces the build-up of cyclic AMP, while insulin inhibits lipolysis through a noncyclic AMP-dependent mechanism. An obvious co - relation between the antilipolytic properties of insulin and adenosine can be observed under the right circumstances. Since there are so many lipolytic agents present, John N. Fain et al. hypothesised that insulin cannot control lipolysis unless cyclic AMP build-up is managed at low adenosine levels. [4]

Adenosine levels will consequently fall due to increased ADA action in insulin-sensitive tissue, which would lessen cells' ability to absorb glucose. Additionally, it has been demonstrated that adenosine increases cyclic AMP (cAMP) via activating hepatic adenylate

cyclase and enhancing glycogenolysis through binding to the adenosine A2a receptor in the liver. As a result, it increases localised insulin resistance and liver glucose production. [58].

INSULIN: -

The islets of Langerhans in the pancreas secrete the peptide hormone insulin, which regulates blood sugar levels. Additionally, insulin helps cells take up glucose and controls how they use fats, carbohydrates, and proteins. Carbohydrates in the circulation cause the secretion of insulin. Under the effect of insulin, these carbs are deposited as glycogen in the muscles and liver. If these carbs are consumed in excess, they will again be processed by insulin into fat and deposited in adipose tissue. The two amino acid chains that make up insulin are joined by two disulphide linkages, which are necessary for insulin to function. Insulin must adhere to insulin receptors on cell surface in order to facilitate the biological effects. A receptor protein is bound and activated by insulin, and the resulting consequences on the cell are brought on by the active receptor. [59,60]

HISTORY AND EVOLUTION OF INSULIN: -

Brockman's body found that insulin is present in most of the β-cells of the pancreas in vertebrates.18 His team proposed that the Sea snails have a variety of insulin toxins that reduce blood glucose. Medical student Paul Langerhans discovered and isolated a unique group of cells from the pancreas in 1869. (Islets of Langerhans). In 1889, Joseph Von Mering and Oscar Minkowski performed a pancreatectomy on a dog to examine the impact of pancreatic enzymes on digestion. In1901, Eugene Opie discovered that the Islets of Langerhans released insulin and that diabetes may result from the loss of these cells. [61]

Nicolae Paulescu synthesized a pancreatic extract in 1916 and demonstrated that diabetic dogs' blood sugar levels were lowered by it. [61] Dr. Frederick Banting and Charles Best, a medical student, conducted research on the canine pancreas in 1921. A biochemist named Bertram Collip joined the study team to assist in the purification of the insulin utilised for testing on humans later. In1922, a 14-year-old adolescent having type 1 diabetes named Leonard Thompson received the first insulin treatment in medicine. [61] Hans Christian Hagedorn suggested in 1936 that the inclusion of protamine could prolong the action of insulin. The intermediate acting insulin Neutral Protamine Hagedorn (NPH), developed by the Danish firm Novo Nordisk, was first sold in 1950.

In 1982, human insulin was given the term to distinguish it from insulin manufactured from animals. The first synthetic, genetically modified "Human Insulin" was produced using E. coli by Arthur Riggs, Keiichi Itakura, and Herbert Boyer in 1978 at the Beckman Research Institute of the City of Hope. [62]

INSULIN SECRETION: -

In reaction to blood glucose levels, the pancreatic beta cells release insulin; glucose reaches the beta cells and stimulates insulin secretion. The beta cells can absorb glucose proportionally to the blood glucose level because they have a large number of glucose transporter proteins (GLUT 2). Glucokinase converts intracellular glucose into glucose-6-phosphate via phosphorylation. It is believed that this phosphorylation mechanism has a role in glucose sensing, which regulates the amount of insulin secreted. The phosphorylation process yields ATP, which closes ATP-sensitive potassium channels and depolarizes the cell membrane. An elevation in intracellular calcium concentration is caused by the depolarization, which also activates voltage-dependent calcium channels. The calcium induces the fusing of the cell membrane with insulin-containing capillaries such that the

insulin is secreted through exocytosis. Some hormones and amino acids encourage calcium consumption to boost the impact of glucose on insulin production. For example, glucose's impact on insulin secretion is promoted by glucagon and cortisol. However, prolonged high-volume production might wear out the beta cells, which can result in diabetes mellitus. (60,63,64)

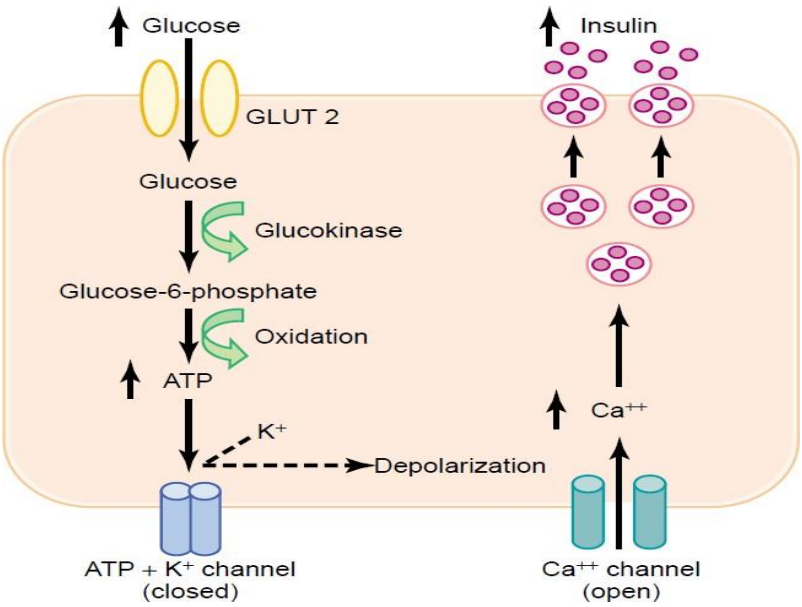

FIGURE -5

PHYSIOLOGICAL ACTION OF INSULIN [65]: -

Effect on liver:

- Reversal of catabolic properties
- Glycogenolysis inhibition
- Prevents conversion of amino acid and fatty acid to keto acids.
- Prevents amino acids from being converted to glucose.
- Promotes glucose storage as glycogen (induces glycogen synthase and glycolysis, inhibits phosphorylase)

- Enhance the synthesis of triglycerides and the production of very low-density lipoproteins

  Effects on the muscle: -

  - Enhanced protein synthesis
  - Enhanced ribosomal protein synthesis
  - Enhanced synthesis of glycogen
  - Enhanced glucose transport
  - Stimulates glycogen synthase and phosphorylase inhibition

Effect on adipose tissue:

- Increases storage of triglyceride
- Insulin activates lipoprotein lipase to hydrolyse triglycerides from lipoproteins.
- To enable the esterification of fatty acids delivered by lipoprotein transport, glucose transfer into the cell supplies glycerol phosphate.
- Intracellular lipase is inhibited by insulin.

FASTING SERUM INSULIN CONCENTRATION: -

Fasting plasma insulin concentrations are a much easier and a less intrusive testing method. Plasma insulin concentrations are assessed by drawing blood samples after the test subject has been fasting for approximately 8 to 12 hours. Insulin resistance is indicated by high plasma insulin levels. Since plasma insulin concentrations are influenced by insulin production, transport, and breakdown in addition to insulin sensitivity, fasting insulin levels can only account for up to 50% of the variance in insulin action. For instance, the pancreatic

beta cells become tired when insulin resistance progresses to type 2 diabetes, and insulin output declines. Since insulin is frequently secreted in pulses, sampling immediately after an insulin pulse could result in measurement values that are not indicative of the real concentration of insulin. [63,66]

INSULIN RESISTANCE: -

Insulin resistance is the absence of insulin's capacity to promote glucose absorption and utilisation in a person as it does in a healthy individual. Genetic predisposition exists for both insulin resistance and reduced insulin production. Various environmental influences, particularly diet and activity, alter this pattern [67]. In 1988, during the Banting lecture at the American Diabetes Association (ADA) Annual Meeting, Dr. Gerald Reaven presented the theory that insulin resistance and hyperinsulinemia were the defining features of a plurimetabolic syndrome. This syndrome also included essential hypertension, hypertriglyceridemia, decreased plasma HDL cholesterol levels, and some degree of glucose intolerance. [68]

The theory, termed as "syndrome X," suggested that there may be a link between the Insulin Resistance syndrome and an increased risk of cardiovascular disease. The general population may contain as many as 2.5% of non-diabetics who exhibit some symptoms of the condition, according to another theory. [69].

PATHOPHYSIOLOGY OF INSULIN RESISTANCE: - [70]

Muscles, fatty tissue, and the liver are the main areas where insulin resistance occurs. According to one concept, an excess of free fatty acids causes ectopic lipid build-up and immune-mediated inflammatory changes in muscle tissue that lead to the development of insulin resistance. Up to 70–75% of the elimination of glucose occurs in muscle. When

muscle uptake is compromised, more glucose is returned to the liver, furthers ectopic fat deposition and insulin resistance enhances de novo lipogenesis and circulating free fatty acids.

Adipose Tissue: -

The hyperinsulinemic euglycemic clamp method was used by researchers to establish that lipolysis is insulin-sensitive. Circulating free fatty acids rise as a result of insulin's inability to stop lipolysis in insulin-resistant adipose tissue, particularly visceral adipose tissue. Increased quantities of plasma free fatty acids have a direct impact on the metabolism of the liver and muscles, further boosting insulin resistance.

