Clinical Implication and Comparative Analysis of Different Biotechnological Medicinal Products in Treatment of Diabetes Mellitus in Pakistan

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DECLARATION

I hereby declare that the thesis titled **"Clinical Implication and Comparative Analysis of Different Biotechnological Medicinal Products in Treatment of Diabetes Mellitus in Pakistan"** submitted at Department of Biotechnology, Quaid-i-Azam University Islamabad for the award of degree of Master of Philosophy in Biotechnology is the result of research work carried out by me under supervision of Dr. Muhammad Zia Associate Professor, Department of Biotechnology, Faculty of Biological Sciences, Quaid-i-Azam University Islamabad, Pakistan, during period 2020-2022. I further declare that the results presented in this thesis have not been submitted for the award of any other degree or fellowship. I am aware of the terms copyright and plagiarism. I shall be responsible for any copyright violation found in this work.

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This is certified that the dissertation titled **"Clinical Implication and Comparative Analysis of Different Biotechnological Medicinal Products in Treatment of Diabetes Mellitus in Pakistan"** submitted by Hafiza Shizza Rafique to the Department of Biotechnology, Faculty of Biological Sciences, Quaid-i-Azam University Islamabad, Pakistan, is accepted in its present form as it is satisfying the dissertation requirement for the degree of **Master of Philosophy in Biotechnology**

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DEDICATION

This thesis is dedicated to my respected parents, husband and family who have been a constant source of inspiration. They have given me the drive and discipline to tackle any task with enthusiasm and determination.
Without their love and support, this thesis would not have been made possible.

Contents

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List of Abbreviations

DM	Diabetes mellitus
T2DM	Type 2 diabetes mellitus
ADA	American Diabetes Association
IDF	International Diabetes Federation
SMBG	Self-Monitoring Blood Glucose
BSF	Blood sugar fasting
BSR	Blood sugar random
RLS	Restless leg syndrome
HbA1c	Glycated Hemoglobin
LADA	Latent autoimmune diabetes of adults
MODY	Maturity onset diabetes of the young
GDM	Gestational Diabetes Mellitus
HI	Human Insulin
MI	Modern Insulin
DPP4I	Dipeptidyl Peptidase 4 Inhibitors
S	Sulfonyl urea
B	Biguanides
TZD	Thiazolidinediones
SGLT2	Sodium Glucose Co-transporter Inhibitors
S	Semaglutide
T0	Initial time point
T5	Final time point
1st Value	Value at the start of study
5th Value	Value at the end of study

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Hafiza Shizza Rafique

Abstract

Diabetes mellitus is comprising of two words; diabetes (Greek word) meaning siphon and mellitus (Latin word) means honey. It is not a new word it was recognized 3500 years ago. Three million people are diagnosed with diabetes and this number increase in every moment. 537 million adults (20-79 years) are living with diabetes. This number is predicted to rise to 643 million by 2030 and 783 million by 2045. Pakistan stands on 3rd number in prevalence of Diabetes among the world. This study was aimed to access the clinical implication and comparative analysis of different biotechnological medicinal products in the treatment of diabetes mellitus in twin cities of Pakistan.

The study was conducted after ethical approval from respective centers along with patients consent. 151 patients having type 2 diabetes mellitus (T2DM) were carefully chosen by random selection method. Data was collected from both diabetic clinics and hospital setups i.e., Umar Diabetes Foundation, Islamabad and Rasheed Nursing Home Hospital, Rawalpindi.

The study shows that 90% of the study patients using Human insulin had blood sugar fasting >126mg/dl at the start of study while at the end of the study 76% shows blood sugar fasting greater than 126mg/dl. 92% study population using human insulin shown blood sugar random greater than 200mg/dl while after the end of study 70% patients shows blood sugar random greater than 200mg/dl. 94.2% type 2 diabetic patients using semaglutide showed Blood sugar random >200mg/dl at start of the study which reduced to 49% after three months. Initially 5.9% patients showed blood sugar random within range 140-199mg/dl which increased to 21.6% at the end of study. Semaglutide showed significant control in blood sugar values. 100% patients show HbA1c >6.5% at the start of the study but at the end of study this rate reduced to 82.4%. In patients using fix-dose combination, data shows that 98% patients have blood sugar fasting greater than 126mg/dl at the start of the study that reduced to 74% at the end of the study. Initially only 2% patients showed blood sugar fasting within acceptable range but after three months use of this product this figure increase to 26%. Similarly, 100% patients showed blood sugar random >200mg/dl but at the end of this study this value reduced to 74%. Initially 0% patients showed blood sugar random within acceptable range (140-199mg/dl) but after end of this study this figure increase to 26%.

The study shows that traditional rDNA insulin is effective for management of diabetes but it increases the body weight which leads to insulin resistance with the passage of time. As compared to traditional rDNA human insulin, modern biotechnological medicinal products are very good options for management of chronic diabetes mellitus. The additional benefit of newly developed weekly acting rDNA semaglutide is weight reduction and very less chances of hypoglycemia which is major side effect of traditional human insulin. Fix-Dose combination (Degludec+Liraglutide) shows very similar effect of diabetes management as semaglutide but less effective in HbA1c and body weight reduction in comparison to semaglutide.

Key Words: Diabetes Mellitus; Blood sugar fasting; HbA1c; Blood sugar Random; Body weight

Chapter 1

Introduction

INTRODUCTION

1.1 Diabetes Mellitus

Diabetes mellitus is comprising of two words; diabetes (Greek word) meaning siphon and mellitus (Latin word) means honey. It is not a new word but recognized 3500 years ago (Ebbell, 1937; Mekala & Bertoni, 2020). Roman and Greek scientists describes the diabetes with increase in urine than the normal routine. Madhumeha was Indian scientist who observed that ants moving towards human urine, she give the name of sweet urine disease to the diabetes. Susrata discovered that diabetes leads to polyuria (Motala, Mbanya, Ramaiya, Pirie, & Ekoru, 2022). American Diabetes Association define diabetes as multifactorial disorder which is due to either insulin deficiency or insulin resistance and Persistent elevation in blood sugar level that leads toward multi-organ failure. Janbon discovered 1st oral anti-hyperglycemic agent i.e., sulfonylurea (Banerjee, Bharaj, & Banerjee, 2019). Eli Lilly and company is a biotechnological firm which produced first human insulin by using rDNA technology (Nagaich, 2015). Newly approved GLP1 analogues i.e. Dulaglutide, liraglutide and Semaglutide by different regulatory authorities are commonly used for management of diabetes(Bucheit et al., 2020).

There are three main types of diabetes mellitus that included type 1 diabetes mellitus which is also called insulin dependent diabetes mellitus. While other type of diabetes is type 2 diabetes mellitus which is non-dependent insulin diabetes mellitus. Type 1 Diabetes Mellitus comprises 5-10% while type 2 diabetes is 90% of all the diabetes type. Type 1 diabetes exact cause is not known. It may be due to viral attack or autoantibody formation against pancreatic beta cell (Roep, Thomaidou, van Tienhoven, & Zaldumbide, 2021). The differential test of type 1 diabetes mellitus is ketone body formation in the blood and urine, type 1 patients always report in emergency(Goddard & Oxlad, 2022). Pancreatic beta cell totally destroyed in this type of diabetes(Eisenbarth, 2005; Secrest, Washington, & Orchard, 2021). Type 2 Diabetes mellitus is the most common form of diabetes which occurs at any age but mostly after 25 years of age. It is multifactorial as exact cause of this type of diabetes is still unknown. A number of studies shows that more than 50% of type 2 diabetes caused by positive family history. Sedentary life style and junk food intake are also major contributing factor of this form of diabetes (Shakoor et al., 2021) (Williams, Jones, & Stephens, 2022). Gestational diabetes is third major type of diabetes which is due to

hormonal changes during pregnancy. After pregnancy ends, this form of diabetes recovers itself. This form of diabetes at later stage of life may be converted to type 2 diabetes mellitus in some patients(Tsakiridis et al., 2021). A number of studies show that this form of diabetes is increasing due to poor food quality, unbalance and unhealthy diet. Baby born in women showing gestational diabetes mellitus are at risk of life if not treated properly(Mustafa et al., 2021)(H. Wang et al., 2022).

There are three minor types of diabetes. Latent autoimmune diabetes of adult (LADA), Maturity onset of diabetes of young (MODY) and neonatal diabetes. Latent autoimmune diabetes of adult is first minor types of diabetes which is due to some unknown causes. Patient's body shows autoantibodies in blood but beta cell not totally destroyed which is checked by c-peptide test. At start of this type body sugar can be controlled by using oral anti-hyperglycemic agents but later on beta cell slowly destroyed which leads LADA toward type 1 diabetes(Jones, McDonald, Shields, Hagopian, & Hattersley, 2021)(Huang, Pearson, Wong, Wen, & Zhou, 2022). Maturity onset diabetes of young's is second minor type of diabetes which can be diagnosed by molecular genetic test. It is like non-insulin dependent diabetes mellitus but age wise patients seem to be a type 1 diabetes mellitus. Positive family history of patients shows this type of diabetes(Hare & Topliss, 2022). Neonatal is third type of minor diabetes mellitus which may be due to gene mutation. It mostly occurs at age of below 6 years. Treatment option includes insulin and sulfonylurea class drugs(Armentrout, 1995; De Franco, 2021).

Diabetes mellitus general complications divided into short term and long-term complications. Short term includes hypoglycemia (blood sugar level <70mg/dl) which is mostly observed in insulin and sulfonylurea class drug user diabetic patients (Cryer, 2016). Diabetic ketoacidosis is second short term complication of diabetes which is mostly observed in type 1 diabetes mellitus. It is emergency situation when body sugar level elevates to >300mg/dl and patient becomes unconscious. Blood test shows the presence of ketone body and blood pH drop to acidic which cause severe belly pain(Sha et al., 2022). Third short term complication of diabetes is non-ketotic hyperosmolar diabetic coma, which is mostly observed in type 2 diabetes mellitus. In this type blood sugar increase to greater than 300mg/dl but blood shows no ketone bodies(Habeeb, 2022).

Diabetes mellitus long term complications may be divided into 3 types; Retinopathy, Nephropathy and neuropathy. These types of complications are developed on later stages of uncontrolled diabetes patients and these changes are not reversible like short term complication. Retinopathy is common type of long term complication and mostly observed in uncontrolled type 2 diabetes mellitus which may be leads toward blindness. (Tamadon, Ghorbani, Rezaei, & Daraei, 2015) Nephropathy is second long term complication which destroy glomerulus of the kidney and ultimately leads patients towards dialysis(Antonetti, Silva, & Stitt, 2021). Neuropathy is third long term complication which cause feet and legs numbness and with the passage of time loss of sensation (Sawaf et al., 2022)(Montenegro, Griz, & Bandeira, 2022). Newly discovered long-term complication of uncontrolled diabetes are restless leg syndrome and anxiety. Restless leg syndrome is most common in women and it leads towards insomnia (Matar et al., 2022). Some studies shows that RLS may be due to some other factors like iron deficiency(Mubeen & Ahsan, 2022). Anderson et al. study shows that glycemic level is directly related with anxiety if blood glucose level of patient is increase its level of anxiety in most of the patients is increase(Duarte-Díaz et al., 2022).

1.2 Diagnosis of Type 2 DM (American Diabetes Association)

Following are the diagnosis criteria for Type 2 DM(Committee, 2021):

Sr.#	Diagnosis Criteria of Type 2 DM
1.	Minimum 8 hours fasting blood glucose ≥ 126mg/dl (7.0mmole/L)
2.	2hours post taking 75g anhydrous oral glucose load, blood glucose random ≥ 200mg/dl (11.1mmole/L)
3.	HbA1c≥ 6.5%
4.	Patients showing classic symptoms of hyperglycemia.

1.3 Treatment of Type 2 DM

Diabetes mellitus can be managed by using different drug classes including both Pharmaceutical and biotechnological.

1.3.1 Pharmaceutically manufactured drugs

Pharmaceutically prepared drugs for the management of diabetes mellitus are divided into different classes. The commonly used molecules of different classes include; i. Metformin (Biguanides class) Most common used pharmaceutical drug for management of type 2 diabetes mellitus at early stage. Its decrease insulin resistance, decrease gluconeogenesis and increase sensitivity of insulin(Mathu, Abarnadevika, & Ariharasivakumar, 2021).

Glimipride and Gliclazide are mostly used molecules of sulfonylurea class drugs. These drugs increase the insulin secretion and often lead to hypoglycemia. Long term use of this class damage pancreases and increase body weight(Strojek et al., 2021). Glinides (meglitinides) are very rare use class of oral anti- hyperglycemic agents due to hypoglycemia, high cost and body weight gain issues(Blahova et al., 2021). Pioglitazone of thiazolidinediones class drug which is not commonly use due to potential cause of bladder cancer(Hurren & Dunham, 2021). Acarbose of alpha glucosidase inhibitor class drug is commonly used for management of type 2 diabetes mellitus. It decreases the absorption of glucose from small intestine so decreases body weight. It is not recommended in GIT disturbed patients (Kaur et al., 2021). Dipeptidyl peptidase inhibitor is most commonly used class of oral anti-hyperglycemic drugs which includes sitagliptin and vildagliptin. It inhibits the incretin metabolism so increase glucose dependent insulin secretion. It mostly available in combination with metformin. There is no hypoglycemic effect with this class of drugs.(Bogdanov et al., 2022). Sodium glucose co-transporter inhibitor (SGLT2) is a newly launched anti-hyperglycemic medicine for treatment of type 2 diabetes mellitus. This class of drugs decrease the reabsorption of glucose from kidneys to control the body glucose via excretion in urine. Following generic fall in this class of drug: Dapagliflozin and Empagliflozin (O'Hara & Jardine, 2022).

1.3.2 Biotechnologically prepared anti-hyperglycemic agents:

Two types of medicinal biotechnological products are used for management of different type of diabetes; which includes; i. Glucagon like Peptide-1 (GLP-1) receptor agonist, ii. Insulin and their combinations. GLP-1 work like natural incretin hormones. These drug increase insulin secretion by glucose dependent mechanism and no chances of hypoglycemia. Satiety feeling, decrease glucagon and gastric emptying time is mechanism of action in addition to insulin secretion. This class of drugs are manufactured by using rDNA technology. Commonly used molecules of this class are; liraglutide (24 hours acting), Dulaglutide (weekly acting) and semaglutide (weekly acting)(Górriz, Romera, Cobo, O'Brien, & Merino-Torres, 2022). Insulin is second class of biotechnological medicinal products which includes different types and combinations(Iwamoto et al., 2022): Traditional rDNA Human Insulin

i. Human NPH (Natural Protamine Hagdrone) Insulin

ii. Regular Human Insulin

Short acting modern Insulin/insulin analogues

i. Lispro
ii. Aspart
iii. Glulisine

Long-acting insulin

i. Glargine
ii. Detemir

Premixed insulin

i. Human Pre-mixed i.e. 70/30= 70% Human NPH (Natural Protamine Hagdrone) 30% Human regular insulin.
ii. Modern pre-mixes i.e. 70/30 & 50/50

Ultra-long acting Insulin

i. Degludec

Fix-dose combination

Liraglutide+Degludec

1.4 Global epidemiology of Diabetes Mellitus

Duran *et al* study demonstrate that 3million people diagnosed with diabetes and this number increasing in every moment.(Duran et al., 2014). In 2017, 415 million diabetic patients diagnosed with diabetes and this figure increasing very rapidly among other chronic diseases, and may reach to 642 million by the end of 2040(IDF). **537 million** adults (20-79 years) are living with diabetes. This number is predicted to rise to **643 million** by 2030 and **783 million** by 2045. (IDF-2021).