Muscle Tissue: -

Muscle is the main organ for the removal of glucose after consumption of a caloric load and conversion to glucose; it accounts for 70% of tissue glucose uptake. With a higher calorie load, muscle can absorb more glucose, and this extra glucose is then transported into the liver, where it causes DNL. FFA production and triglyceride subsequently rises, resulting in ectopic fat deposition in the liver, adipose tissue, and muscle. The outcome, both insulin resistance and the generation of inflammatory markers are elevated. Insulin resistance in muscle tissue is also influenced by other variables like physical inactivity and genetic risk.

Hepatic Tissue: -

Increased glucose substrate delivery to the liver as a result of insulin resistance in muscles causes DNL, which is accompanied by increased lipid deposition and inflammation. Increased lipolysis by adipocytes owing to insulin resistance in adipose tissue raises the levels of circulating FFA, which in turn causes an increase in steatosis and insulin resistance in muscle tissue. In conjunction with caloric intake, insulin limits the postprandial rise in

glucose by inhibiting glycogenolysis and lowering hepatic glucose synthesis. Because insulin resistance disrupts this feedback system, postprandial glucose levels rise as hepatic glucose synthesis increases. Insulin resistance is further exacerbated by glucotoxicity, which is connected to high blood glucose levels.

MEASUREMENT OF INSULIN RESISTANCE [71]: -

Two categories of insulin sensitivity indexes exist: 1) Indices calculated using triglyceride, insulin, and glucose concentrations in fasting plasma. 2) Indices calculated using plasma glucose and insulin concentrations measured during the course of a conventional 120-minute experiment (75 g glucose) OGTT.

The former category consists of the QUICKI INDEX and homeostasis model assessment-insulin resistance (HOMA-IR).

Homeostasis model assessment-insulin resistance (HOMA-IR) [72]: -

Matthews et al. created the homeostasis model assessment for the first time in 1985. It is a technique for evaluating beta-cell activity and insulin resistance based on baseline fasting glucose, insulin, or C-peptide concentrations. The HOMA model predicts fasting steady state insulin and glucose levels by simulating the dynamics of glucose and insulin. While insulin-mediated glucose production via the liver regulates blood glucose levels, insulin levels are dependent on the pancreatic cells' response to glucose concentrations. As a result, the compromised cell function reflects a decreased cellular response to insulin secretion that is induced by glucose. Similar to this, decreased insulin's inhibitory effect on hepatic glucose synthesis is a sign of insulin resistance The HOMA model has demonstrated to be an useful epidemiological and clinical technique for assessing insulin resistance. HOMA uses a

collection of straightforward, scientifically developed nonlinear equations to describe this glucose–insulin balance. It is created by dividing the insulin glucose product by a fixed factor. An indicator of hepatic insulin resistance is obtained by multiplying FPG by FPI.

Matthews et al suggested equation is as follows:

$HOMAIR$ = FI× FG/ 22.5

FI -fasting insulin (FI in µU/mL)

FG- fasting glucose (FG in mmol/L) concentrations

Quantitative insulin sensitivity check index (QUICKI) [73]:

The quantitative insulin sensitivity check index (QUICKI) is a discrete mathematic translation of plasma insulin concentrations and fasting blood sugar that was developed empirically. It is a variant of the HOMA-IR equation because it alters the data by using both the reciprocal and the logarithm of the glucose–insulin product, somewhat skewing the distribution of fasting insulin readings. QUICKI is identical to the HOMA model's simple equation form in every way except that QUCKI is calculated using a log transform of the insulin and glucose product. The QUICKI can be determined from fasting insulin (µIU/ml) and fasting plasma glucose (mg/dl) concentrations.[5] QUICKI = 1/ (logI0 + logG0)

AIMS AND OBJECTIVES

AIMS AND OBJECTIVE OF THE STUDY:

To estimate and correlate serum adenosine deaminase levels and serum fasting insulin level in clinically diagnosed type 2 diabetes Mellitus patients and to determine the role of ADA in the development and progression of type-2 Diabetes mellitus.

MATERIALS AND METHODS

STUDY DESIGN:

Cross-sectional study.

SOURCE OF DATA:

The study included outpatients and inpatients of B.L.D.E. (D.U.) Shri B.M.Patil Medical College hospital and research center, Vijayapura.

- The patients were informed about the study in all respects, and informed consent was obtained.
- Period of study was from January 2021 to June 2022

METHODS:

Patients attending BLDE (DEEMED TO BE UNIVERSITY) SHRI BM PATIL MEDICAL COLLEGE HOSPITAL diagnosed with type 2 Diabetes Mellitus were selected for the study.

Nature and purpose of the study were explained to patients, and informed consent was taken from those who were willing to participate. A structural format was given to each subject to record personal details.

Patient's present and past medical history was recorded, and a detailed physical examination was done.

METHOD OF COLLECTION OF DATA

Study patients:

A detailed history, general physical examination, systemic examination, and investigation were performed on all patients who fulfilled inclusion criteria, both male and female, who

attended outpatient and admitted in BLDE (DEEMED TO BE UNIVERSITY) Shri B M Patil medical college hospital and research center, vijayapura.

SAMPLE SIZE: With anticipated Mean±SD of Serum Insulin level in Diabetes 9.2217±2.088[(ref)], the study would require a sample size of 70 patients with a 95**%** level of confidence and a precision of 0.5**.**

Formula used

- $\mathbf{n=\frac{z^2\ p*q}{d^2}}$

Where Z= Z statistic at α level of significance

d^2= Absolute error

P= Proportion rate

q= 100-p

- **Statistical Analysis**
- The data obtained were entered in a Microsoft Excel sheet, and statistical analysis were performed using a statistical package for the social sciences (Version 20).
- Results were presented as Mean (Median) ±SD, counts and percentages, and diagrams.
- Categorical variables were compared using the Chi-square test.
- Correlation between variables were calculated by Person's/Spearman's correlation.
- $p<0.05$ was considered statistically significant. All statistical tests were performed two-tailed.

INCLUSION CRITERIA:

Patients with newly diagnosed or known cases of type 2 diabetes mellitus.

Type-2 Diabetes Mellitus diagnostic criteria: - [13]

Fasting blood sugar levels more than or equivalent to 126 mg/dl (7.0 mmol/l)

OR

Increased frequency of urination, extreme thirst, unanticipated weight loss, and other symptoms, along with a random blood sugar measurement of 200 mg/dl (11.1 mmol/l) or higher

OR

Blood glucose level ≥200 mg/dl (11.1 mmol/l), measured two hours after a 75-gram glucose load

OR

HbA1c ≥ 6.5%.

EXCLUSION CRITERIA: -

- Diagnosed cases of Tuberculosis
- Leprosy
- Acute lymphadenitis
- Infectious mononucleosis
- Enteric fever
- Hepatitis A and B
- Chickenpox
- Hematopoietic malignancies like Hodgkin's lymphoma and drug-induced lymphadenitis
- Type 1 diabetes mellitus
- Patient on insulin therapy

OBSERVATION AND RESULTS

OBSERVATIONS AND RESULTS

Seventy (N=70) patients satisfying the inclusion criteria were assessed in our study.

PATIENT'S DISTRIBUTION ACCORDING TO AGE

Age (Years)	No. of patients	Percentage
< 20	1	1.4
20 - 29	1	1.4
30 - 39	1	1.4
40 - 49	10	14.3
50 - 59	17	24.3
60 - 69	28	40.0
70 - 79	9	12.9
80+	3	4.3
Total	70	100.0

TABLE – 1

	AGE
Mean	58.93
Median	60.00
Std. Deviation	11.994

TABLE -2

Table -1 shows number of patients according to age distribution with maximum number of patients in age group of 60-69 28 with distribution of 40 % of patients in same age group.

Table -2 shows age group based on mean and median with value of 58.93 and 60 respectively with standard deviation of 11.994.

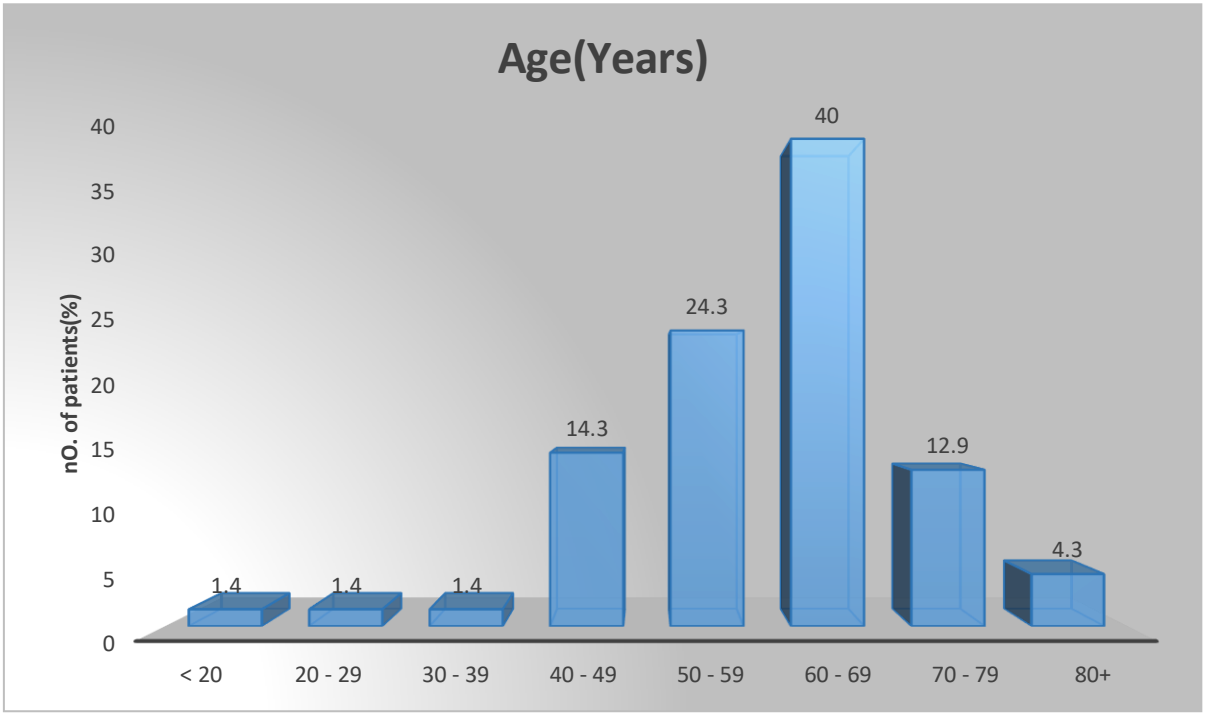

FIGURE- 6

Figure -6 shows number of patients in each age group with maximum number in age of 60-69.