1.5 Epidemiology of Type2 Diabetes Mellitus in Pakistan

A study published in Lancet which shows that 33 millions of people in Pakistan are living with diabetes(Bhutta, Haq, & Basit, 2022). IDF atlas 10th addition shows that in 2021 Pakistan shows comparative highest prevalence rate of Diabetes, 30% in among top ten countries showing highest diabetes cases. Rank wise Pakistan on 3rd number in prevalence of Diabetes among the world. Disease progress shows that in 2045 Pakistan would be on rank 1(Sun et al., 2022).

1.6 Literature Review:

LK Billings *et al.* conducted multicenter (USA, Spain, Turkey, Denmark and Greece) study evaluated the effect of once daily degludec/liraglutide to Basal Bolus in different patients to evaluate the effect of diabetes management. Degludec/liraglutide is a fix dose combination which seems to be more beneficial in managementof type 2 diabetes mellitus and control of body weight than basal bolus insulin. (Billings et al., 2021).

In 2020 there is another comparative study which shows that GLP-1 receptor agonists efficacy of liraglutide in comparison with dulaglutide conducted in Italy. This study reveals that once weekly dulaglutide is more effective than 24 hours acting liraglutide in addition to patients compliance(Morieri et al., 2020).

Very recent comparative research in Asia shows the metformin or sulfonylurea verses dulaglutide effect to manage the diabetes. This study reveal that biotechnological medicinal product dulaglutide is more effective for diabetes management in patient who fails to response pharmaceutical products like metformin or sulfonylurea for blood sugar control(W. Wang et al., 2019).

LS Matza *et al* study conducted in USA reveals that two biotechnological product dulaglutide and liraglutide behave differently for management of diabetes. Dulaglutide is weekly acting GLP-1 agonist while liraglutide is 24 hours acting GLP-1 agonist. Patient using once weekly acting product dulaglutide are more compliant than once daily acting liraglutide as a result better glycemic control was observed with once weekly acting dulaglutide(Matza et al., 2018).

S Ghosal and B Sinha study shows that a number of treatments are available for management of type 2 diabetes mellites. But addition of glucagon like peptide with sodium-glucose link transport inhibitor is very effective for management of diabetes. This combination is more effective in those patients having HbA1c level greater than 7%(Ghosal & Sinha, 2018).

A Viljoen *et al.* study in USA reveals that GLP-1 are more effective for management of diabetes along with weight management. GLP-1 agonist has lower chances of hypoglycemia. Semaglutide once weekly acting GLP-1 agonist which is more effective if patient not respond to metformin(Viljoen et al., 2019).

In 2018 Pratley *et al.* conducted multicenter (USA, UK Denmark and Germany) a head-to-head comparative study which shows that Semaglutide seems to be better treatment option than dulaglutide between GLP-1 analogues. As both drugs belong to same class but glycemic control along with weight management is better achieved with semaglutide than dulaglutide(Pratley et al., 2018).

Japanese study shows that diabetes management with long-acting insulin i.e. glargine is more expensive than dulaglutide. This study shows the better glycemic control with dulaglutide than insulin glargine(Ishii, Madin-Warburton, Strizek, Thornton-Jones, & Suzuki, 2018).

Recently a study published by Nature which reveals the cardioprotective effect of GLP-1 in addition to management of diabetes. GLP-1 another off-label use which is seems in most of the patients was weight loss. This additional benefit of newly biotechnological product helpful for management of diabetes mellitus in obese type 2 diabetes patients.(Sivalingam et al., 2021).

A retrospective study published by Springer shows that injectable newly developed biotechnological product i.e. semaglutide is more beneficial for management of diabetes than old GLP-1 agonist. Semaglutide not only better option for diabetes management but also patient compliant as it is weekly acting(Jain et al., 2021).

AM Nada *et al.* conducted a study in Egypt which reveals the effect of incretin mimetic after glucose increase in blood. This study shows the effect of different GLP-1 receptor agonist i.e., Liraglutide and Dulaglutide on bloog glucose management and body weight reduction. Dulaglutide shows more weight reduction than liraglutide.(Nada & Younan, 2021).

In 2021 a study was conducted in Japan in which two biotechnological products were compare for management of diabetes. It was observed that both liraglutide and dulaglutide are effective in management of diabetes as both reduces the HbA1c(Tanaka, Okada, Tokutsu, & Tanaka, 2021).

A comparative analysis was conducted to check the effect of biotechnological product liraglutide in addition to traditional metformin. It was observed that with addition of

liraglutide the management of diabetes was improve in addition to body weight loss. This treatment option is better in obese diabetic patients(Kavyasree, Geetha, & Shanmugasundaram, 2021).

Daniel J *et al.* performed a survey type investigation to find the association of sleep time, activeness and diabetes mellitus. In this study, Daniel concluded that sleep time has strong association with activeness and diabetes mellitus. He observed that patients whose sleeping time is between 7-8 hours are more active and healthy than those whose sleeping time is more than 8 hour or less than 7 hours(Jyoti, Ali, & Abbas, 2022).

Malaysian study shows there are many other factors also contribute to sleep disorder in diabetic patients. The most common factors are peripheral neuropathy, age, and duration of diabetes. It was observed that 27% patients are victims of restless leg syndrome and 45% have poor quality of sleep(Nasir, Draman, Zulkifli, Muhamad, & Draman, 2022).

Shaista A. Siddiqi *et al.* study in Karachi reveals that the frequency of restless leg syndrome and contributing factors, which can increase the severity of this disorder. It was interesting to know that not only diabetes mellitus is involved in restless leg syndrome but some other factor are also involved, like age, peripheral neuropathy, life style and poor glycemic control(Siddiqi et al., 2015).

In Netherland, a study regarding depression association with HbA1C was conducted. It was observed that higher HbA1C level not only causes depression but also leads towards insomnia, mood disturbance and decrease appetite(Zahra, Ramadhani, Mamfaluti, Pamungkas, & Firdausa, 2022).

1.7 Hypothesis of the Study

Hypothesis:

Ho= Biotechnological medicinal products have no role for management of Diabetes (T2DM)

H1= Biotechnological medicinal products have a role for management of Diabetes (T2DM).

1.8 Objectives:

Measuring the outcome

The following are the outcome measures that were used to check the impact of Biotechnological medicinal products for treatment of diabetes.

1.8.1 Primary Objective:

To observe the effectiveness of different biotechnological medicinal products in management of Blood sugar fasting and body weight.

1.8.2 Secondary Objective:

To observe the effectiveness of different biotechnological medicinal products in management of Blood sugar random, HbA1c and weight.

1.9 Funding:

Self

This study is the first study to check the effectiveness of different biotechnological medicinal products on managements of T2DM patients by visiting different hospitals and special diabetic setups in twin city of Pakistan.

1.10 Study Questions

i. What is the use of biotechnological medicinal products for diabetes management in twin city of Pakistan?

ii. What is the relationship of age, Blood sugar fasting, Blood sugar random, HbA1c, gender, family history and lifestyle with different types of Biotechnological medicinal products?

iii. Is there any association between age, Blood sugar fasting, Blood sugar random, HbA1c, gender, lifestyle and family history with different types of Biotechnological products?

iv. Either ADA/IDF/PES guidelines are followed for type 2 diabetes management?

Chapter 2

Material and Method

METERIALS AND METHODS

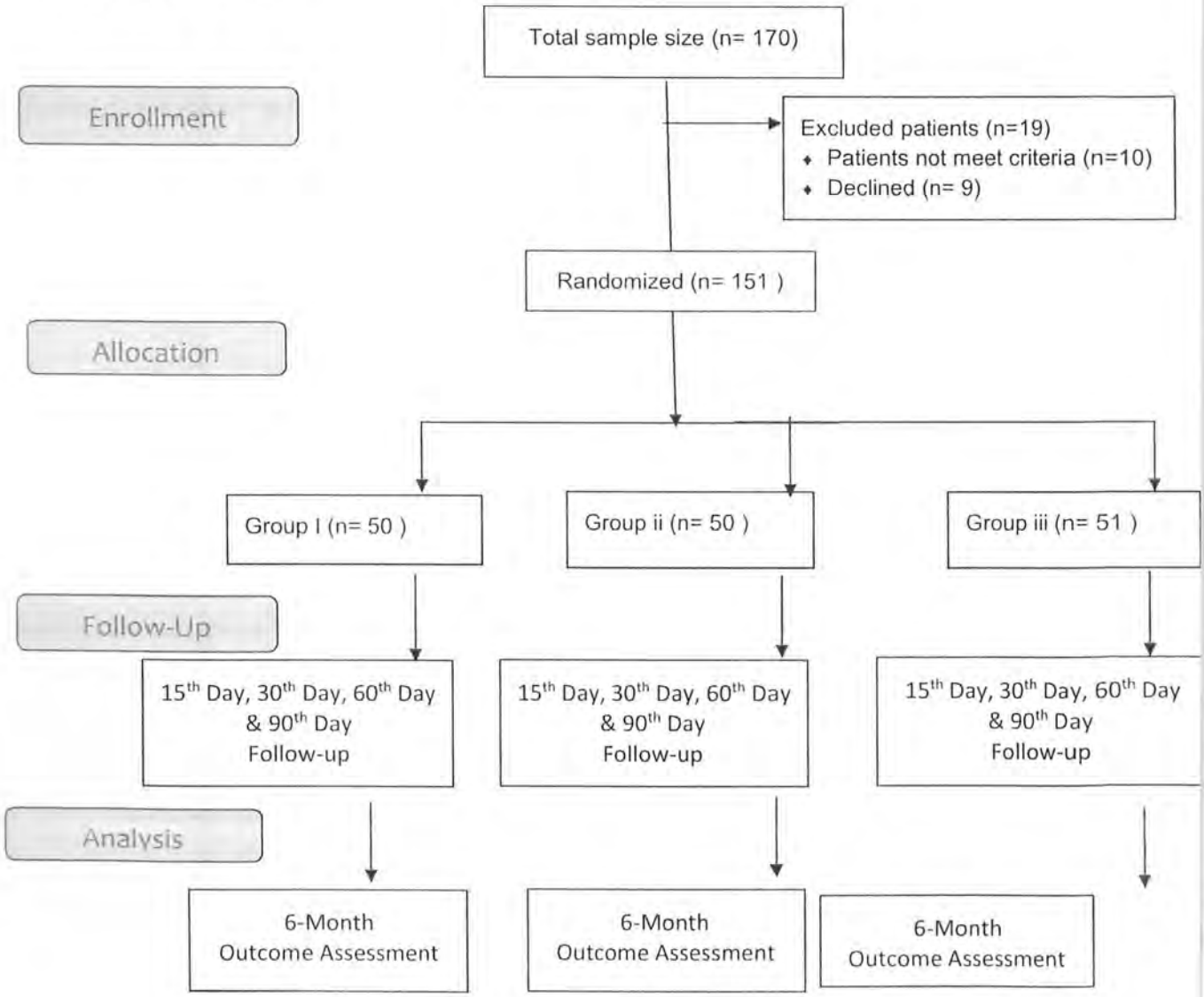
2.1 Study design

A randomized control prospective, open label six months study was conducted in Pakistan in which 151 patients having T2DM were carefully chosen by random selection method. Study show the clinical implication and comparative analysis of different biotechnological medicinal products in treatment of diabetes mellitus in Pakistan.

2.2 Sample Recruitment procedure

Outdoor patient selected for this study and after written inform consent they were randomly numbered. The ratio of each group is 1:1:1

Flow Diagram



2.3 Study setting

For six months, data was collected (October 2021 to April 2022). The qualified Nutritionist interviewed and invited each person living with T2DM who attended a designated diabetic center to take part in the study. The nature and objective of the research were explained to those who volunteered to participate. The lead investigator evaluated each consented patient against pre-determined inclusion and exclusion criteria.

Data was collected from both diabetic clinics and hospital setup i.e., Umer Diabetes Foundation, Islamabad and Rasheed Nursing Home Hospital, Rawalpindi. The data was collected in two parts: the first covered the individuals' socio-demographic and clinical information, and the second includes the Lab values of the patients. In Part 1 of the data collecting tool, eligible participants were asked to fill in their socio-demographic variables (gender, age, educational level, diabetes family history, and co-morbidities), which were gathered from their medical records. In part 2 of the data collection instrument, they were also asked to show their medical lab reports. The investigator collecting the data supported participants who couldn't understand any element of the questionnaire or couldn't self-administer it. The accuracy of the participants' knowledge was ensured, and they were encouraged to answer all of the questions in order, without skipping any. It took 10-15 minutes to complete the task in total. The clinical parameters of the participants were gathered from the patients' medical records, including blood sugar fasting, blood sugar random and HbA1c. Due to a lack of financing for the study, no monetary or other incentives were given to study participants except blood sugar checking.

2.4 Recruiting patients

The Qualified Nutritionist provided information on the trial's conduct and operating protocols to uncontrolled T2DM patients (HbA1c > 8%). The participants were gathered between October 2021 to April 2022. 170 patients were selected after proper patient's informed consent, but 151 participants fulfilled the study criteria. 50 patients in each group. Patient's education related to diet, exercise and medicine intake was served by direct sitting in health care facility and telephonically.

2.5 Evaluation of the starting point

The participants underwent an overall and gender-specific baseline evaluation was performed, which comprised lab values i.e. Blood sugar fasting, Blood sugar random, HbA1C, and physical assessment.

2.6 Dropouts or missing data

In the group i, there were 7 dropouts (4 withdrew and 3 were lost to follow up) and 8 in the group ii, (5 withdrew and 3 were lost to follow up) and 4 in group iii,(1 withdrew and 3 were lost to follow up). As a result, the final analysis included 50 patients in the group i and 50 patients in group ii and 51 patients in the group iii. (Fig. 1). The patients in each group continued to take part in the study until the second follow-up.

2.7 Population to be studied

HbA1c readings above 8%, according to ADA criteria (Baranwal, Maskey, Chaudhari, & Sherchand, 2020), indicated uncontrolled diabetes. As a result of the study's inclusion and exclusion criteria, only 151 patients were careful about baseline examination (Fig. 1).

2.8 Criteria for inclusion

Patients with uncontrolled T2DM over the age of 25-70, regardless of ethnicity, gender, or social status, must have visited the diabetic setup in the previous three months and willing to participate in the study.

2.9 Criteria for exclusion

Patients under the age of 25 or above 70, those with cognitive impairment, those who have missed appointments in the past three months and those who are unwilling

2.10 Study Way

In current study participant were not inform about grouping. All groups of this study were provided structural education by qualified dietitian about diet (low glycemic index diet, low calories diet, low glycemic load diet and healthy diet options) and disease, sign and symptoms of hyperglycemia, hypoglycemia, physical activity, blood sugar normal values, importance of good glycemic control and importance of medicine intake.

Nutritionist provided education to all groups during first visit face-to-face and follow-up on 15th Day, 30th Day, 60th Day & 90th Day under supervision of consultant. Printed educational hand outs were provided for medicine intake, dietary, SMBG and exercise as per American Diabetes Association guidance document.

2.11 Plan of action

Principle investigator evaluated patient with diabetes unique requirements for the revision of the Therapeutic Care Plan at each visit after 2 weeks, based on the patient's glycemic records. This was done through progress notes and talks with the research physician about treatment modifications, if any were needed.

The average patient encounter time was between 10 and 15 minutes at each 2-week follow-up visit.

2.12 Intervention by Investigator

All Participants were separated into three groups, i, ii, and iii, based on the number of follow-up appointments they attended, as shown in Figure 1. Investigator followed the international guidelines of IDF and ADA for patients with diabetes.

2.13 Biotechnological medicinal products administration and oral hypoglycemic agents

Patients were informed about the administration time, which could be with or 15–30 minutes before a meal, the dose plan, potential adverse effects, and often interfering medicines. Insulin administration was discussed with the patient, with emphasis on injection technique, syringe use, insulin pen use, and proper administration methods. The optimal injection sites, such as the thighs and abdomen, were explained to the participants, along with the need of changing the injection site.

2.14 Treatment goals and medicine adherence

Patients were questioned systematically by asking open-ended questions like, "Have you ever forgotten to take your dose?" Are you sometimes sloppy with your medication? and did you stop medicine if you feel better? Patients were questioned regarding the occurrence of any adverse effects likes Hypoglycemia and taught how to recognize the episode of hypoglycemia or hyperglycemia along with its management. All patients with diabetes were also informed about the glycemic targets that are required to achieve glycemic control, such as blood sugar fasting levels of 126 mg/dL, blood sugar random levels of 180 mg/dL, and HbA1c less than 7%, which should be evaluated every three months.

2.15 Modifications to one's diet and way of life

According to ADA Guidelines(Aamir et al., 2019), dietary regimens were adjusted to the need of patients, taking into account factors such as weight loss, hypertension, kidney disease, liver disease and socioeconomic issues. Patients were advised to engage in 20–30

minutes of exercise each day in the morning or when ever feel free but follow the same time every day, depending on their capacities, in order to promote weight loss and maintain a healthy BMI (BMI).

2.16 Sample size:

Sample size planned on the basis of previous studies(Butt, Ali, Bakry, & Mustafa, 2016; Moreira, de Fátima Mantovani, & Soriano, 2015).

The sample size computed was found to be 50 for each group.

2.17 Ethical consideration:

The study was approved by Quaid-i-Azam University Ethical Committee and respective data collection center. Informed consent was signed by all participants before start of a participant's interview.

2.18 Pilot Study

A pilot study was conducted on 15 participants 10% of the sample size. The purpose of pilot study design was to check to feasibility of the study which needs to be conducted. Additional confirmation was made by the supervisor.

2.19 Statistical Analysis Tool

Data after collection was analyzed by using different statistical tools in SPSS data analyzing software (SPSS version 22).

2.20 Variables

List of dependent and independent variables

Dependent Variables	Independent Variables
Blood Sugar fasting	Age
Blood Sugar Random	Gender
Body Mass Index	Family History
HbA1c	Life style
	Occupation
	Medication
	Comorbidities

2.21 Statistical Analysis Test

Following tests were used to analyze data collected for the research study.

i. Frequency and percentages for demographic variables.

ii. One sample t-test and Paired sample t-test (compare two population means 'before-after' studies).

iii. Significance level: $\alpha \leq 0.05$

Chapter 3

Results

Results

3.1 Patients related parameters

3.1.1 Demographic characteristics of patients using Human Insulin

The demographics variables of study populations include age, gender, education, occupation and body mass index. Results shows that there were 50 patients including both males (29) and female (21). Age wise patients were divided into five groups. There were 3(6%) between 25 to 34 years, 35 to 44 years were 9(18%), 45 to 54 years were 21(42%), 55 to 64 years were 9(18%) and 65 to 70 years were 8(16%). Mean age of patients was 51. Maximum patients lie between age of 45 to 54 years and minimum between 25 to 34 years. From Educational point of view, patients were divided into five groups. Illiterate 12(24%), primary 5(10%), secondary 8(16%), higher secondary 9(18%) and graduate 16(32%). Data show that maximum diabetic patients were found graduate 16(32%) and minimum were primary 5(10%). Occupation wise patients were divided into seven groups: jobless1 (2%), housewife 1(2%), student 26(52%), businessman 2(4%) private job 15(30%) government job 1(2%) and 4(8%) retired. As per Center for Disease Control, Body Mass Index wise patients were categorized into five groups: underweight 2(4%), normal weight 14(28%), overweight 15(30%), obese 13(26%) and extreme obese 6(12%).

Table 3.1.1: Demographic variables of type 2 diabetes patients (n=50)

Parameters	***n (%)***
Patients Age (Years) Means 51	
25 to 34 years	*3(6%)*
35 to 44 years	*9(18%)*
45 to54 years	*21(42%)*
55 to 64 years	*9(18%)*
65 to 70 years	*8(16%)*
Gender	
Male	*29(58%)*
Female	*21(42%)*
Education	
Illiterate	*12(24%)*
Primary	*5(10%)*
Secondary	*8(16%)*
Higher Secondary	*9(18%)*
Graduate	*16(32%)*
Occupation	
Jobless	*1(2%)*
Housewife	*1(2%)*
Student	*26(52%)*

	Businessman	*2(4%)*
	Private Job	*15(30%)*
	Govt. Job	*1(2%)*
	Retired	*4(8%)*
BMI		
	Under weight	*2(4%)*
	Normal Weight	*14(28%)*
	Overweight	*15(30%)*
	Obese	*13(26%)*
	Extreme Obese	*6(12%)*

Laboratory Parameters were blood sugar fasting, blood sugar random, HbA1c and body weight of the patients. Out of 50 patients 45(90%) patients had blood sugar fasting > 126mg/dl, 4(8%) had between 100-125mg/dl and 1(2%) had <100mg/dl whereas at the end of study significant patients increase in control range 100-125mg/dl i.e., 11(22%).

Random blood sugar level show that 46(92%) patients had >200mg/dl and 4(8%) had between 140-199mg/dl while at the end of study blood sugar random show significant increase in number within range group: 140-199mg/dl i.e., 13(26%) and 2(4%) <140mg/dl. Initially 50(100%) patients had HbA1c >6.5% but at the end of the study 49(98%) showed > 6.5% and 1(2%) lies between 5.7-6.4%.

Minor difference in HbA1c reduction before and after study was observed. Body weight shows increase in healthy weight.

Table 3.1.2: Laboratory values of type 2 diabetes patients (n=50)

Parameters	***n (%)***	***n (%)***
Blood Sugar Fasting	*T0*	*T5*
<100mg/dl	*1(2%)*	*1(2%)*
100-125mg/dl	*4(8%)*	*11(22%)*
>126mg/dl	*45(90%)*	*38(76%)*
Blood Sugar Random		
<140mg/dl	*0(0%)*	*2(4%)*
140-199mg/dl	*4(8%)*	*13(26%)*
>200mg/dl	*46(92%)*	*35(70%)*
HbA1c		
<5.7	*0(0%)*	*0(0%)*
5.7-6.4	*0(0%)*	*1(2%)*
>6.5	*50(100%)*	*49(98%)*
Weight		
40-50kg	*3(6%)*	*1(2%)*
51-60kg	*7(14%)*	*7(14%)*

61-70kg	*12(24%)*	*15(30%)*
71-80kg	*14(28%)*	*13(26%)*
81-90kg	*11(22%)*	*8(16%)*
91-100kg	*2(4%)*	*6(12%)*
>100kg	*1(2%)*	

3.1.2 Blood Sugar Fasting

Figure shows that 90% of T2DM patients had blood sugar fasting >126mg/dl, 8% had between 100-125mg/dl and 2% had <100mg/dl while at the end of study 76% had >126mg/dl and 22% between 100-125mg/dl. Figure show patients having blood sugar fasting within therapeutic range increase by using traditional biotechnological product i.e., Human Insulin.

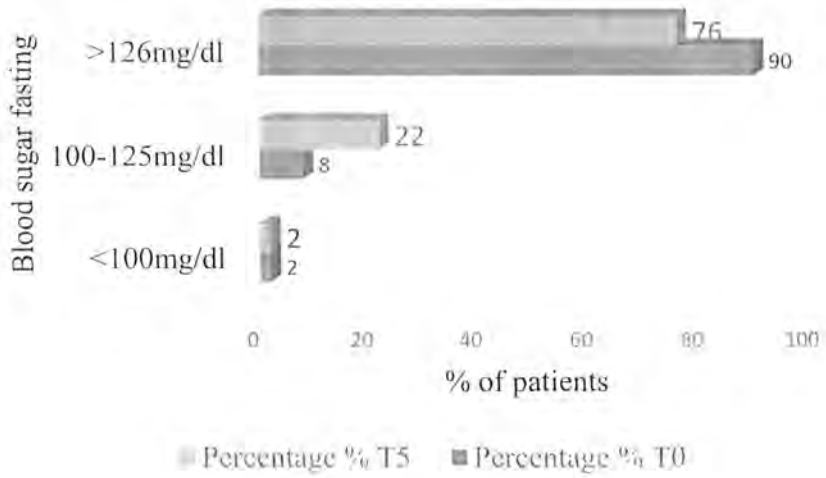


Figure 3.1.1: Graphically presentation of Blood Sugar Fasting

3.1.3 Blood Sugar Random

Figure shows that 92% of T2DM patients had blood sugar random >200mg/dl, 8% had between 140-199mg/dl and 0% had <140mg/dl while at the end of study 70% had >200mg/dl, 26% between 140-199mg/dl and 4% had <140mg/dl. Figure show patients having blood sugar random within therapeutic range increase by using traditional biotechnological product i.e., Human Insulin.

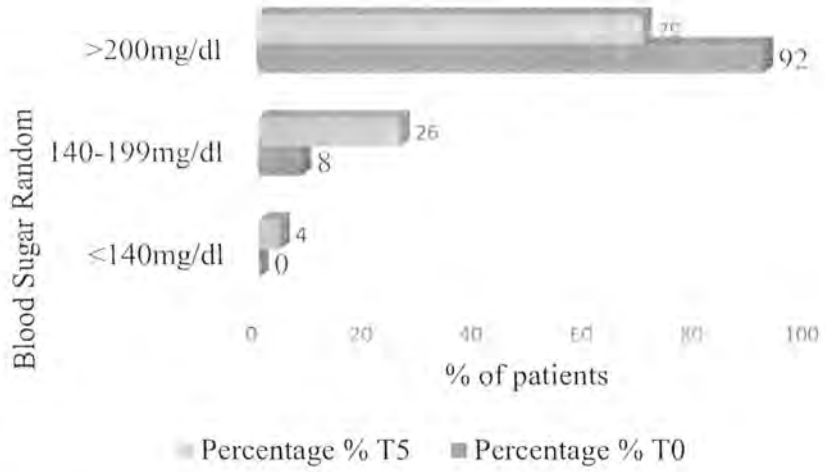


Figure 3.1.2: Graphically presentation of Blood Sugar Random

3.1.4 Weight

Figure shows weight wise distribution of T2DM patients. This figure clearly depicts the increase in healthy body weight of diabetic patients using human insulin.

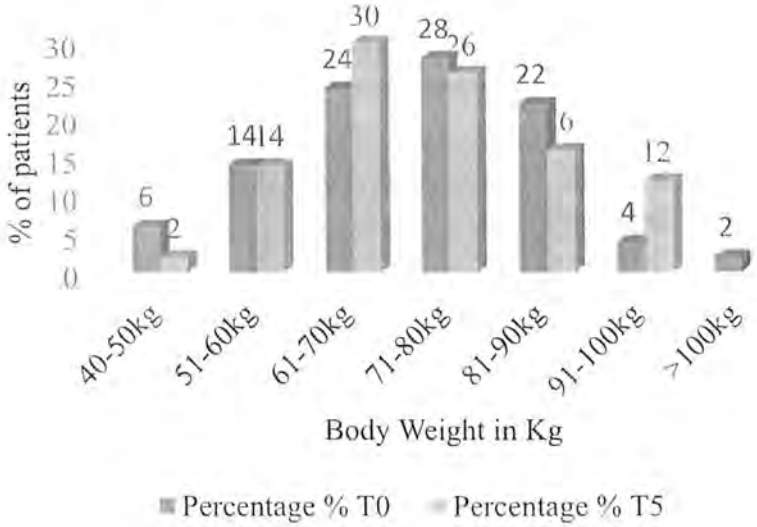


Figure 3.1.3: weight wise distribution of T2DM patients

3.1.5 Gender Distribution

Figure reveal that 58% of type 2 diabetes patients using human insulin were male while 42% were female. This shows more than 50% male using human insulin for management of diabetes mellitus.

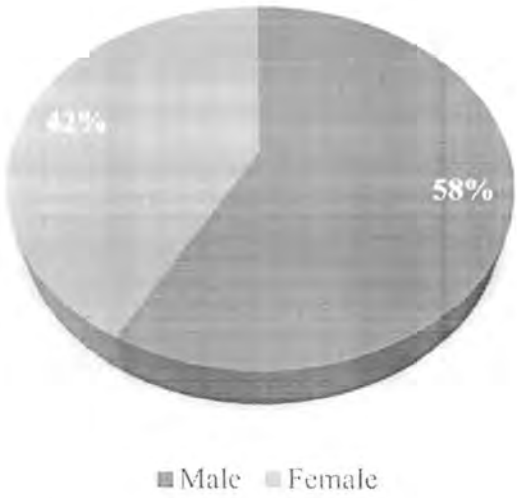


Figure 3.1.4: Gender wise distribution of T2DM patients

3.1.6 Age

Figure shows age wise patients' distribution which reveals that maximum patients fall within age of 45 to 54 years of an age while minimum fall within range of 25-34 years of an age.