PATIENT'S DISTRIBUTION ACCORDING TO SEX

Gender	No. of patients	Percentage
Female	30	42.9
Male	40	57.1
Total	70	100.0

Table -3

Table-3 shows distribution of patients according to gender with 40 number of patient in male gender and 30 in female gender respectively.

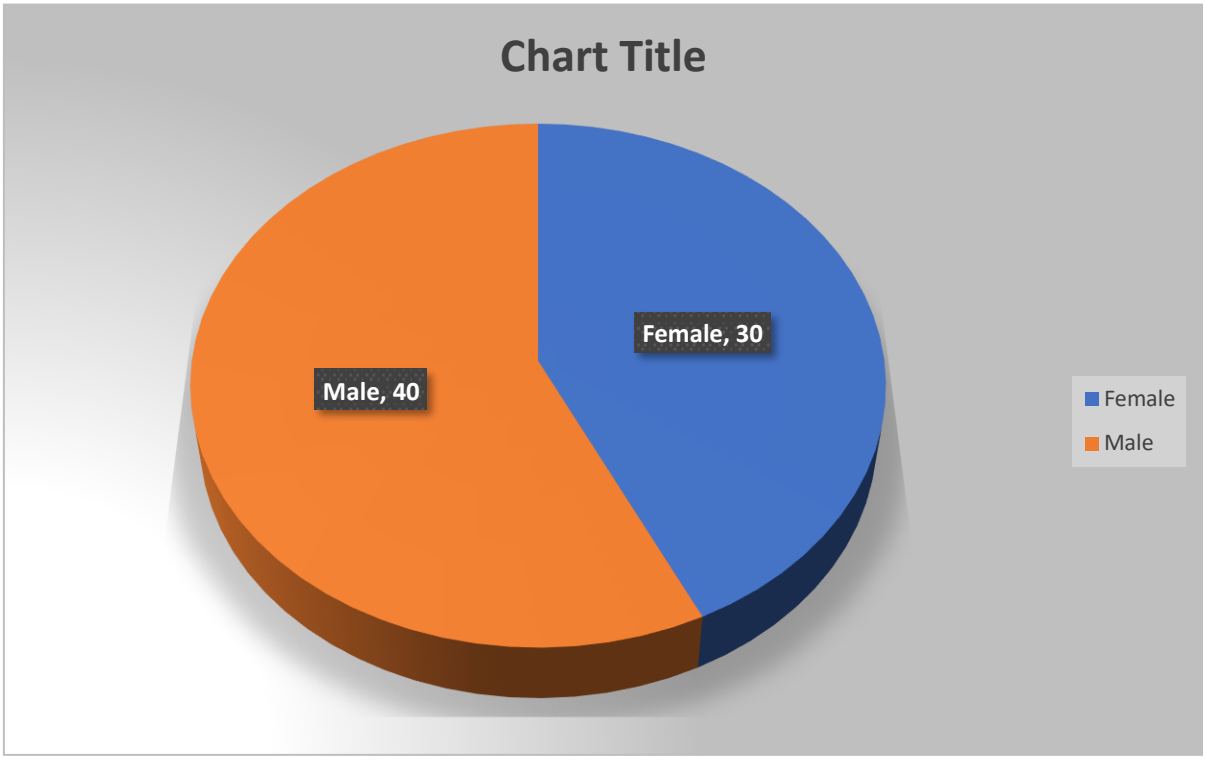

Figure – 7

Gender distribution of study subjects are depicted in the figure 7.

	Age	**ADA**	**F.INSULIN**	**FBS**	**PPBS**	**HbA1C**
Mean	58.93	22.8109	13.7349	174.31	219.86	8.4586
Median	60.00	20.5050	11.8650	165.50	207	7.9500
Std. deviation	11.994	10.318	10.61600	51.903	59.384	2.18920

TABLE -4

Table - 4 shows mean and median and standard deviation value of serum ADA, serum Fasting insulin, Fasting blood sugar , Post prandial blood sugar and HbA1C.

Comparison of serum ADA and Fasting blood glucose:

		FBG
ADA	Correlation Coefficient	-.085
	Sig. (2-tailed)	.483
	N	70

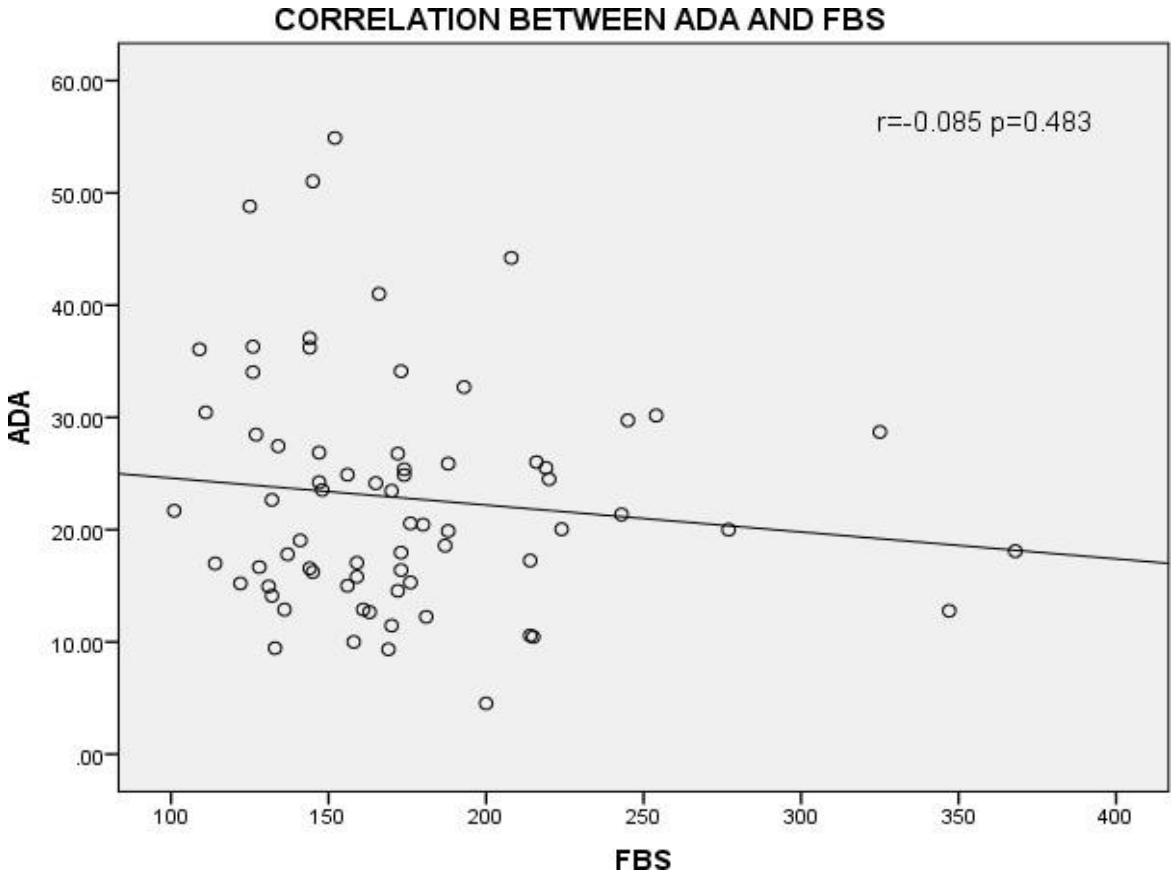

Figure -8

Figure -7 represent mild positive correlation between serum ADA and serum fasting blood glucose level with p value = 0.483 and r= 0.085

Comparison of serum ADA and Post prandial blood glucose:

		PPBS
ADA	Correlation Coefficient	.103
	Sig. (2-tailed)	.395
	N	70

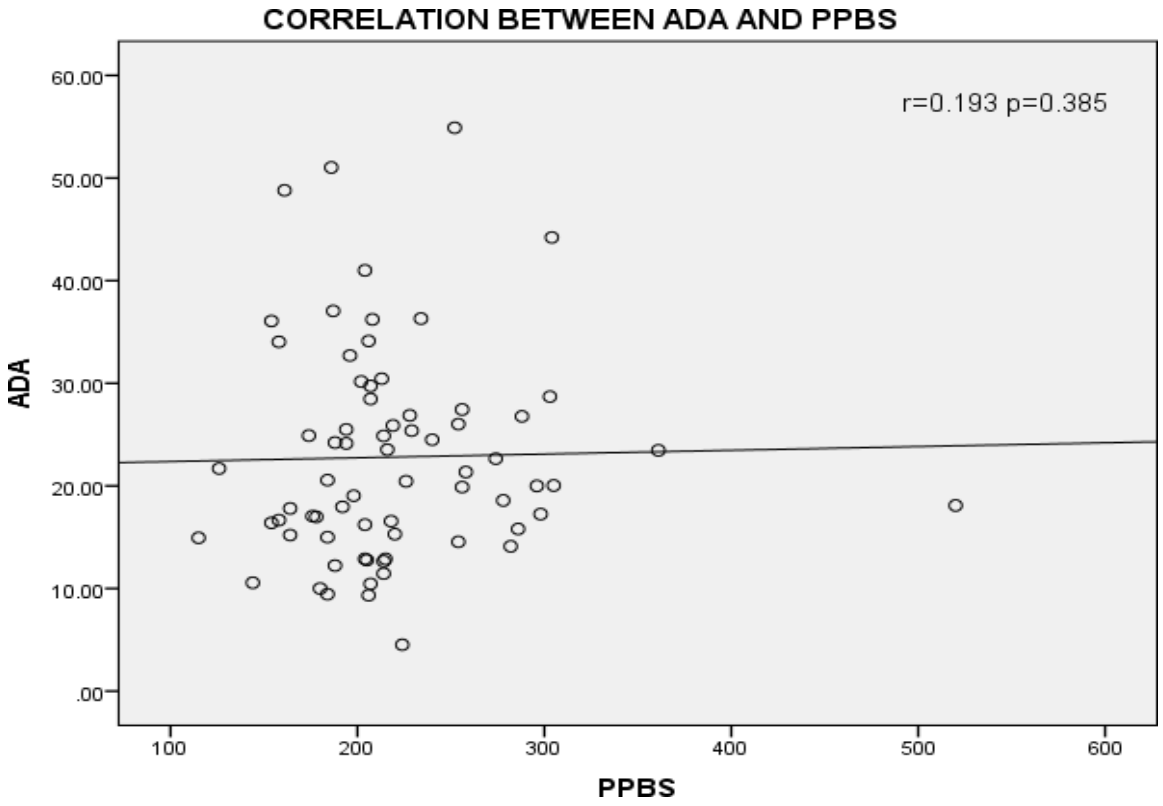

Figure -9

Figure -8 represent mild positive correlation between serum Adenosine deaminase and PPBS with r value=0.193 and p value=0.385.

Comparison of serum ADA and Glycosylated haemoglobin

		ADA
HBA1C	Correlation Coefficient	.157
	Sig. (2-tailed)	.194
	N	70

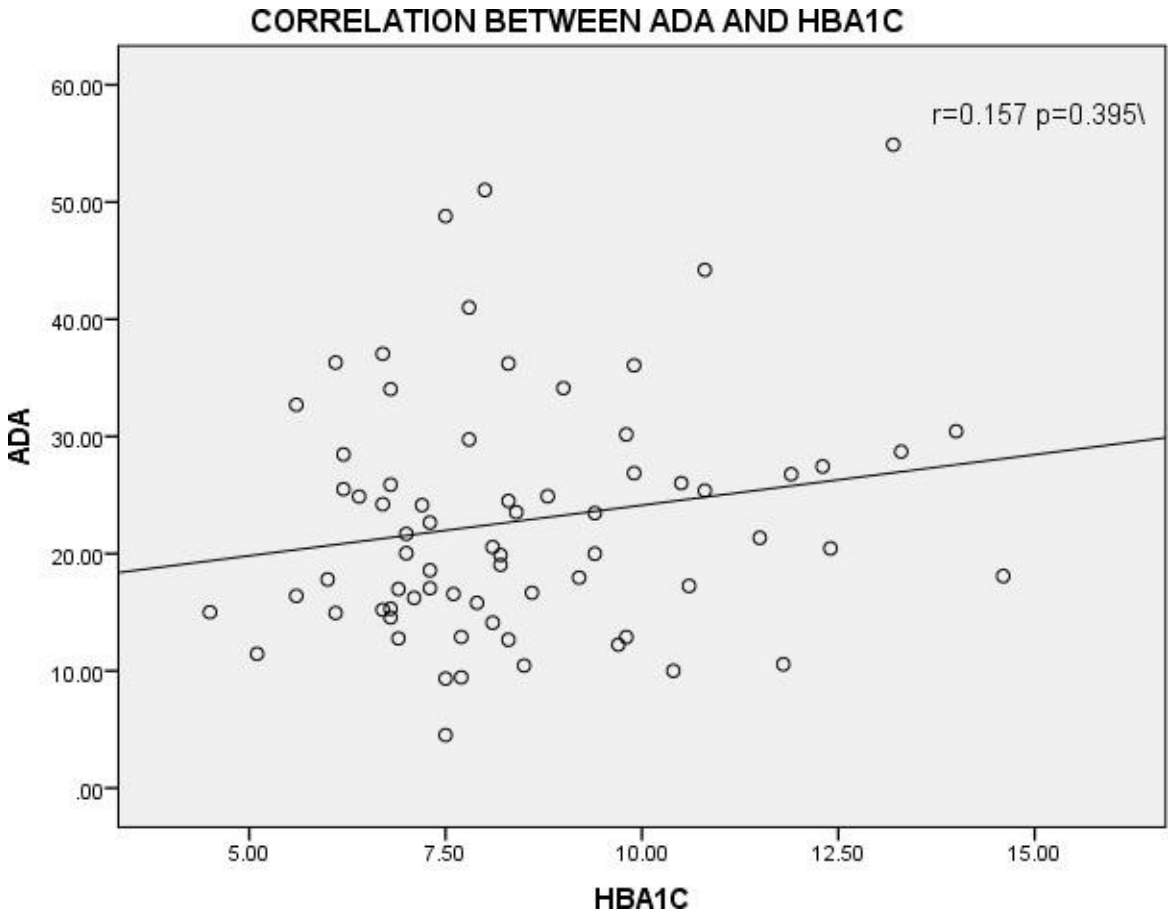

Figure -11

Figure -11 represent mild positive correlation between serum ADA and HbA1C with r value= 0.157 and p value = 0.395.

Comparison of Fasting serum insulin and Fasting blood glucose:

		FBG
F. INSULIN	Correlation Coefficient	.144
	Sig. (2-tailed)	.234
	N	70

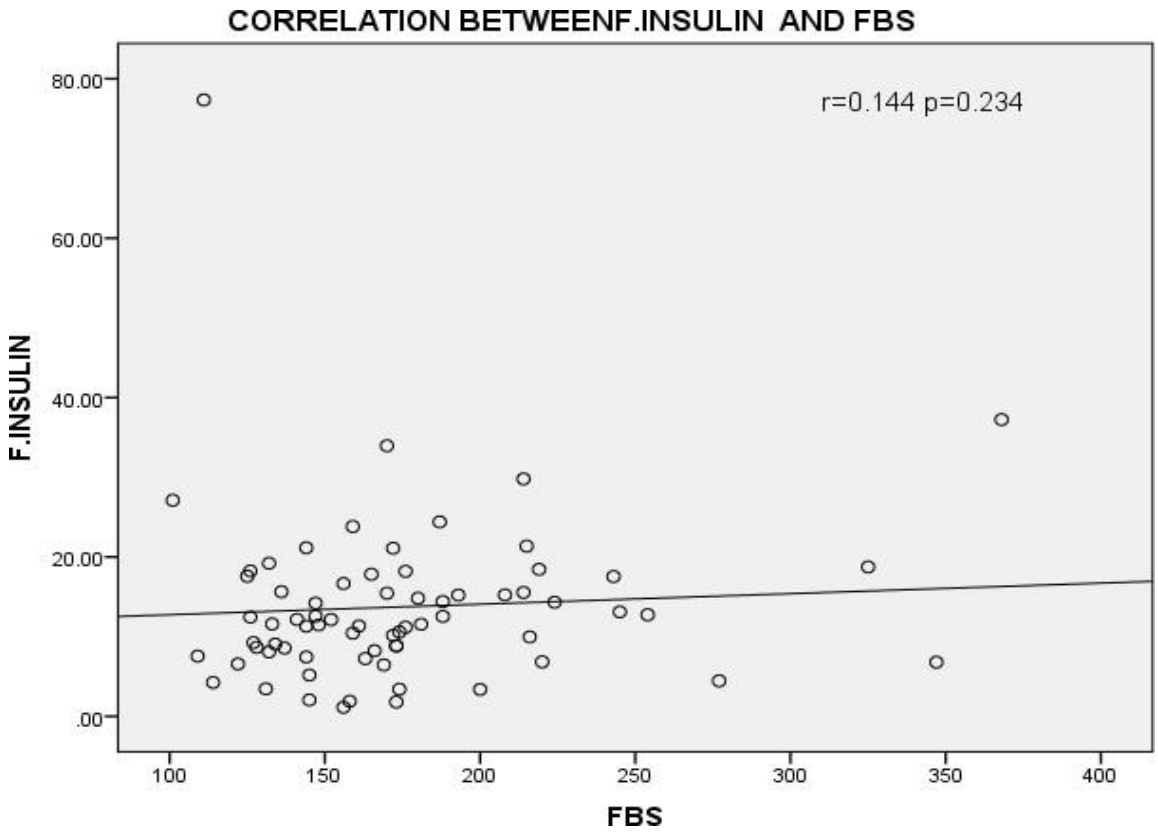

Figure -12

Figure – 12 represent mild positive correlation between serum fasting insulin level and serum fasting blood glucose level with r value= 0.144 and p value = 0.234.