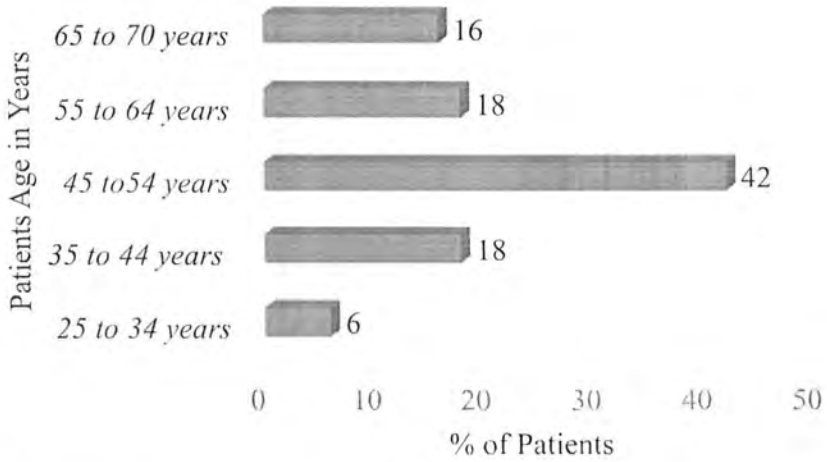
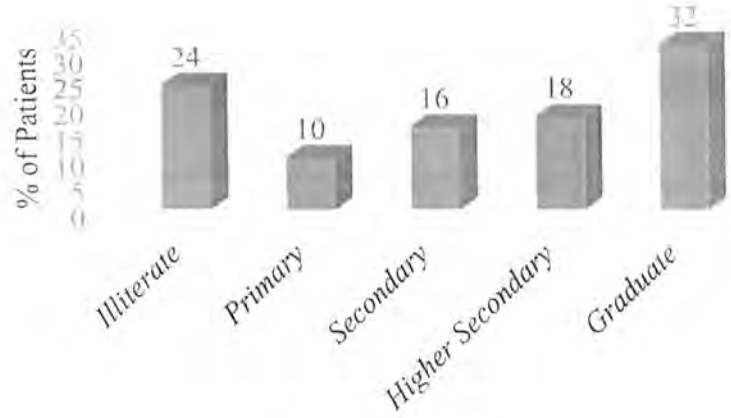


Figure 3.1.5: Age wise distribution of T2DM patients

3.1.7 Education

Figure 3.1.6: shows the Education wise distribution of patients. Out of 50 patients 32 were graduate, 18 higher secondary, 16 secondary, 10 primary and 24 were illiterate.



3.1.8 Occupation

Figure shows occupation wise distribution of patients. Figure reveals that more than 50% of the patients using traditional Biotechnological product for management of TDM were students.

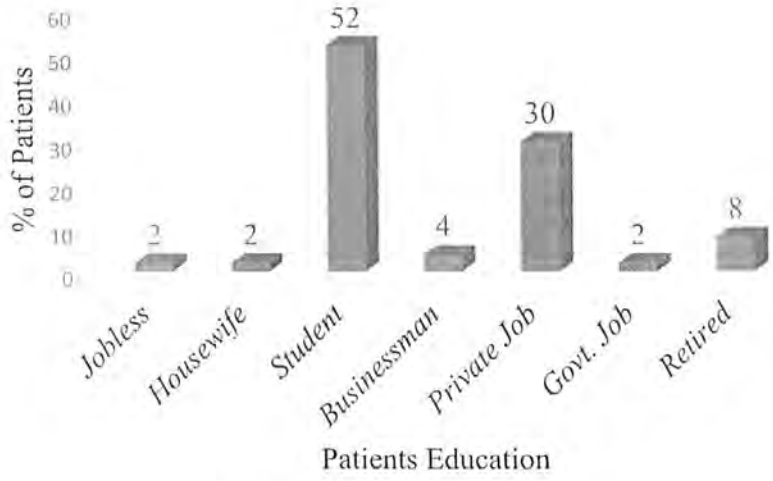


Figure 3.1.7: Occupation wise distribution of T2DM patients

3.1.9 Body Mass Index

Figure shows BMI wise distribution of patients. Body weight usually decreased in type 2 diabetes patients if it remains uncontrolled but if patients use traditional rDNA human insulin than body weight increases.

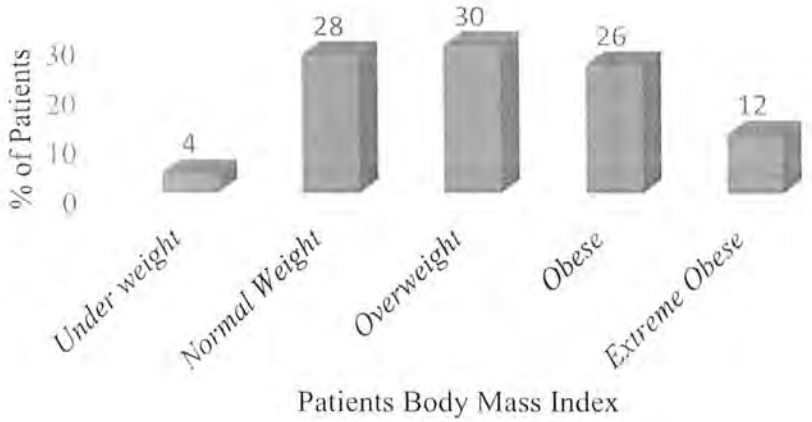


Figure 3.1.8: BMI wise distribution of T2DM patients.

3.1.10 HbA1c

Figure shows the comparison of HbA1c reduction at start and end of study in patients using human insulin. It was clearly observed the HbA1c reduced by using human insulin in most of the patients.

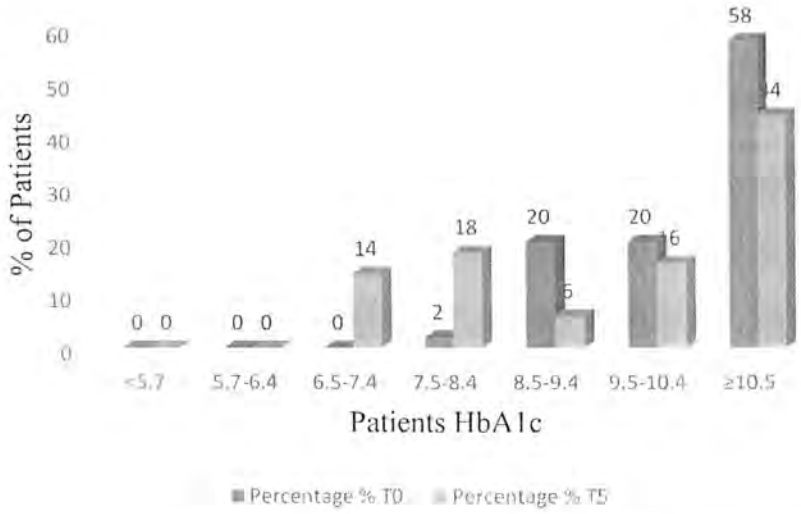


Figure 3.1.9: HbA1c at start of study and at end of patients using Human Insulin.

3.1.10 Pair Sample T test on different variables

Table 3.1.3 shows Paired t-test analysis (P<0.05) for Blood sugar fasting after 15 days use of human insulin. This table shows P-value <0.05 which indicate significant reduction in blood sugar fasting after 15 days use of human insulin.

	Paired Differences							
BSF-1 - BSF_2	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	T	df	Sig. (2-tailed)
	1.36200E1	47.69755	6.74545	.06450	27.17550	2.019	49	.049

Table 3.1.4 Paired t-test analysis (P<0.05) for Blood sugar fasting after 30 days use of human insulin. Below table shows P-value 0.000 which indicate very significant reduction in blood sugar fasting after 30 days use of human insulin.

	Paired Differences							
BSF_1 - BSF_3	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
	3.17600E1	46.76889	6.61412	18.46843	45.05157	4.802	49	.000

Table 3.1.5 Paired t-test analysis (P<0.05) for Blood sugar fasting after 60 days use of human insulin. Below table shows P-value 0.00 which indicate significant reduction in blood sugar fasting after 60 days use of human insulin.

	Paired Differences							
BSF_1 - BSF_4	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	Df	Sig. (2-tailed)
	3.69800E1	46.80811	6.61967	23.67728	50.28272	5.586	49	.000

Table 3.1.6 Paired t-test analysis (P<0.05) for Blood sugar fasting after 90 days use of human insulin. This table shows P-value 0.00 which indicate significant reduction in blood sugar fasting after 90 days use of human insulin.

	Paired Differences							
BSF_1 - BSF_5	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
	4.23200E1	46.98825	6.64514	28.96609	55.67391	6.369	49	.000

Table 3.1.7 Paired t-test analysis (P<0.05) for Blood sugar random after 15 days use of human insulin. This table shows P-value 0.000 which indicate significant reduction in blood sugar random after 15 days use of human insulin.

	Paired Differences							
BSR_1 - BSR_2	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
	4.78600E1	88.97994	12.58366	22.57218	73.14782	3.803	49	.000

Table 3.1.8 Paired t-test analysis (P<0.05) for Blood sugar random after 30 days use of human insulin. This table shows P-value 0.000 which indicate significant reduction in blood sugar random after 30 days use of human insulin.

Paired Samples Test

	Paired Differences							
BSR_1 - BSR_3	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
	8.13400E1	94.94351	13.42704	54.35735	108.32265	6.058	49	.000

Table 3.1.9 Paired t-test analysis (P<0.05) for Blood sugar random after 60 days use of human insulin. This table shows P-value 0.000 which indicate significant reduction in blood sugar random after 60 days use of human insulin.

Paired Samples Test

	Paired Differences							
BSR_1 - BSR_4	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
	8.37800E1	104.37868	14.76138	54.11591	113.44409	5.676	49	.000

Table 3.1.10 Paired t-test analysis (P<0.05) for Blood sugar random after 90 days use of human insulin. This table shows P-value 0.000 which indicate significant reduction in blood sugar random after 90 days use of human insulin.

	Paired Differences							
BSR_1 - BSR_5	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	T	Df	Sig. (2-tailed)
	6.83600E1	126.27452	17.85791	32.47318	104.24682	3.828	49	.000

Table 3.1.11 Paired t-test analysis (P<0.05) for HbA1c after 90 days use of human insulin. This table shows P-value 0.000 which indicate significant reduction in HbA1c after 90 days use of human insulin.

	Paired Differences							
HBA1c_1 - HbA1C_2	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	T	Df	Sig. (2-tailed)
	1.32160	1.81839	.25716	.80482	1.83838	5.139	49	.000

Table 3.1.12 Paired t-test analysis (P<0.05) for body weight after 15 days use of human insulin. This table shows P-value greater than 0.05 which indicate non-significant reduction in body weight after 15 days use of human insulin.

	Paired Differences							
Weight_1 - Weight_2	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	T	df	Sig. (2-tailed)
	-.02000	.99980	.14139	-.30414	.26414	-.141	49	.888

Table 3.1.13 Paired t-test analysis (P<0.05) for body weight after 30 days use of human insulin. This table shows P-value greater than 0.05 which indicate non-significant reduction in body weight after 30 days use of human insulin.

	Paired Differences							
Weight_1 - Weight_3	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
	-.16000	1.59540	.22562	-.61341	.29341	-.709	49	.482

Table 3.1.14 Paired t-test analysis (P<0.05) for body weight after 60 days use of human insulin. This table shows P-value greater than 0.05 which indicate non-significant reduction in body weight after 60 days use of human insulin.

	Paired Differences							
Weight_1 - Weight_4	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
	-.46000	2.38370	.33711	-1.13744	.21744	-1.365	49	.179

Table 3.1.15 Paired t-test analysis (P<0.05) for body weight after 90 days use of human insulin. This table shows P-value greater than 0.05 which indicate non-significant reduction in body weight after 90 days use of human insulin.

	Paired Differences							
Weight_1 - Weight_5	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
	-.74000	3.24390	.45876	-1.66190	.18190	-1.613	49	.113

3.2 Demographic characteristics of patients using Modern Biotechnological product i.e., Semaglutide

The demographics variables of study populations include age, gender, education, occupation and body mass index. Results shows that there were 50 patients including both males (21) and female (30). Age wise patients were divided into five groups. There were 4(7.8%) between 25 to 34 years, 35 to 44 years were 10(19.6%), 45 to 54 years were 19(37.3%), 55 to 64 years were 16(31.4%) and 65 to 70 years were 2(3.9%). Maximum patients lie between age of 45 to 54 years and minimum between 65 to 70 years. Education point of view patients were divided into five groups. Illiterate 6(11.8%), primary 6(11.8%), secondary 6(11.8%), higher secondary 9(17.6%) and graduate 24(47%). Data shows that maximum diabetic patients were found graduate 24(47%). Occupation wise patients were divided into seven groups: jobless2 (3.9%), housewife 19(37.3%), student 0(0%), businessman 13(25.5%), private job 6(11.8%) government job 10(19.6%) and 1(2%) retired. As per Center for Disease Control, Body Mass Index wise patients were categorized into five groups: underweight 0(0%), normal weight 2(3.9%), overweight 23(45.1%), obese 10(19.6%) and extreme obese 16(31.4%).

Table 3.2.1: Demographic variables of type 2 diabetes patients. (n=51)

Parameters	***n (%)***
Patients Age (Years)	
25 to 34 years	*4(7.8%)*
35 to 44 years	*10(19.6%)*
45 to54 years	*19(37.3%)*
55 to 64 years	*16(31.4%)*
65 to 70 years	*2(3.9%)*
Gender	
Male	*21(41.2%)*

	Female	*30(58.8%)*
Education		
	Illiterate	*6(11.8%)*
	Primary	*6(11.8%)*
	Secondary	*6(11.8%)*
	Higher Secondary	*9(17.6%)*
	Graduate	*24(47%)*
Occupation		
	Jobless	*2(3.9%)*
	Housewife	*19(37.3%)*
	Student	*0(0%)*
	Businessman	*13(25.5%)*
	Private Job	*6(11.8%)*
	Govt. Job	*10(19.6%)*
	Retired	*1(2%)*
BMI		
	Under weight	*0(0%)*
	Normal Weight	*2(3.9%)*
	Overweight	*23(45.1%)*
	Obese	*10(19.6%)*
	Extreme Obese	*16(31.4%)*

Laboratory Parameters were blood sugar fasting, blood sugar random, HbA1c and body weight of the patients. Out of 50 patients 50(98%) patients had blood sugar fasting > 126mg/dl and 1(2%) had between 100-125mg/dl whereas at the end of study significant patients increase in control range 100-125mg/dl i.e., 18(35.3%) and 5(9.8%) below 100mg/dl.

Random blood sugar level show that 48(94.2%) patients had >200mg/dl and 3(5.9%) had between 140-199mg/dl while at the end of study blood sugar random show significant increase in number within range group: 140-199mg/dl i.e., 11(21.6%) and 14(27.5%) <140mg/dl.

Initially 50(100%) patients HbA1c was >6.5% but at the end of the study 42(82.4%) was > 6.5%, 8(15.7%) between 5.7-6.4 and 1(2%) below 5.7%. Significant difference in HbA1c reduction before and after study was observed. Body weight table show decrease in overweight.

Table 3.2.2: Laboratory values of type 2 diabetes patients

Parameters	***n (%)***	***n (%)***
Blood Sugar Fasting	*T0*	*T5*
<100mg/dl	*0(0%)*	*5(9.8%)*

	100-125mg/dl	*1(2%)*	*18(35.3%)*
	>126mg/dl	*50(98%)*	*28(54.9%)*
Blood Sugar Random			
	<140mg/dl	*0(0%)*	*14(27.5%)*
	140-199mg/dl	*3(5.9%)*	*11(21.6%)*
	>200mg/dl	*48(94.2%)*	*25(49%)*
HbA1c			
	<5.7	*0(0%)*	*1(2%)*
	5.7-6.4	*0(0%)*	*8(15.7%)*
	>6.5	*50(100%)*	*42(82.4%)*
Weight			
	40-50kg	*0(0%)*	*0(0%)*
	51-60kg	*1(2%)*	*4(7.8%)*
	61-70kg	*6(11.8%)*	*5(9.8%)*
	71-80kg	*7(13.7%)*	*13(25.5%)*
	81-90kg	*16(31.4%)*	*16(31.4%)*
	91-100kg	*7(13.7%)*	*7(13.7%)*
	>100kg	*14(27.5%)*	*6(11.8%)*

3.2.1 Blood Sugar Fasting

Figure show that 98% of T2DM patients had blood sugar fasting >126mg/dl, 2% had between 100-125mg/dl and 0% had <100mg/dl while at the end of study 54.9% had >126mg/dl, 35.3% between 100-125mg/dl and 9.8% below 100mg/dl. Figure show patients having blood sugar fasting within therapeutic range increase by using modern biotechnological product i.e., Semaglutide.

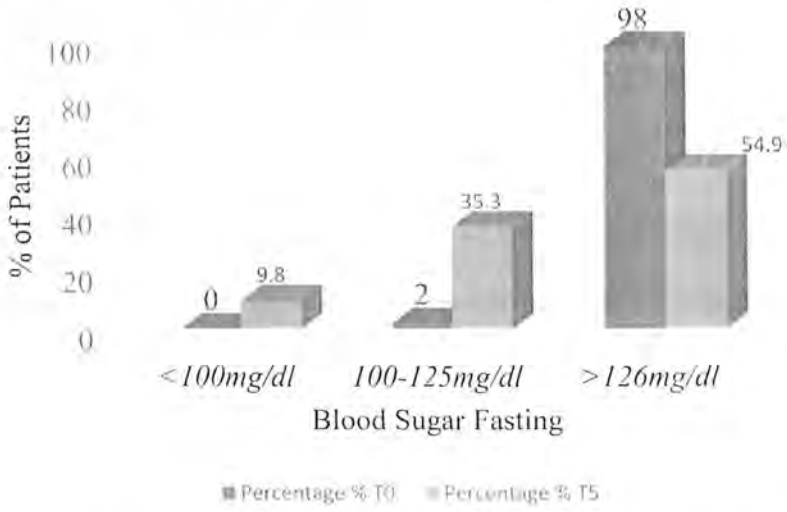


Figure 3.2.1: Graphically presentation of Blood Sugar Fasting

3.2.2 Blood Sugar Random

Figure show that 94.2% of T2DM patients had blood sugar random >200mg/dl and 5.9% had between 140-199mg/dl while at the end of study 49% had >200mg/dl, 21.6% between 140-199mg/dl and 27.5% had <140mg/dl. Figure show patients having blood sugar random within therapeutic range increase by using modern biotechnological product i.e., Semaglutide.

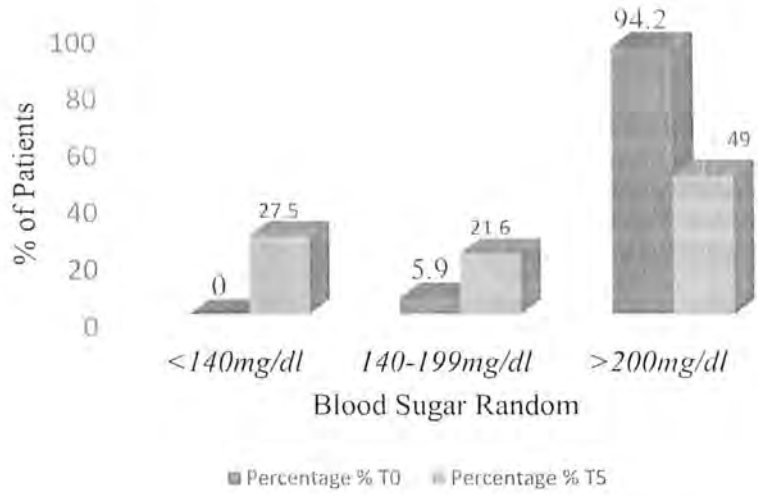


Figure 3.2.2: Graphically presentation of Blood Sugar Random

3.2.3 HbA1c

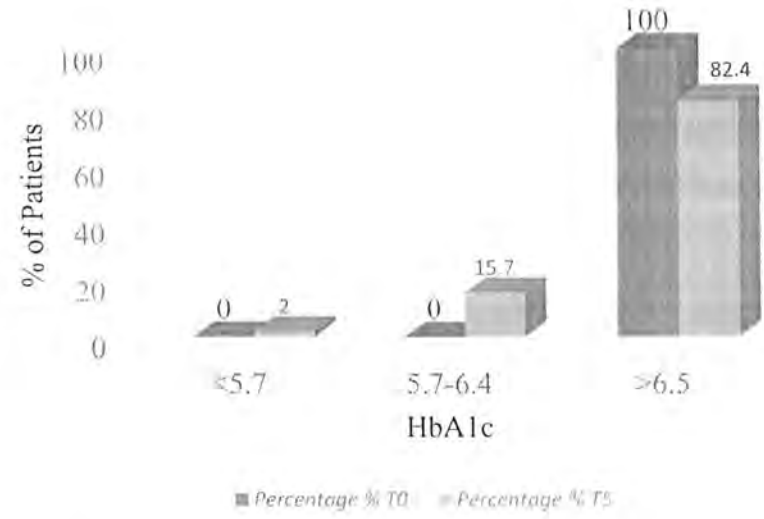


Figure 3.2.3: HbA1c wise distribution of T2DM patients

3.2.4 Weight

Figure show weight wise distribution of T2DM patients. This figure shows that initially 27% patients have body weight greater than 100kg but at the end of study this value reduced to 11.8% which indicate the significant reduction in body weight.

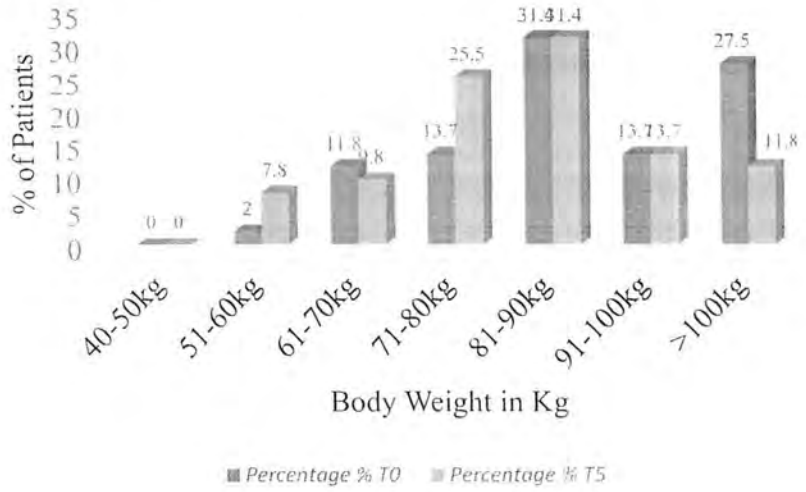


Figure 3.2.4: Weight wise distribution of T2DM patients

3.2.5 Gender Distribution

Figure reveal that 58% patients were male while 42% were female. 59% female use semaglutide the reason is that most of the diabetic females are obese so, semaglutide work dually to manage diabetes as well as body weight.

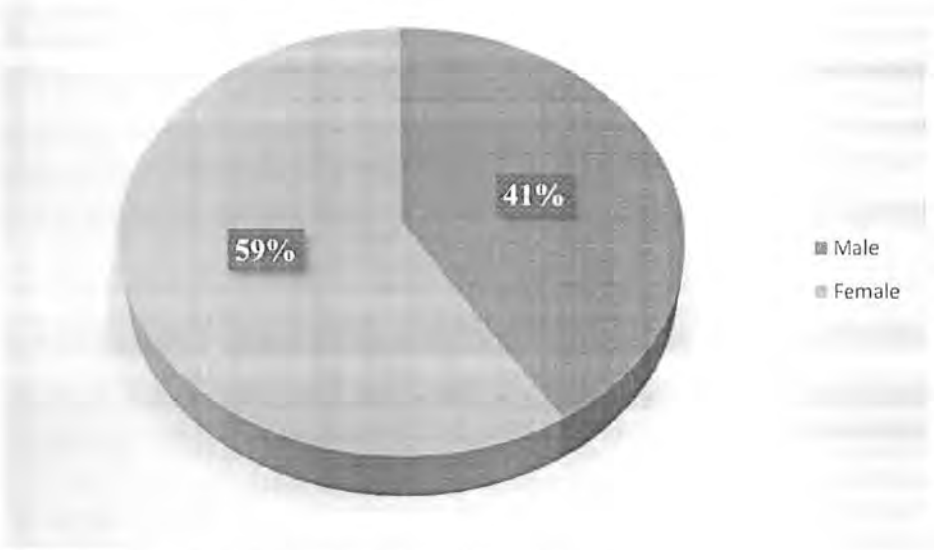


Figure 3.2.5: Gender wise distribution of T2DM patients

3.2.6 Age

Figure show age wise patients' distribution which reveal that maximum patients fall within age of 45 to 54 years of an age.

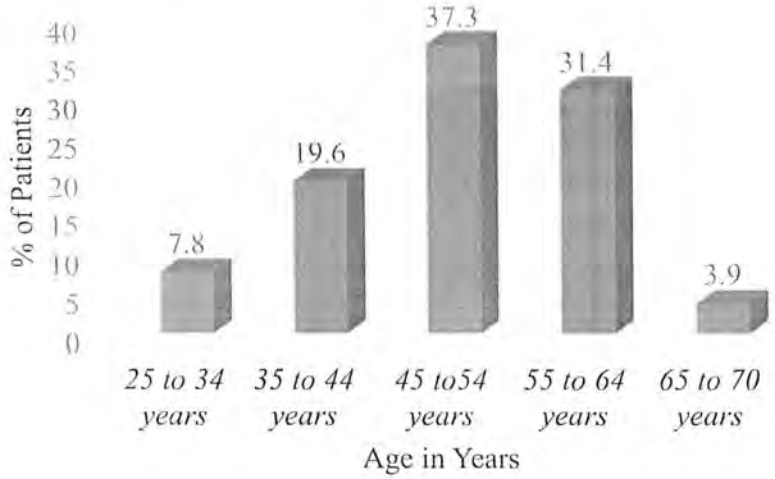


Figure 3.2.6: Age wise distribution of T2DM patients

3.2.7 Education

Figure show the Education wise distribution of patients. This figure shows that most of the semaglutide user are graduate i.e. 47%.

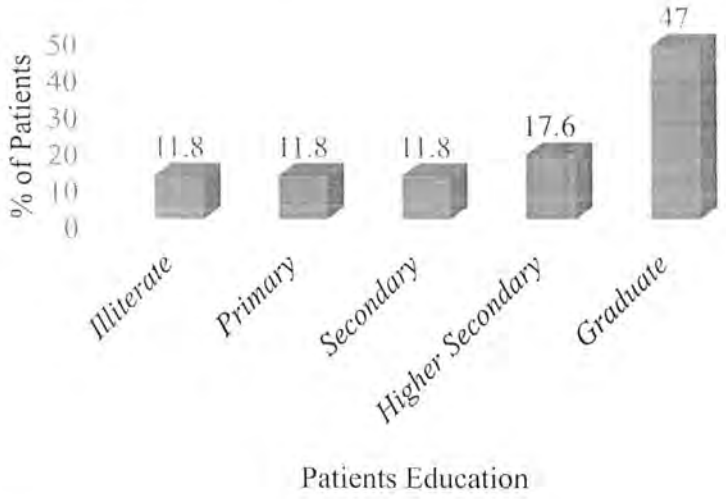
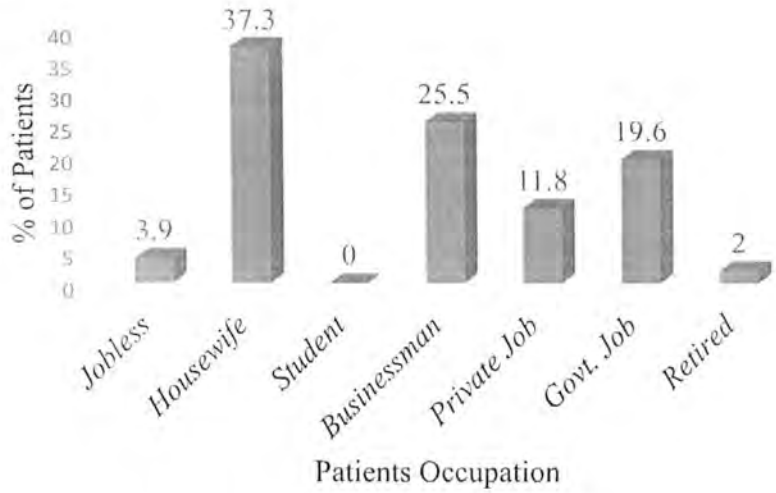


Figure 3.2.7: Education wise distribution of T2DM patients

3.2.8 Occupation

Figure 3.2.8 show occupation wise distribution of patients. Occupation wise 37.3% user of semaglutide are housewives, 2nd major user are business man while none of the student use this product.



3.2.9 Body Mass Index

Figure 3.2.9 show modern Biotechnological product used in such patients which were overweight. This figure shows that 45.1% semaglutide user are overweight while none of the patient is under weight. This product reduced body weight and better treatment option for obese diabetic patients.

Figure 3.2.10: Shows that HbA1c reduction clearly observed by using rDNA semaglutide glucagon like peptide.

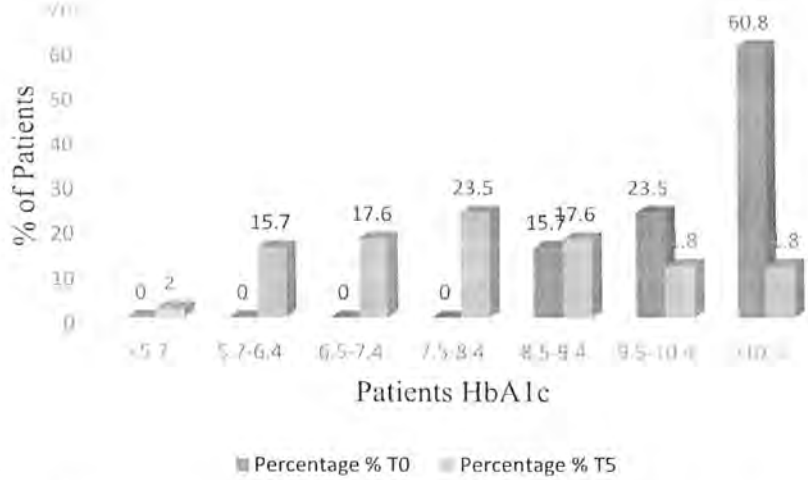


Figure 3.2.9: HbA1c at start of study and at end of patients using semaglutide

3.2.10 Pair sample T test on different parameters

Table 3.2.3 Pair sample t-test analysis (P<0.05) for Blood sugar fasting after 15 days use of Semaglutide. Table shows P-value 0.042 which is greater than 0.05, this shows not significant reduction in blood sugar fasting after 15 days use of rDNA Semaglutide.