Comparison of Fasting serum insulin and post prandial blood glucose:

		PPBG
F. INSULIN	Correlation Coefficient	.265*
	Sig. (2-tailed)	.027
	N	70

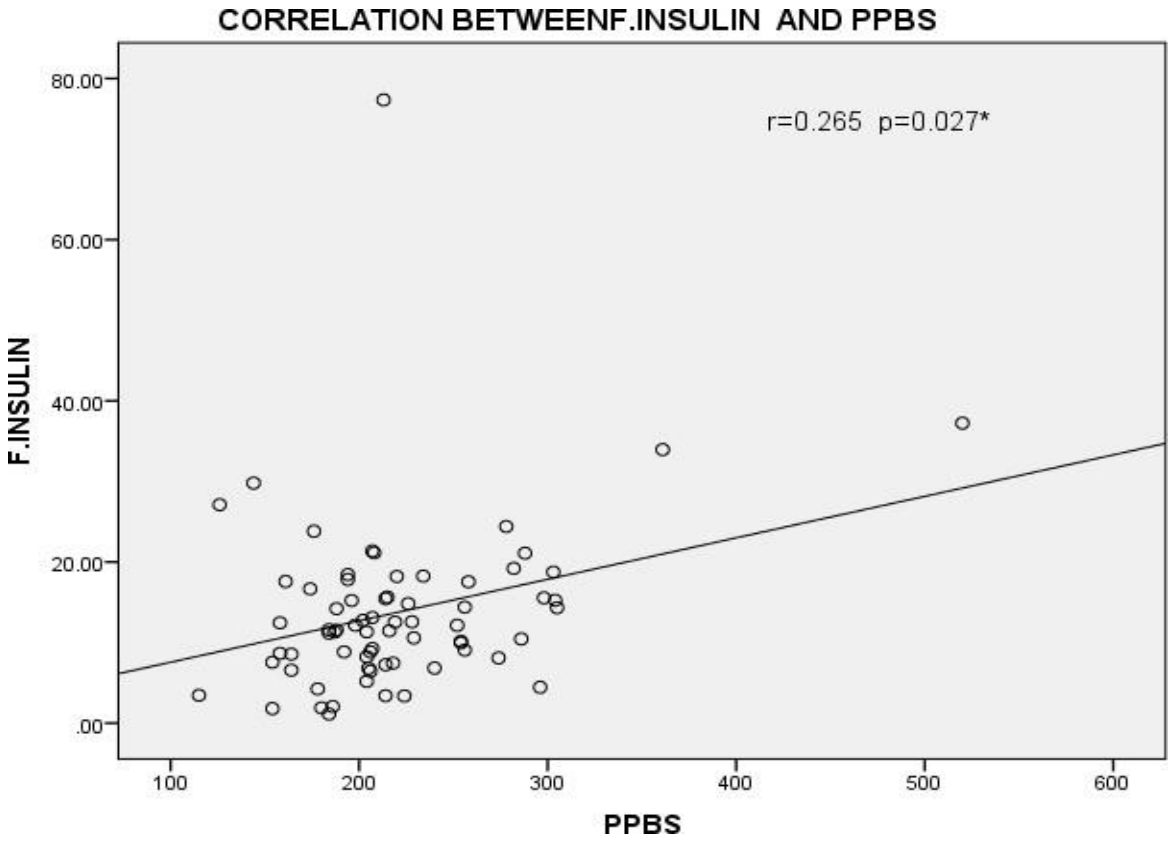

Figure - 13

Figure – 13 represent mild positive and significant correlation between serum fasting insulin level and serum PPBS level with r value= 0.265 and p value =0.027.

Comparison of Fasting serum insulin and Glycosylated hemoglobin:

		HbA1C
F. INSULIN	Correlation Coefficient	.273*
	Sig. (2-tailed)	.022
	N	70

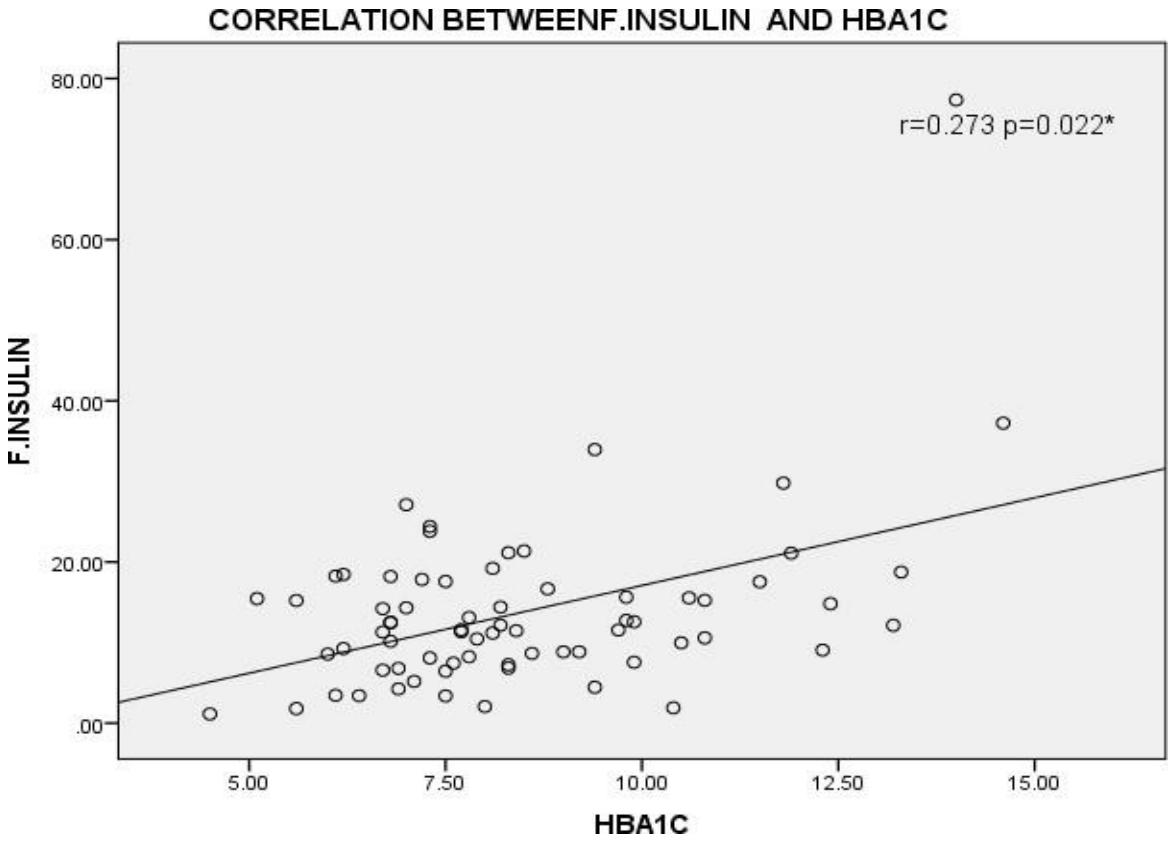

Figure - 14

Figure -14 represent mild positive and significant correlation between serum fasting insulin level and HbA1C level with r value = 0.273 and p value= 0.022.

Comparison of serum ADA and Fasting serum insulin:

		F. INSULIN
ADA	Correlation Coefficient	.199
	Sig. (2-tailed)	.099
	N	70

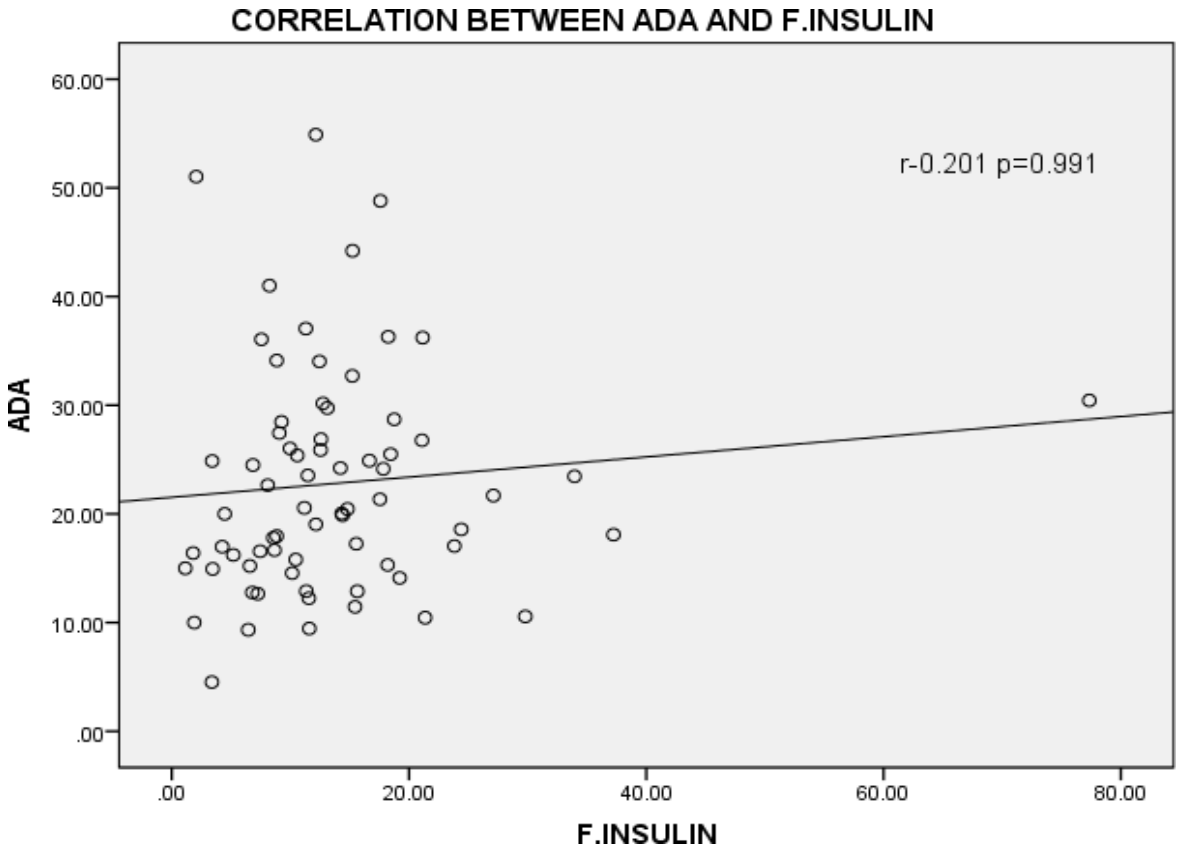

Figure – 15

Figure -15 suggest mild positive correlation between serum ADA and serum fasting insulin levels with r value= 0.201 and p value = 0.991.

Correlation QUICKI with serum ADA and serum fasting insulin:-

		ADA	FASTING INSULIN
QUICKI	Correlation Coefficient	-0.126	-0.901
	Sig. (2-tailed)	0.299	0.000
	N	70	70

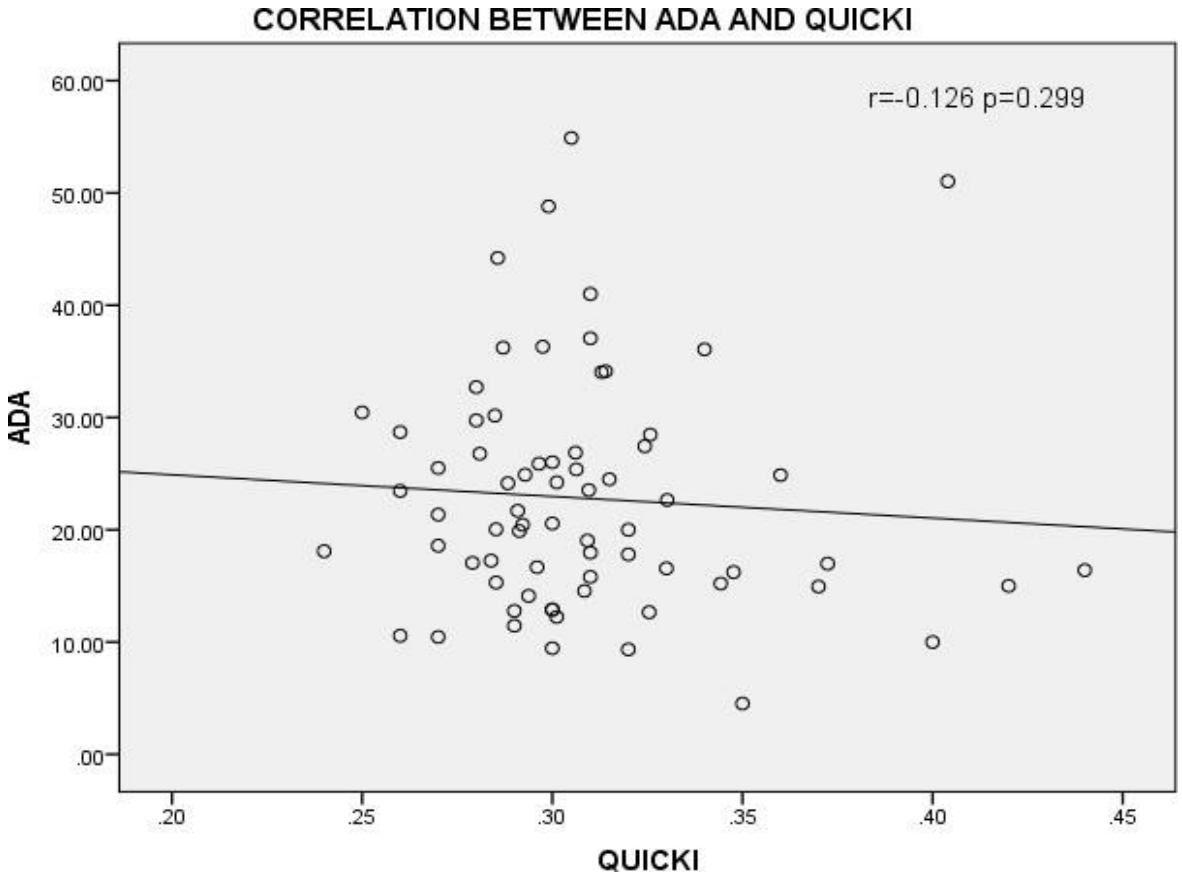

FIGURE -16

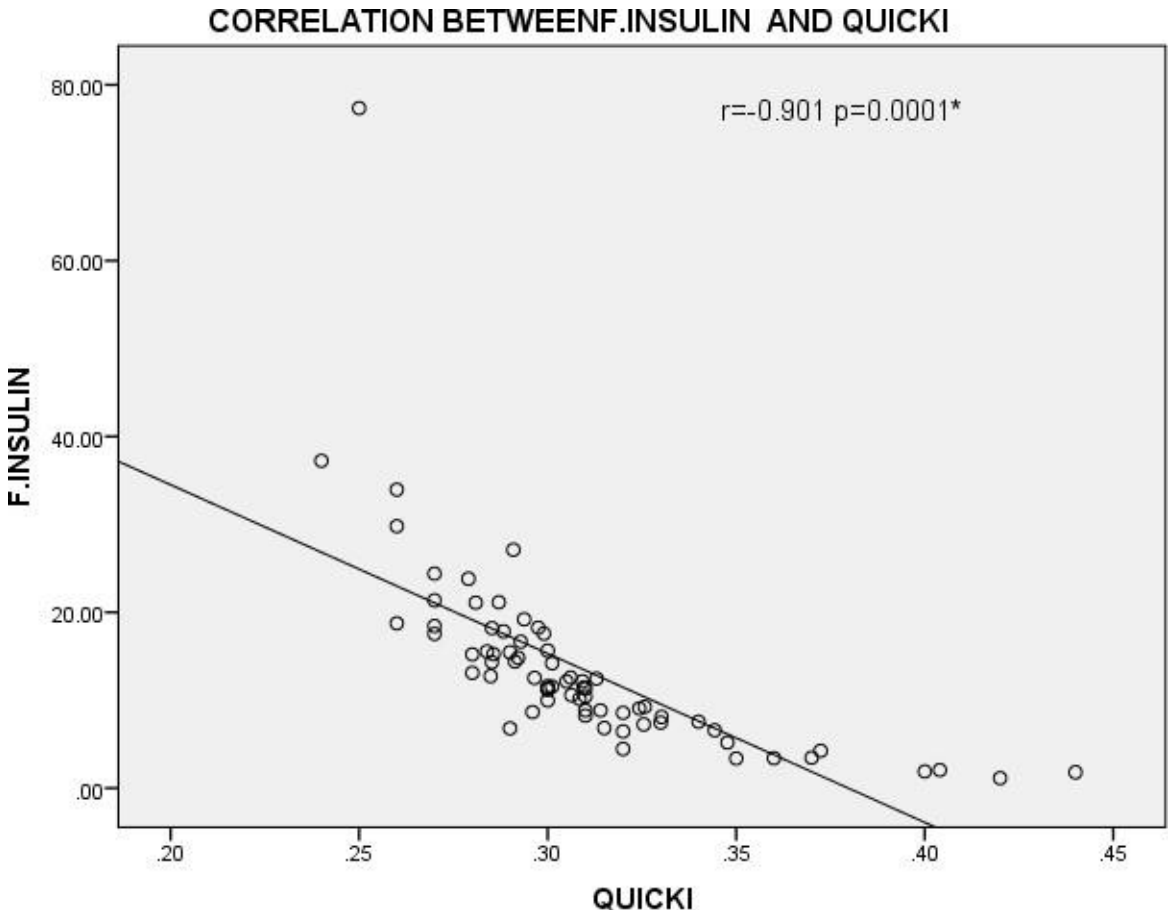

FIGURE -17

Figure -16 suggest negative correlation between serum ADA and QUCKI (marker of insulin resistance) with r=0.126 and p=0.299.

Figure -17 suggest strong significant negative correlation between serum fasting insulin and QUICKI with r=0.901 and p= 0.0001*

DISCUSSION

In this study we included 70 samples with known case or newly diagnosed patients of type 2 diabetes mellitus to study the correlation between serum adenosine deaminase level and serum fasting insulin levels in patient of type 2 diabetes mellitus.

We also correlated serum ADA and serum fasting insulin levels with other parameters such as serum FBS, PPBS and HbA1c.