	Paired Differences							
BSF_1 - BSF_2	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
	7.19608	24.59514	3.44401	.27858	14.11357	2.089	50	.042

Table 3.2.4 Pair sample t-test analysis (P<0.05) for Blood sugar fasting after 30 days use of Semaglutide. Table shows P-value 0.000 which is less than 0.05, this shows significant reduction in blood sugar fasting after 30 days use of rDNA Semaglutide.

	Paired Differences							
	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
BSF_1 - BSF_3	1.63137E1	28.10729	3.93581	8.40842	24.21903	4.145	50	.000

Table 3.2.5 Pair sample t-test analysis (P<0.05) for Blood sugar fasting after 60 days use of Semaglutide. Table shows P-value 0.000 which is less than 0.05, this shows significant reduction in blood sugar fasting after 60 days use of rDNA Semaglutide.

	Paired Differences							
	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
BSF_1 - BSF_4	2.88824E1	27.80838	3.89395	21.06112	36.70359	7.417	50	.000

Table 3.2.6 Pair sample t-test analysis (P<0.05) for Blood sugar fasting at the end of study. Table shows P-value 0.000 which is less than 0.05, this that Blood Sugar Fasting after 90 days use of semaglutide was statistically significant reduced.

	Paired Differences							
BSF_1 - BSF_5	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
	4.51176E1	31.91028	4.46833	36.14274	54.09256	10.097	50	.000

Table 3.2.7 Pair sample t-test analysis (P<0.05) for Blood sugar random after 15 days use of Semaglutide. Table shows P-value 0.000 which is less than 0.05, this shows significant reduction in blood sugar random after 15 days use of rDNA Semaglutide.

	Paired Differences							
BSR_1 - BSR_2	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
	4.62157E1	79.03476	11.06708	23.98680	68.44457	4.176	50	.000

Table 3.2.8 Pair sample t-test analysis (P<0.05) for Blood sugar random after 30 days use of Semaglutide. Table shows P-value 0.000 which is less than 0.05, this shows significant reduction in blood sugar random after 30 days use of rDNA Semaglutide.

	Paired Differences							
	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
BSR_1 - BSR_3	9.20784E1	91.01799	12.74507	66.47921	117.67765	7.225	50	.000

Table 3.2.9 Pair sample t-test analysis (P<0.05) for Blood sugar random after 60 days use of Semaglutide. Table shows P-value 0.000 which is less than 0.05, this shows significant reduction in blood sugar random after 60 days use of rDNA Semaglutide.

	Paired Differences							
BSR_1 - BSR_4	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
	1.06706E2	86.36534	12.09357	82.41524	130.99653	8.823	50	.000

Table 3.2.10 Pair sample t-test analysis (P<0.05) for Blood sugar random at the end of study. Table shows P-value 0.00 which is less than 0.05, this shows significant reduction in blood sugar random at the end of study in patients using rDNA Semaglutide.

	Paired Differences							
BSR_1 - BSR_5	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
	1.20941E2	122.23738	17.11666	86.56136	155.32099	7.066	50	.000

Table 3.2.11 Pair sample t-test analysis (P<0.05) for Body weight after 15 days use of Semaglutide. Table shows P-value 0.785 which is greater than 0.05, this shows not significant reduction in body weight after 15 days use of rDNA Semaglutide.

	Paired Differences							
Weight1 - Weight_2	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
	.03922	1.01903	.14269	-.24739	.32582	.275	50	.785

Table 3.2.12 Pair sample t-test analysis (P<0.05) for Body weight after 30 days use of Semaglutide. Table shows P-value 0.106 which is greater than 0.05, this shows not significant reduction in body weight after 30 days use of rDNA Semaglutide.

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
weight -1 Weight_3	3.27451	14.18743	1.98664	-.71577	7.26479	1.648	50	.106

Table 3.2.13 Pair sample t-test analysis (P<0.05) for Body weight after 60 days use of Semaglutide. Table shows P-value 0.000 which is less than 0.05, this shows significant reduction in body weight after 60 days use of rDNA Semaglutide.

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
weight -1 Weight_4	3.27451	2.54620	.35654	2.55838	3.99064	9.184	50	.000

Table 3.2.14 Pair sample t-test analysis (P<0.05) for Body weight after 90 days use of Semaglutide. Table shows P-value 0.000 which is less than 0.05, this shows significant reduction in body weight after 90 days use of rDNA Semaglutide.

	Paired Differences					t	df	Sig. (2-tailed)
weight -1 Weight_5	Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
	6.62745	5.22670	.73188	5.15742	8.09749	9.055	50	.000

Table 3.2.15 Pair sample t-test analysis (P<0.05) for HbA1c at the end of study. Table shows P-value 0.00 which is less than 0.05, this shows significant reduction in HbA1c the end of study in patients using rDNA Semaglutide.

	Paired Differences					t	df	Sig. (2-tailed)
HBA1c_1 - HbA1C_2	Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
	2.98275	1.47426	.20644	2.56810	3.39739	14.449	50	.000

3.3 Demographic characteristics of patients using Modern Biotechnological product i.e., Fix-Dose Combination (Degludec+Liraglutide)

The demographics variables of study populations include age, gender, education, occupation and body mass index. Results show that there were 50 patients including both males (23)

and female (27). Age wise patients were divided into five groups. There were 0(0%) between 25 to 34 years, 35 to 44 years were 10(20%), 45 to 54 years were 18(36%), 55 to 64 years were 20(40%) and 65 to 70 years were 2(4%). Maximum patients lie between age of 55 to 64 years and minimum between 25 to 34 years. Education point of view patients were divided into five groups. Illiterate 3(6%), primary 3(6%), secondary 10(20%), higher secondary 11(22%) and graduate 23(46%). Data shows that maximum diabetic patients were found graduate 23(46%). Occupation wise patients were divided into seven groups: jobless 1(2%), housewife 17(34%), student 0(0%), businessman 10(15%), private job 18(36%) government job 3(6%) and 6(12%) retired. As per Center for Disease Control, Body Mass Index wise patients were categorized into five groups: underweight 0(0%), normal weight 17(34%), overweight 18(36%), obese 8(16%) and extreme obese 7(14%).

Table 3.3.1: Demographic variables of type 2 diabetes patients. (n=50)

Parameters	***n (%)***
Patients Age (Years)	
25 to 34 years	*0(0%)*
35 to 44 years	*10(20%)*
45 to54 years	*18(36%)*
55 to 64 years	*20(40%)*
65 to 70 years	*2(4%)*
Gender	
Male	*23(46%)*
Female	*27(54%)*
Education	
Illiterate	*3(6%)*
Primary	*3(6%)*
Secondary	*10(20%)*
Higher Secondary	*11(22%)*
Graduate	*23(46%)*
Occupation	
Jobless	*1(2%)*
Housewife	*17(34%)*
Student	*0(0%)*
Businessman	*5(10%)*
Private Job	*18(36%)*
Govt. Job	*3(6%)*
Retired	*6(12%)*
BMI	
Under weight	*0(0%)*
Normal Weight	*17(34%)*
Overweight	*18(36%)*
Obese	*8(16%)*

Extreme Obese	*7(14%)*

Laboratory Parameters were blood sugar fasting, blood sugar random, HbA1c and body weight of the patients. Out of 50 patients 49(98%) patients had blood sugar fasting >126mg/dl and 1(2%) had between 100-125mg/dl whereas at the end of study significant patients increase in control range 100-125mg/dl i.e., 13(26%).

Random blood sugar level show that 50(100%) patients had >200mg/dl while at the end of study blood sugar random show significant increase in number within range group: 140-199mg/dl i.e., 13(26%).

Initially 50(100%) patients had HbA1c >6.5% but at the end of the study there is no reduction in HbA1c apparently as per reference value but Blood Sugar Fasting and Blood Sugar Random clearly show significant reduction in blood sugar values by using Biotechnological medicinal products. Body weight also decreased in overweight type 2 diabetes patients.

Table 3.3.2 : Laboratory values of type 2 diabetes patients

Parameters	***n (%)***	***n (%)***
Blood Sugar Fasting	*T0*	*T5*
<100mg/dl	*0(0%)*	*0(0%)*
100-125mg/dl	*1(2%)*	*13(26%)*
>126mg/dl	*49(98%)*	*37(74%)*
Blood Sugar Random		
<140mg/dl	*0(0%)*	*0(0%)*
140-199mg/dl	*0(0%)*	*13(26%)*
>200mg/dl	*50(100%)*	*37(74%)*
HbA1c		
<5.7	*0(0%)*	*0(0%)*
5.7-6.4	*0(0%)*	*0(0%)*
>6.5	*50(100%)*	*50(100%)*
Weight		
40-50kg	*0(0%)*	*0(0%)*
51-60kg	*2(4%)*	*3(6%)*
61-70kg	*16(32%)*	*23(46%)*
71-80kg	*16(32%)*	*9(18%)*
81-90kg	*5(10%)*	*9(18%)*
91-100kg	*5(10%)*	*1(2%)*
>100kg	*6(12%)*	*5(10%)*

3.3.1 Blood Sugar Fasting

Figure show that 98% of T2DM patients had blood sugar fasting >126mg/dl and 2% had between 100-125mg/dl while at the end of study 74% had >126mg/dl and 26% between 100-125mg/dl. Figure show patients having blood sugar fasting within therapeutic range

increase by using modern biotechnological product i.e., Fix-Dose Combination (Degludec+Liraglutide)

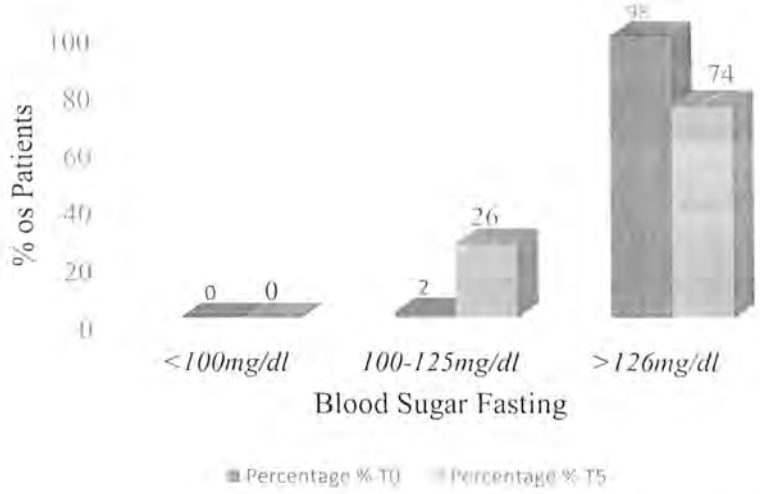


Figure 3.3.1: Graphically presentation of Blood Sugar Fasting

3.3.2 Blood Sugar Random

Figure show that 100% of T2DM patients had blood sugar random >200mg/dl while at the end of study 74% had >200mg/dl and 26% between 140-199mg/dl. Figure show patients having blood sugar random within therapeutic range increase by using modern biotechnological product i.e., Fix-Dose Combination (Degludec+Liraglutide)

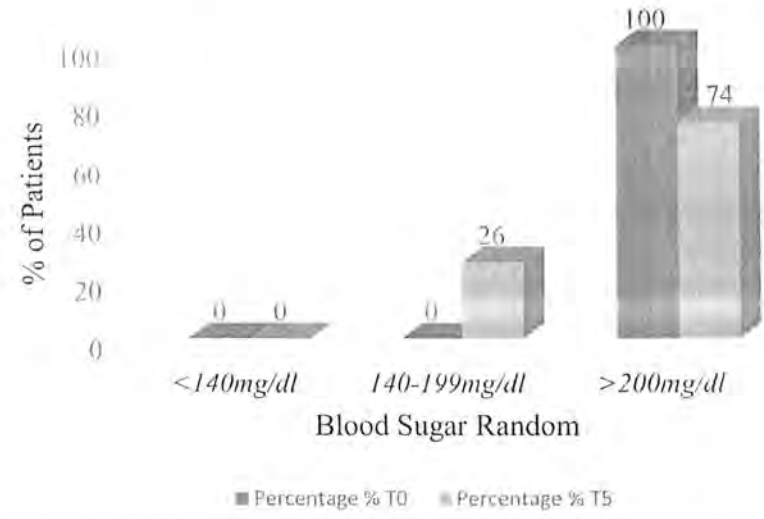


Figure 3.3.2: Graphically presentation of Blood Sugar Random

3.3.3 Weight

Figure show weight wise distribution of T2DM patients. This table shows that more than half patients body weight fall within category of 61-80kg using fix-dose combinations.

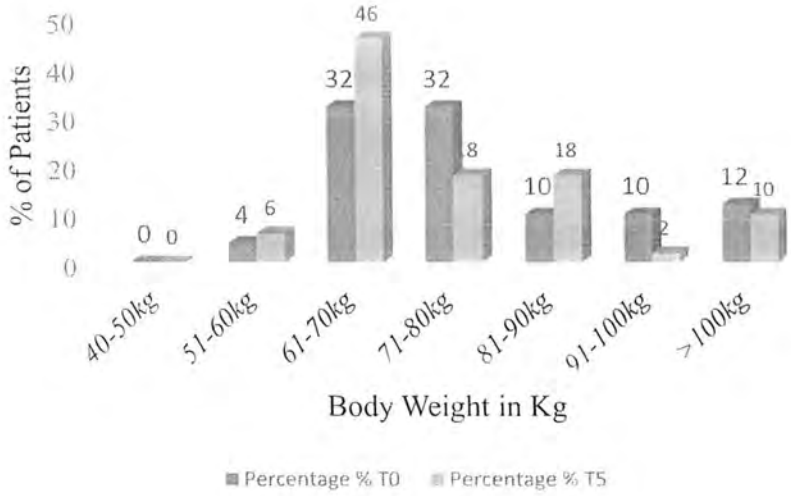


Figure 3.3.3: Weight wise distribution of T2DM patients

3.3.4 Gender Distribution

Figure reveal that 46% patients were male while 54% were female. 54% males use fix-dose combination while 46% female use this product in this study.

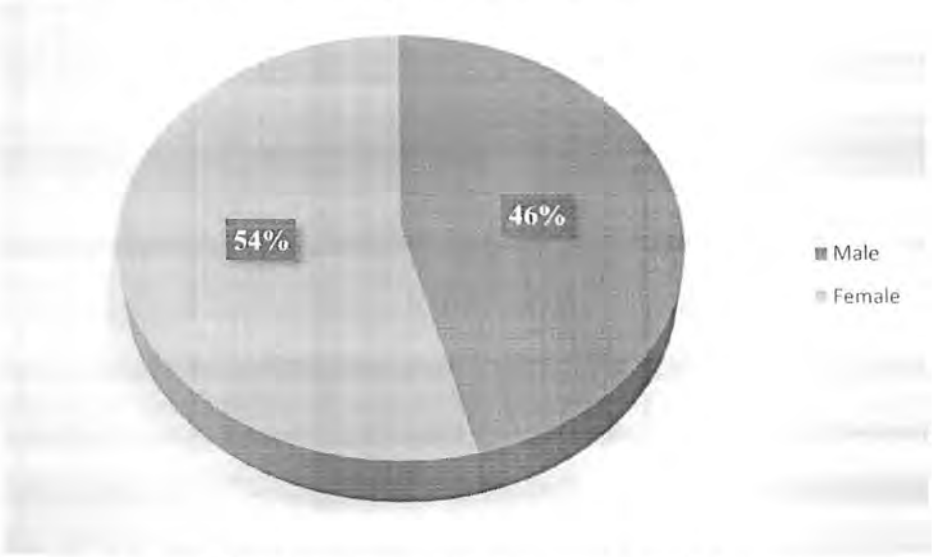


Figure 3.3.4: Gender wise distribution of T2DM patients

3.3.5 Age

Figure show age wise patients' distribution which reveal that maximum patients fall within age of 55 to 64 years of an age while minimum patients within 65 to 70 years of an age. None patients using fix-dose combination fall within category of 25-34 years of an age.