In our study out of 70 samples, 30 were females and 40 were males with highest number of patients 27 in the age group of 60-69 (table 1 and figure-6 and 7) with mean and median value of 58.93 and 60 respectively with standard deviation of 11.994 (table -2).

The mean and median value of serum ADA was 22.8109 and 20.5050; serum fasting insulin was 13.7349 and 11.8650; FBS was 174.31 and 165.50; PPBS was 219.86 and 207; HbA1C was 8.45 and 7.95. (Table – 4).

Association between serum ADA and Fasting blood glucose levels: -

In this study we found mild positive correlation between serum ADA and serum fasting blood glucose level with p value = 0.483 and r= 0.085 in contrast to study done by Dharamveer et al, Jae-GeunL et al, Nisha et al and Amandeep et al. [74-77]

Association between serum ADA and Post prandial blood glucose levels: -

In this study we found mild positive correlation between serum ADA and PPBS level with with r value=0.193 and p value=0.385, which was also shown in previous studies by Dharamveer et al, Jae-GeunL et al, Nisha et al and Amandeep et al. [74-77]

Association between serum ADA and HbA1C: -

In this study we found mild positive correlation between serum ADA and HbA1C with r value= 0.157 and p value = 0.395. figure also suggest increase level of serum ADA with increase in HbA1C level which was also shown in previous studies by Dharamveer et al, Jae-GeunL et al, Nisha et al, Amandeep et al 25-28 and also by Muthiah et al and Adarsh et al. [78]

Association between fasting serum insulin and fasting blood glucose level: -

In this study we found mildly positive correlation between fasting serum insulin and fasting blood glucose level with r value= 0.144 and p value = 0.234.

Association between fasting serum insulin and PPBS: -

In this study we found mild positive and significant correlation between serum fasting insulin level and serum PPBS level with r value= 0.265 and p value =0.027*. this result can be determined from study done by Gopalratnam Raman et al which states that Increased PPBG produces dyslipidaemia, which activates prothrombotic activity and reduces insulin sensitivity which further cause insulin resistance and increase in fasting serum insulin. [79]

Association of fasting serum insulin level and HbA1C level: -

In this study we found mild positive and significant correlation between serum fasting insulin level and HbA1C level with r value = 0.273 and p value= 0.022* this can be determined from study done by Sitasuwan et al, which shows HbA1c was significantly correlated with and insulin resistance. [80]

Association between fasting serum insulin and serum ADA levels: -

In this study we found mild positive correlation between serum ADA and serum fasting insulin levels with r value= 0.201 and p value = 0.991 which is also shown by Dharamveer et al, Jae-GeunL et al, Nisha et al, Amandeep et al [74-77] and also by Muthiah et al and Adarsh et al. [78]

Association between serum ADA and QUCKI: -

In this study we found mild negative correlation between serum ADA and QUCKI with r=0.126 and p=0.299 which is also shown by Muthiah et al and Adarsh et al [78] which shows reduction in ADA levels in patients with increasing QUICKI.

Association of serum fasting insulin with QUCKI: -

In this study we found strong and significant correlation between serum fasting insulin and QUCKI with with r=0.901 and p= 0.0001*which is also shown by
Katz et al [81] , Navneet et al and Gitanjali et al [82] . this study shown lower the value of QUICKI higher is the insulin resistance

Numerous biochemical and clinical conditions, including hypertension, central obesity, atherosclerosis, hypertriglyceridemia, high LDL, and lowered HDL, are signs of type 2 diabetes. Early identification of insulin resistance reduces the severity of its effects. ADA is distributed differently in various tissues, with lymphoid and fatty tissues containing the most of it. [83]. In skeletal muscle, adenosine is known to take role in insulin-mediated glucose uptake; when its activity rises, cells are less likely to take up glucose, which contributes to insulin resistance. [84]. Adenosine enhances gluconeogenesis and glycogenolysis in some both

in vitro and in vivo research, boosts glucose synthesis, and affects cardiac functions through its receptors, primarily A1 and A2 adenosine receptors. (85)

In T2DM, an increase in ADA blood levels affects insulin metabolism, particularly in adipose tissues where it increases lipolysis, disrupts anti-lipolysis function, and intensifies hyperlipidemia. Adipocytes' oxidative phosphorylation and ATP retention are brought on by the substantial amount of fatty free acids (FFA) brought on by enhanced lipolysis activity. (86). Additionally, ADA decreases sensitivity to insulin in adipocytes and affects PKB (protein kinase B) synthesis in the insulin post receptor phase. (87). Additionally, GLUT4 accessibility to the cell surface for glucose transporters is decreased by ADA (88). As a result, diabetic adipocyte cells might demand greater insulin concentration. (89) By interacting with CD3 and functioning as a co-stimulator for CD4+ T cells, the significant enzyme DPP-4 controls immune function and glucose homeostasis by hydrolyzing integrins. The interactivity of ADA with DPP-4 may enable T cells to proliferate and produce more cytokines, which can disrupt insulin signalling because adenosine induces apoptosis and prevents T lymphocyte differentiation by activating P1 adenosine receptors (90). Proteins in the acute phase are stimulated by cytokines. The acute phase protein regulates homeostasis and has survival benefits in the short term, but it also causes problems in the long run. (91) Insulin resistance and hyperglycemia boost the production of a certain proinflammatory cytokines, such as IL-6 and TNF-alpha, which are produced by monocytes, macrophages, and adipose tissue.[92]. In addition to smoking, sedentary behaviour, diet, and obesity, other significant risk factors for T2DM include ageing, sedentary lifestyle, food intake, smoking, and increased peripheral cytokines (93). Additionally, ADA deficiency is linked to compromised immunological processes. Thus, by decreasing ADA activity, T-lymphocyte activity, inflammation, and insulin sensitivity—all of which are linked to the pathophysiology of T2DM—can be improved. Additional evidence suggests that ADA modifies insulin's bioactivity.

Only a few trials have demonstrated an association between higher serum ADA levels in T2DM patients with glycaemic control [90]. Additionally, greater blood ADA levels have been observed in nonobese T2DM individuals and have been linked to fasting plasma glucose levels [95]. Additionally, metformin reduces blood ADA levels and has been linked positively to insulin resistance [96]. Additionally, it has been noted that ADA levels are higher in those with type 2 diabetes and are favourably connected with fasting blood sugar and insulin levels. Our study demonstrates higher serum ADA levels in T2DM participants, which is consistent with other studies. Additionally, a favourable association between the fasting blood insulin level and the serum ADA level was observed, which may contribute to the pathogenesis of T2DM patients.

CONCLUSION

In this study we correlated of Serum Adenosine Deaminase (ADA) levels and serum fasting insulin in patients of type 2 diabetes mellitus through a cross sectional study. Patients with known case or newly diagnosed type 2 diabetes mellitus were taken.

In this study we found: -

- Serum adenosine deaminase levels were found significantly higher in type 2 diabetes patients
- Mildly positive correlation was found between serum ADA with FBG, PPBG and HbA1C.
- Serum fasting insulin was mildly correlated with FBG and significant correlation with PPBG and HbA1C.
- Serum adenosine deaminase level and serum fasting insulin were mildly correlated with each other.
- Serum adenosine deaminase was positively correlated with QUICKI
- Serum fasting insulin was strongly and significantly correlated with QUICKI

These findings suggest increase of serum ADA in type 2 diabetes mellitus patient and raised serum fasting insulin level which could help in pathogenesis of insulin resistance in type 2 diabetes mellitus patient

SUMMARY

Diabetes mellitus is a heterogenous disease typified by an anomalous or inadequacy of insulin and insulin resistance which cause higher morbidity and death; thus, diabetes must be identified early and managed. Adenosine deaminase is an enzyme involved in purine metabolism which is present in all human tissue and plays crucial role in bioactivity of insulin. Insulin resistance is the greatest risk factor for development of type 2 diabetes mellitus. One of the distinguishing characteristics of insulin resistance is an elevated insulin level. A simple method for monitoring the development of insulin resistance is to estimate fasting serum insulin levels. In this study we correlated serum ADA and serum fasting insulin in patient of newly diagnosed or known case of type 2 diabetes mellitus.

In this study we included 70 samples out of which 30 were females and 40 were males with highest number of patients 27 in the age group of 60-69. We also correlated serum ADA and serum fasting insulin levels with other parameters such as serum FBS, PPBS and HbA1c.

In this study we found,

- Serum adenosine deaminase levels were found significantly higher in type 2 diabetes patients.
- Mild positive correlation was found between serum ADA with FBG, PPBG and HbA1C.
- Serum fasting insulin was mildly correlated with FBG and significant correlation with PPBG and HbA1C. with p value of 0.027* and 0.022*.
- Serum adenosine deaminase level and serum fasting insulin were mildly correlated with each other.
- Serum adenosine deaminse as positively correlated with QUICKI

- Serum fasting insulin was strongly and significantly correlated with QUICKI with p =0.001

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ANNEXURE – I

ETHICAL COMMITTEE CLEARANCE

B.L.D.E. (DEEMED TO BE UNIVERSITY) IEC/no-09/2021 Date-22/01/2021

(Declared vide notification No. F.9-37/2007-U.3 (A) Dated. 29-2-2008 of the MHRD, Government of India under Section 3 of the UGC Act, 1956)

The Constituent College

SHRI. B. M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The e Institutional ethical committee of this college met on 11-01-2021 at 11am to scrutinize the synopsis of Postgraduate students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected and revised version synopsis of the Thesis has been accorded Ethical Clearance

Title: A study of correlation between serum adenosine deaminase level and serum insulin levels in type 2 diabetes mellitus

Name of PG student: Dr Vivaan Vyas, Department of Medicine

Name of Guide/Co-investigator: Dr S S Devarmani, Professor of Medicine

DR .S.V.PATIL
CHAIRMAN
Institutional Ethical Committee
L D E (Deemed to be University)
Shri B.M. Patil Medical College,
VIJAYAPUR-586103 (Karnataka)

Following documents were placed before Ethical Committee for Scrutinization:

1. Copy of Synopsis / Research project
2. Copy of informed consent form
3. Any other relevant documents.

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ANNEXURE - II

INFORMED CONSENT FORM:

TITLE OF RESEARCH: A study of the correlation between serum adenosine deaminase level and serum insulin levels in type 2 diabetes mellitus.