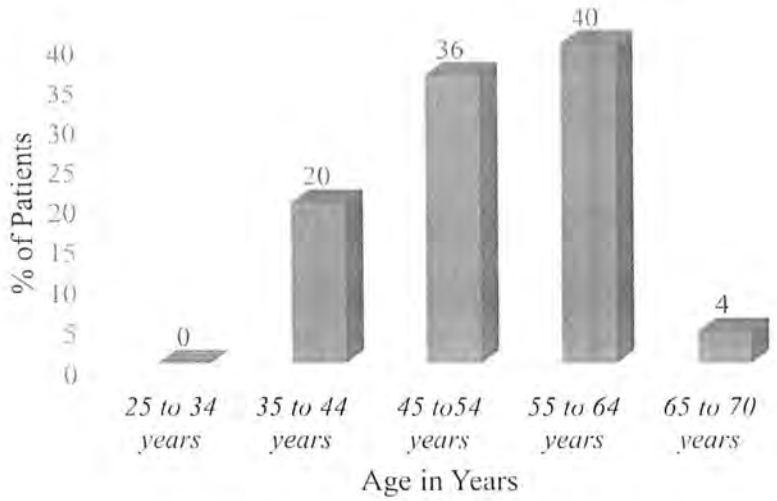


Figure 3.3.5: Age wise distribution of T2DM patients

3.3.6 Education

Figure show the Education wise distribution of patients. 46% graduate, 22% higher secondary, 20% secondary, 6% primary and 6% illiterate type 2 diabetes mellitus using fix-dose combination.

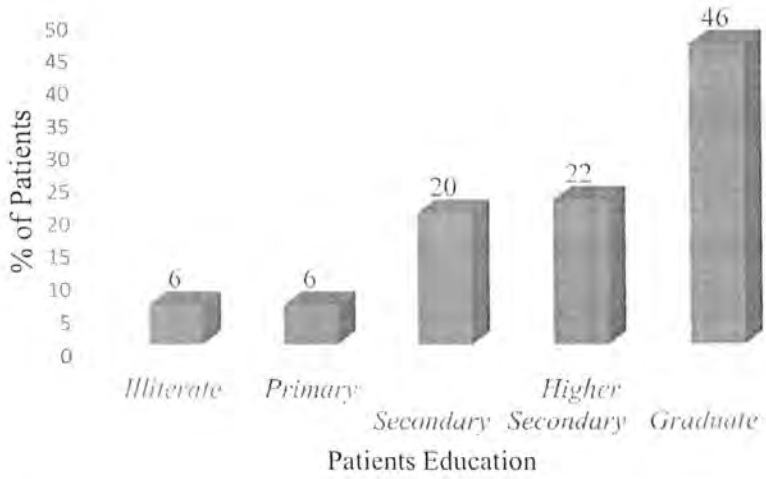


Figure 3.3.6: Education wise distribution of T2DM patients

3.3.7 Occupation

Figure show occupation wise distribution of patients. Maximum patients holding private job 36% of the total using this product.34% housewives, 12% retired, 10% business man, 6% govt-job holder and 2% jobless using this biotechnological product in current study sample.

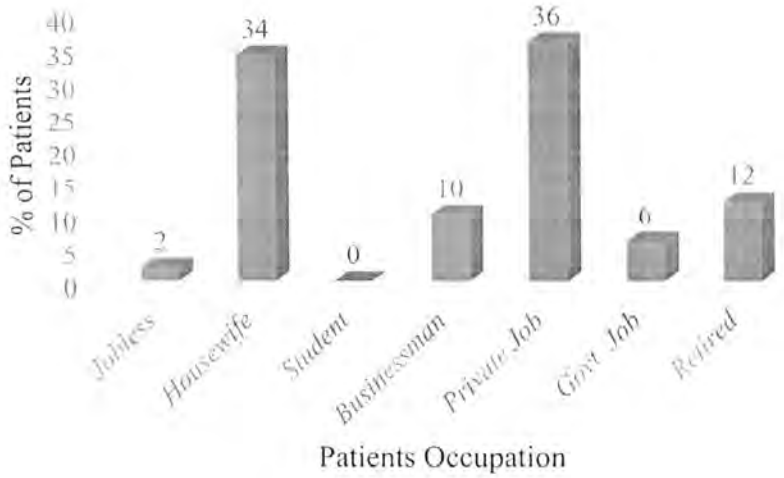


Figure 3.3.7: Occupation wise distribution of T2DM patients

3.3.8 Body Mass Index

Figure show modern Biotechnological product used in such patients which were overweight. 36% overweight, 34% normal weight 16% obese and 14% extreme obese patients using fix-dose combination.

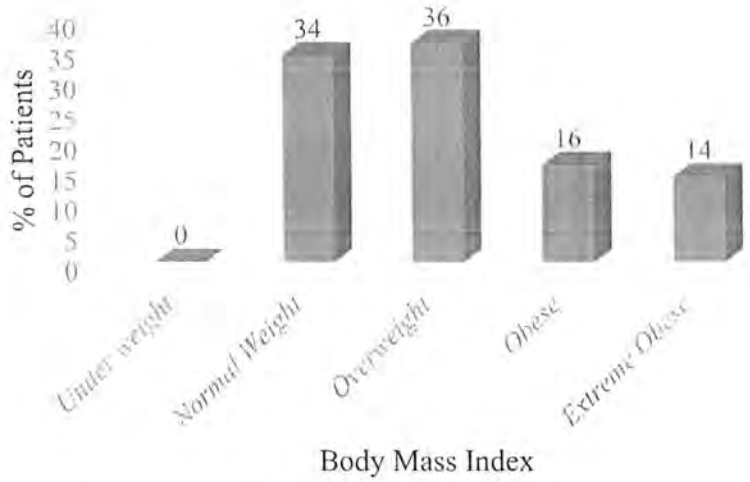


Figure 3.3.8: BMI wise distribution of T2DM patients

Figure 3.3.9 shows HbA1c reduction after using fix-dose combination in type 2 diabetes mellitus patients. This graphically presentation of HbA1c reduction seems to be statistically significantly by using fix-dose combination.

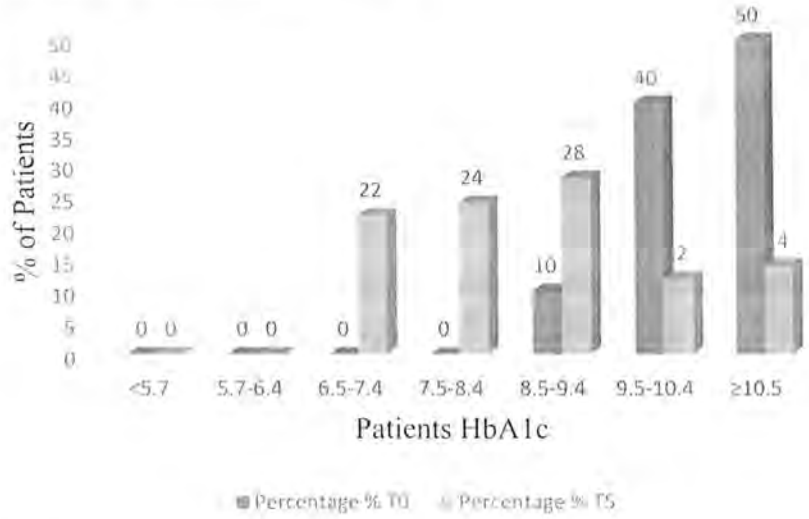


Figure 3.3.9: HbA1c at start of study and at end of patients using fix dose combination

3.3.9 Pair sample T test on different variables

Table 3.3.3 Pair sample t-test analysis (P<0.05) for blood sugar fasting after 15 days use of fix dose combination. Table shows P-value 0.001 which is less than 0.05, this shows significant reduction in blood sugar fasting in patients using fix-dose combination.

	Paired Differences							
BSF_1 - BSF_2	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
	2.16600E1	43.24001	6.11506	9.37133	33.94867	3.542	49	.001

Table 3.3.4 Pair sample t-test analysis (P<0.05) for blood sugar fasting after 30 days use of fix dose combination. Table shows P-value 0.000 which is less than 0.05, this shows significant reduction in blood sugar fasting in patients using fix-dose combination.

	Paired Differences							
BSF_1 - BSF_3	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
	3.54600E1	52.67351	7.44916	20.49035	50.42965	4.760	49	.000

Table 3.3.5 Pair sample t-test analysis (P<0.05) for blood sugar fasting after 60 days use of fix dose combination. Table shows P-value 0.000 which is less than 0.05, this shows significant reduction in blood sugar fasting in patients using fix-dose combination.

	Paired Differences							
BSF_1 - BSF_4	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
	3.67200E1	46.60649	6.59115	23.47458	49.96542	5.571	49	.000

Table 3.3.6 Pair sample t-test analysis (P<0.05) for blood sugar fasting at the end of study. Table shows P-value 0.00 which is less than 0.05, this shows significant reduction in blood sugar fasting at the end of study in patients using fix-dose combination.

	Paired Differences							
BSF_1 - BSF_5	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
	4.70000E1	55.05063	7.78533	31.35479	62.64521	6.037	49	.000

Table 3.3.7 Pair sample t-test analysis (P<0.05) for blood sugar random after 15 days use of fix dose combination. Table shows P-value 0.000 which is less than 0.05, this shows significant reduction in blood sugar random in patients using fix-dose combination.

	Paired Differences							
BSR_1 - BSR_2	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
	4.76600E1	48.98838	6.92800	33.73766	61.58234	6.879	49	.000

Table 3.3.8 Pair sample t-test analysis (P<0.05) for blood sugar random after 30 days use of fix dose combination. Table shows P-value 0.000 which is less than 0.05, this shows significant reduction in blood sugar random in patients using fix-dose combination.

	Paired Differences							
BSR_1 - BSR_3	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
	6.88400E1	55.57689	7.85976	53.04522	84.63478	8.759	49	.000

Table 3.3.9 Pair sample t-test analysis (P<0.05) for blood sugar random after 60 days use of fix dose combination. Table shows P-value 0.000 which is less than 0.05, this shows significant reduction in blood sugar random in patients using fix-dose combination.

	Paired Differences							
BSR_1 - BSR_4	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
	9.09000E1	70.43169	9.96054	70.88354	110.91646	9.126	49	.000

Table 3.3.10 Pair sample t-test analysis (P<0.05) for blood sugar random at the end of study. Table shows P-value 0.00 which is less than 0.05, this shows significant reduction in blood sugar random at the end of study in patients using fix-dose combination.

	Paired Differences							
BSR_1 - BSR_5	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
	1.03760E2	81.77859	11.56524	80.51878	127.00122	8.972	49	.000

Table 3.3.11 Pair sample t-test analysis (P<0.05) for body weight after 15 days use of fix dose combination. Table shows P-value 0.166 which is greater than 0.05, this shows non-significant reduction in body weight in patients using fix-dose combination.

	Paired Differences							
weight -1 Weight_2	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
	-.34000	1.70964	.24178	-.82587	.14587	-1.406	49	.166

Table 3.3.12 Pair sample t-test analysis (P<0.05) for body weight after 30 days use of fix dose combination. Table shows P-value 0.002 which is less than 0.05, this shows significant reduction in body weight in patients using fix-dose combination.

	Paired Differences							
	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
weight -1 Weight_3	.86000	1.90606	.26956	.31830	1.40170	3.190	49	.002

Table 3.3.13 Pair sample t-test analysis (P<0.05) for body weight after 60 days use of fix dose combination. Table shows P-value 0.000 which is less than 0.05, this shows significant reduction in body weight in patients using fix-dose combination.

weight -1 Weight_4	Paired Differences							
	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
	1.98000	1.88971	.26725	1.44295	2.51705	7.409	49	.000

Table 3.3.14 Pair sample t-test analysis (P<0.05) for body weight at the end of study. Table shows P-value 0.00 which is less than 0.05, this shows significant reduction in body weight at the end of study in patients using fix-dose combination.

	Paired Differences							
weight - Weight_5	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
	2.98000	2.09460	.29622	2.38472	3.57528	10.060	49	.000

Table 3.3.15 Pair sample t-test analysis (P<0.05) for HbA1c at the end of study. Table shows P-value 0.00 which is less than 0.05, this shows significant reduction in HbA1c at the end of study in patients using fix-dose combination.

	Paired Differences							
HBA1c_1 - HbA1C_2	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
	2.18760	.95501	.13506	1.91619	2.45901	16.197	49	.000

3.4 Demographic wise distribution of patients using Human Insulin, Semaglutide and fix dose combination Descriptive Statistics

Table: 3.4.1: Table shows the comparison of Human Insulin, semaglutide and fix-dose combination biotechnological products uses patient's demographics comparatively. It was observed that most of the patients fall within range of 45-64 years of an age. Data shows that more than 50% female uses modern biotechnological drug products than men. Education point of view near to 50% diabetic patients using biotechnological injectable are graduate. Occupation wise human insulin user were student, semaglutide user were housewives and

fix-dose combination user were private job holder. Semaglutide uses in overweight diabetic patient was comparatively have more ration than two.

Table: 3.4.1:

Parameters		***n (%)***	***n (%)***	***n (%)***
Patients Age		*HI*	*Semaglutide*	*Fix-Dose*
	25 to 34 years	*3(6%)*	*4(7.8%)*	*0(0%)*
	35 to 44 years	*9(18%)*	*10(19.6%)*	*10(20%)*
	45 to54 years	*21(42%)*	*19(37.3%)*	*18(36%)*
	55 to 64 years	*9(18%)*	*16(31.4%)*	*20(40%)*
	65 to 70 years	*8(16%)*	*2(3.9%)*	*2(4%)*
Gender				
	Male	*29(58%)*	*21(41.2%)*	*23(46%)*
	Female	*21(42%)*	*30(58.8%)*	*27(54%)*
Education				
	Illiterate	*12(24%)*	*6(11.8%)*	*3(6%)*
	Primary	*5(10%)*	*6(11.8%)*	*3(6%)*
	Secondary	*8(16%)*	*6(11.8%)*	*10(20%)*
	Higher Secondary	*9(18%)*	*9(17.6%)*	*11(22%)*
	Graduate	*16(32%)*	*24(47%)*	*23(46%)*
Occupation				
	Jobless	*1(2%)*	*2(3.9%)*	*1(2%)*
	Housewife	*1(2%)*	*19(37.3%)*	*17(34%)*
	Student	*26(52%)*	*0(0%)*	*0(0%)*
	Businessman	*2(4%)*	*13(25.5%)*	*5(10%)*
	Private Job	*15(30%)*	*6(11.8%)*	*18(36%)*
	Govt. Job	*1(2%)*	*10(19.6%)*	*3(6%)*
	Retired	*4(8%)*	*1(2%)*	*6(12%)*
BMI				
	Under weight	*2(4%)*	*0(0%)*	*0(0%)*
	Normal Weight	*14(28%)*	*2(3.9%)*	*17(34%)*
	Overweight	*15(30%)*	*23(45.1%)*	*18(36%)*
	Obese	*13(26%)*	*10(19.6%)*	*8(16%)*
	Extreme Obese	*6(12%)*	*16(31.4%)*	*7(14%)*

Table: 3.4.2: Comparative table of Laboratory Parameters of different patients using different Biotechnological products.