GUIDE : **DR S.S. DEVARMANI**

M.D GENERAL MEDICINE

P.G. STUDENT : **DR. VIVAAN VYAS**

All aspects of this consent form are explained to the patient in the language understood by him or her.

PURPOSE OF STUDY:

I have been informed that the purpose of this study is to study for the presence of an elevated level of adenosine deaminase levels in a patient with type 2 diabetes mellitus.

PROCEDURE:

I understand that I will undergo a detailed history and clinical examination and investigations.

BENEFITS:

I understand that my participation in this study will have no direct benefit tome other than the potential benefit of treatment, which is planned to prevent further morbidity and mortality in me.

CONFIDENTIALITY:

I understand that the medical information produced by the study will become a part of

hospital records and will be subjected to confidentiality and privacy regulation of the hospital. If the data is used for publication, the identity will not be revealed.

REQUEST FOR MORE INFORMATION:

I understand that I may ask for more information about the study at any time.

REFUSAL OR WITHDRAWAL OF PARTICIPATION:

I understand that my participation is voluntary, and I may refuse to participate or withdraw from the study at any time.

(Signature of Guardian) (Signature of patient)

STUDY SUBJECT CONSENT FORM:

I confirm that Dr. Vivaan Vyas explained to me the purpose of this research, the study procedure that I will undergo, and the possible discomforts and benefits that I may experience in my own language.

I have been explained all the above in detail in my own language, and I understand the same. I agree to give my consent to participate as a subject in this research project.

DATE SIGNATURE OF PARTICIPANT

DATE SIGNATURE OF WITNESS

ANNEXURE-III

PROFORMA

Name: I.P./OPNo.:

Age: Hospital:

Sex: Address:

HISTORY

Chief complaint

BRIEF HISTORY OF PRESENTING ILLNESS

PAST AND ASSOCIATED ILLNESS

FAMILY HISTORY:

PERSONAL HISTORY:

Diet

Appetite

Sleep

Bowel and bladder

General physical examination

Pulse

BP

Temp.

RR

Height

Weight

SYSTEMIC EXAMINATION

Cardiovascular system: -

Central nervous system: -

Respiratory system: -

Per abdomen examination: -

PROVISIONAL DIAGNOSIS

Treatment detail

INVESTIGATION:

FBS: mg/dL

PPBS: mg/dL

HbA_1c: %

Urea: mg/dL

Creatinine: mg/dL Urine Routine:

Lipid Profile: Total Cholesterol: mg/dL

Triglyceride: mg/dL

HDL Chol.:

mg/dL

VLDL Chol.: mg/dL

LDL Chol.: mg/dL

Other Investigations**:**

Serum Adenosine Deaminase: U/L

Serum fasting insulin: -

Hematological

Hemoglobin

TLC/DLC

Glycosylated hemoglobin

X-ray chest (P.A.) view

Electrocardiogram

CONCLUSION:

DATE:

SIGNATURE

MASTER CHART

NAME	AGE	SEX	ADA	F.INSULIN	FBS	PPBS	HBA1C	QUICKI	
SHIVANAND	56	M	12.89	11.34	161	204	7.7	0.3	
MALLESHAYYA	55	M	11.45	15.45	170	214	9.1	0.29	
MOTILAL	75	M	10.561	29.8	214	144	11.8	0.26	
GADIGEPPA	71	M	10	1.9	138	180	10.4	0.4	
BASAPPA	74	M	48.8	17.58	125	161	7.5	0.299	
RAJU	40	M	14.983	3.45	131	115	6.1	0.37	
BHOGAPPA	52	M	10.45	21.36	215	207	8.5	0.27	
HANAMAWWA	61	F	12.77	6.8	347	205	6.9	0.28	
GEETA	49	F	16.685	8.86	128	158	8.6	0.296	
LAXMIBAI	54	F	17.05	23.82	139	176	7.3	0.279	
TUKARAM	68	M	9.34	6.45	165	206	7.5	0.32	
SHIVABAI	80	F	12.854	7.25	183	214	8.3	0.32547	
MAMTAJ	64	F	19.895	4.46	277	286	8.4	0.32	
RANJANA	49	F	20.45	14.82	180	226	12.4	0.2922	
GUNDAWWA	65	F	4.52	3.38	200	224	7.5	0.35	
SUCHANANDA	48	F	15.8	10.45	159	286	7.9	0.31	
SIDAWWA	66	F	9.45	11.59	133	184	7.7	0.3	
BHVANI	20	F	32.7	15.22	193	196	5.6	0.28	
SOMALA	82	M	24.9	16.66	136	174	8.8	0.29284	
SHARDA	65	F	16.4	<1	173	154	5.6	0.44	
BABU	50	M	12	1.14	136	184	4.5	0.42	
SIDDAPPA	67	M	24.5	6.84	220	240	8.3	0.315	
AMEENSAB	70	M	25.5	18.45	219	194	6.2	0.27	
NINGAPPA	73	M	12.24	11.36	181	188	9.7	0.30115	
YALLAPPA	58	M	34.03	12.45	126	158	6.8	0.31294	
BORAWA	70	F	27.44	9.07	134	256	12.3	0.3243	
BHIMRAY	58	M	17.8	8.57	137	164	6	0.32	
KENCHAWWA	46	F	36.22	21.15	144	208	8.3	0.28705	
IBRAHIM	60	M	21.35	17.55	243	258	11.5	0.27	
FIRAM	62	F	21.69	27.11	101	126	7	0.2908	
RAMU	64	M	15.21	6.58	122	164	6.7	0.3443	
LAKSHMIBAI	60	F	23.54	11.47	148	216	8.4	0.3096	
MOUNESH	30	F	29.74	13.11	245	207	7.8	0.28	

AISHWARYA	19 F	23.46	33.95	170	361	9.4	0.26		
KASAVVA	70 F	28.47	9.25	127	207	6.2	0.32374		
KANTABAI	65 F	20.56	11.18	176	184	8.1	0.3		
MALLIKARJUN	49 M	18.09	37.24	388	520	14.6	0.24		
MAHANANDA	45 F	44.21	15.24	208	304	10.8	0.28563		
BASAPPA	56 M	12.88	15.64	136	215	9.8	0.3		
YAMANAPPA	60 M	26.57	12.58	147	228	9.9	0.3061		
NIYAZ	34 M	54.9	12.14	152	252	13.1	0.305		
VAMANAPPA	60 M	16.98	4.25	114	178	6.9	0.3724		
LAKSMI	58 F	37.05	11.3	144	187	6.7	0.31		
DEVU	78 M	51.03	2.06	145	186	8	0.404		
SHAMALA	68 F	30.17	12.74	254	202	9.8	0.2849		
VEERBHADRAPPA	65 M	25.38	10.58	174	229	10.8	0.3063		
SHASHIKALA (PR)	48 F	28.7	18.75	325	303	13.3	0.26		
SANGAPPA	61 M	17.96	8.85	173	192	9.2	0.31		
BHUTALI	55 M	24.87	3.41	174	214	6.4	0.36		
HANAMANTH	52 M	41	8.24	166	204	7.8	0.31		
KASTURI	64 F	36.07	7.56	109	154	9.9	0.34		
HANAMANTH	55 M	30.49	77.34	111	213	14	0.25		
SANGAPPA	61 M	34.12	8.85	173	206	9	0.314		
Irappa	58 M	19.88	14.4	188	256	8.2	0.26133		
LAXMI	60 F	24.14	17.83	165	194	7.2	0.2883		
RAMCHANDRA	60 M	16.22	5.19	145	204	7.1	0.34784		
TOPPANNA	65 M	19.04	12.15	141	188	8.2	0.30823		
BHEMAPPA	57 M	26.02	9.57	216	254	10.5	0.3		
CHAUDAWWA	68 F	24.22	14.21	147	188	6.7	0.3012		
LAXMIBAI	65 F	36.3	18.25	136	234	6.1	0.2975		
SUDHINDRA	64 M	25.88	12.54	188	219	6.8	0.2665		
SIDAMMA	60 M	16.56	7.45	144	218	7.6	0.33		
VAISHALI	44 F	14.11	19.21	132	182	8.1	0.29376		
ALLABI	70 M	15.3	18.2	176	220	6.8	0.28524		
SHIVSHANKAR	50 M	26.77	21.09	172	288	11.9	0.28093		
CHANDRASHEKHAR	80 M	17.25	15.55	214	298	10.6	0.2889		
MALLAMMA	68 F	20.04	14.32	234	305	7	0.2852		

ASHOK	55 M	22.65	8.09	132	274	7.3	0.3302	
RENUKA	48 F	14.55	10.15	172	254	6.8	0.30845	
RAMGOUNDA	66 M	18.58	24.4	187	278	7.3	0.27	