This table shows that maximum number of patients fall within acceptable therapeutic range at the end of study were semaglutide user (35.3%) than fix-dose combination user (26%) and lastly human insulin user (22%). While in case of blood sugar random maximum patient at the end of study fall within therapeutic range were Fix-dose combination user & human insulin user (26%), while least 21.6% semaglutide user. Maximum HbA1c

Table: 3.4.2:

Parameters	*n (%)*	*n (%)*	*n (%)*	*n (%)*	*n (%)*	*n (%)*
Blood Sugar Fasting	*T0 HI*	*T5 HI*	*T0 Semaglutide*	*T5 Semaglutide*	*T0 Fix-Dose*	*T5 Fix-Dose*
<100mg/dl	*1(2%)*	*1(2%)*	*0(0%)*	*5(9.8%)*	*0(0%)*	*0(0%)*
100-125mg/dl	*4(8%)*	*11(22%)*	*1(2%)*	*18(35.3%)*	*1(2%)*	*13(26%)*
>126mg/dl	*45(90%*	*38(76%)*	*50(98%)*	*28(54.9%)*	*49(98%)*	*37(74%)*
Blood Sugar Random						
<140mg/dl	*0(0%)*	*2(4%)*	*0(0%)*	*14(27.5%)*	*0(0%)*	*0(0%)*
140-199mg/dl	*4(8%)*	*13(26%)*	*3(5.9%)*	*11(21.6%)*	*0(0%)*	*13(26%)*
>200mg/dl	*46(92%)*	*35(70%)*	*48(94.2%)*	*25(49%)*	*50(100%)*	*37(74%)*
HbA1c						
<5.7	*0(0%)*	*0(0%)*	*0(0%)*	*1(2%)*	*0(0%)*	*0(0%)*
5.7-6.4	*0(0%)*	*1(2%)*	*0(0%)*	*8(15.7%)*	*0(0%)*	*0(0%)*
>6.5	*50(100%)*	*49(98%)*	*50(100%)*	*42(82.4%)*	*50(100%)*	*50(100%)*
Weight						
40-50kg	*3(6%)*	*1(2%)*	*0(0%)*	*0(0%)*	*0(0%)*	*0(0%)*
51-60kg	*7(14%)*	*7(14%)*	*1(2%)*	*4(7.8%)*	*2(4%)*	*3(6%)*
61-70kg	*12(24%)*	*15(30%)*	*6(11.8%)*	*5(9.8%)*	*16(32%)*	*23(46%)*
71-80kg	*14(28%)*	*13(26%)*	*7(13.7%)*	*13(25.5%)*	*16(32%)*	*9(18%)*
81-90kg	*11(22%)*	*8(16%)*	*16(31.4%)*	*16(31.4%)*	*5(10%)*	*9(18%)*
91-100kg	*2(4%)*	*6(12%)*	*7(13.7%)*	*7(13.7%)*	*5(10%)*	*1(2%)*
>100kg	*1(2%)*		*14(27.5%)*	*6(11.8%)*	*6(12%)*	*5(10%)*

Table 3.4.3 Comparative HbA1c reduction by using different Biotechnological products.

This table shows the reductions in HbA1c value using different drug products. It was observed that patients having HbA1c greater than 10.5% using human insulin at start were 58% while at end of study reduced to 44%. Similarly HbA1c reduction observed from 60.8% to 11.8% by using semaglutide and from 50% to 14% reduction observed by using fix dose combination. Comparative analysis shows maximum reduction of HbA1c by using semaglutide in this study.

Parameters		***Human Insulin***		***Semaglutide***		***Fix-Dose***	
HbA1c		*T0*	*T5*	*T0 S*	*T5 S*	*T0*	*T5*
1	*<5.7*	*0(0%)*	*0(0%)*	*0(0%)*	*1(2%)*	*0(0%)*	*0(0%)*
2	*5.7-6.4*	*0(0%)*	*1(0%)*	*0(0%)*	*8(15.7%)*	*0(0%)*	*0(0%)*
3	*6.5-7.4*	*0(0%)*	*7(14%)*	*0(0%)*	*9(17.6%)*	*0(0%)*	*11(22%)*

4	*7.5-8.4*	*1(2%)*	*9(18%)*	*0(0%)*	*12(23.5%)*	*0(0%)*	*12(24%)*
5	*8.5-9.4*	*10(20%)*	*3(6%)*	*8(15.7%)*	*9(17.6%)*	*5(10%)*	*14(28%)*
6	*9.5-10.4*	*10(20%)*	*8(16%)*	*12(23.5%)*	*6(11.8%)*	*20(40%)*	*6(12%)*
7	*≥10.5*	*29(58%)*	*22(44%)*	*31(60.8%)*	*6(11.8%)*	*25(50%)*	*7(14%)*

Graphically presentation of above table Table: 3.4.2 & 3.4.3 is as under:

Figure 3.4.1: Figure show the comparative analysis of blood sugar fasting in different patient using different biotechnological products

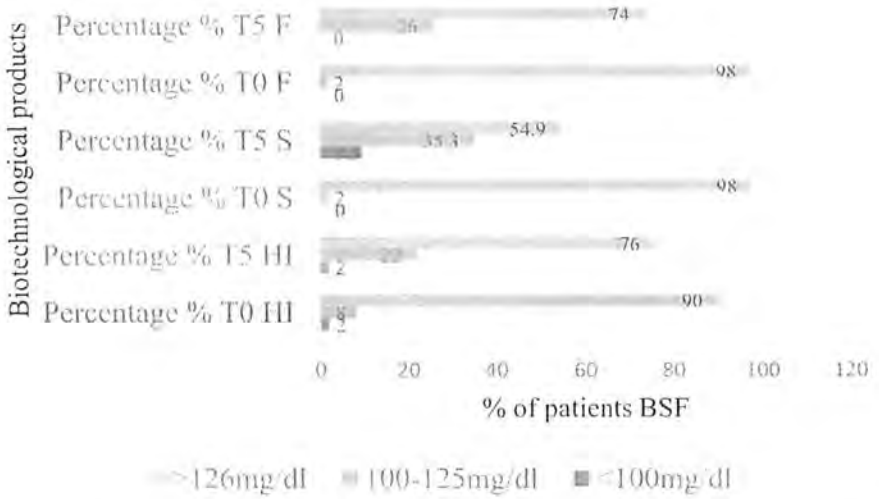


Figure 3.4.2: Figure show the comparative analysis of blood sugar random in different patient using different biotechnological products

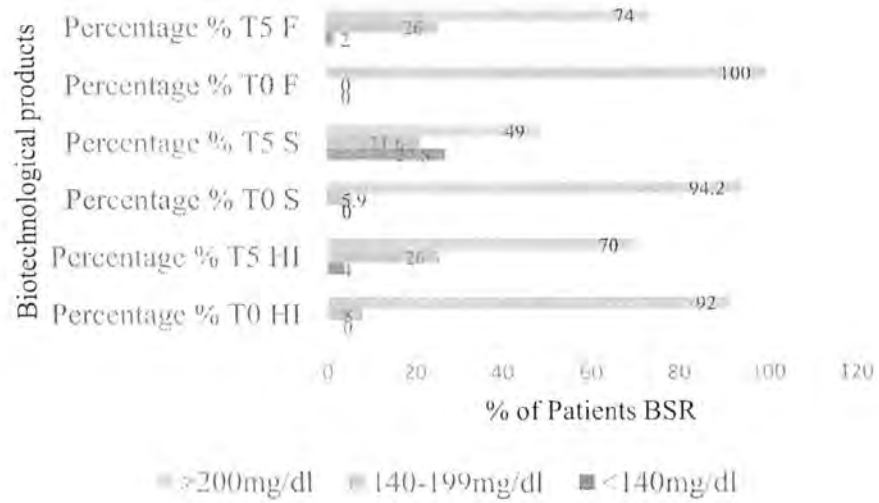
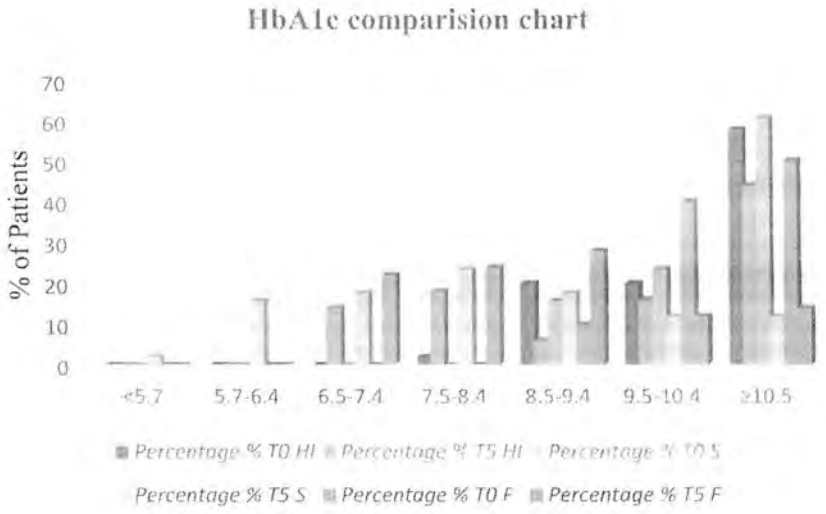


Figure 3.4.3: Figure show the comparative analysis of HbA1c in different patient using different biotechnological products



Chapter 4

Discussion

4.1 Discussion

This prospective study shows that traditional biotechnological and modern biotechnological medicinal products are very beneficial for management of diabetes mellitus. Clinical impact of different biotechnological medicinal products can be explained only with prospective study. Healthcare professionals currently treating most of the chronic disease by using different biotechnological medicinal products.

4.2 Human Insulin:

Human Insulin is very commonly used for management of diabetes mellitus for a long time. Before the invention of rDNA technology insulin filtered from animal blood than injected to diabetes patients for their blood glucose management. The diabetes management approach was totally changed after the invention of biotechnology. Riggs et al stated that first time Eli Lilly developed first human insulin by using E-coli as a vector using fermentation technology(Riggs, 2021). It works similarly as human body produced insulin to control body sugar levels. Bahera et al stated that NovoNordisk develop fist biosimilar human insulin by using yeast as a vector(Bahera, Prasad, & Behera, 2021). Now a days a number of biotechnological firm both national and international manufacturing human insulin.

This study shows that human insulin can be prescribed to underweight patients due to its anabolic role, 22% patients' blood sugar level shows within range after using human insulin which is mark able difference from initial values i.e., 8%.

Current study shows that 90% of the study patients had blood sugar fasting >126mg/dl at start of study while at the end of the study 76% shows blood sugar level greater than 126mg/dl. The study clearly shows that after using rDNA product blood sugar fasting can be managed. Very similar study of Retnakaran et al shows blood sugar manage by using human insulin. 92% study population shows blood sugar random greater than 200mg/dl while after the end of study 70% patients shows blood sugar random greater than 200mg/dl. These results show significant control in blood sugar level after using human insulin. HbA1c before and after study shows minor difference but body weight shows that human insulin increases the body weight of diabetic patients.

4.3 Semaglutide:

Semaglutide is modern biotechnological medicinal product. Pharmacologically it is long acting glucagon like peptide which is first time produced by NovoNordisk using

Saccharomyces cerevisiae (yeast) as a vector. It mimics the role of incretin which is naturally produced in small intestine after food intake. Natural incretin work for 1.5 -2.1 minutes after its secretion from small intestine but biotechnologically produced semaglutide work for a week.

Current study shows that doctor prescribe this biotechnological medicinal product to most of the overweight, obese and extreme obese patients. Due to its cost issue most of the businessman and housewives take this product. From Educational point of view mostly user of this product was graduate. Out of 51 enrolled patients 98% shows blood sugar fasting greater than 126mg/dl but after the use of this product this value reduced to 54%. Initially only 2% selected patients' blood sugar fasting was within therapeutic range but after using this magical product 35.3% patients' blood sugar fasting fall within range. Japanese study shows that semaglutide is very effective in management of blood sugar fasting, blood sugar random and body weight(Masaki et al., 2022).

94.2% type 2 diabetic patients show Blood sugar random >200mg/dl at start of the study which reduced to 49% after three months. Initially 5.9% patients show blood sugar random within range 140-199mg/dl which increase to 21.6%. Semaglutide shows significant control in blood sugar values. 100% patients show HbA1c >6.5% at the start of the study but at the end of study this rate reduced to 82.4%. Semaglutide shows markable reduction in body weight. Currently enrolled patients >100kg were 27.5% but at the end of study they decreased to 11.8%. Very similar study conducted in Italy by Marzullo et al which shows that semaglutide is very effective in reducing HbA1c(Marzullo et al., 2022).

4.4 Fix-Dose Combination (Degludec+Liraglutide)

Fix-dose combination is the innovation of NovoNordisk in which to different biotechnologically manufactured products were used in a fix dose combination for management of Type 2 diabetes mellitus. This combination contains ultra-long acting modern insulin (degludec) along with 24hours acting glucagon like peptide i.e., liraglutide. This innovation of biotech company provides both insulin and GLP-1 receptor agonist to the patients in a single injection with single prick.

Mostly patients using this product fall within range of 55 to 64 years of an age. From Educational point of view 46% sample size are graduate. Occupation wise data shows that 36% holding private job. 36% of the total patients were overweight. This biotechnological

product is costly and can be administer to obese diabetic patients for management of diabetes along with body weight.

Clinically data showed that blood sugar fasting before the start of this product of study population greater than 126mg/dl were 98% which reduced to 74% at the end of the study. Initially only 2% patients show blood sugar fasting within acceptable range but after three months use of this product this figure increase to 26%. Similarly, 100% patients show blood sugar random >200mg/dl but at the end of this study this value reduced to 74%. Initially 0% patients showed blood sugar random within acceptable range (140-199mg/dl but after study end this figure increase to 26%. Basu et al study shows that fix-dose combination of Degludec and Liraglutide is very good for management of diabetes and weight reduction(Basu, Shanthi, & Shirisha, 2022). Apparently HbA1c value shows no changes before and after study from reference value, but raw data shows remarkable reduction in HbA1c at the end of the study. Body weight of the patients also reduced by using this magical biotechnological product.

4.5 Comparative Analysis:

Demographically comparison shows that age wise most of the patients fall within the category of 45 to 64 years of an age. Gender wise most of the females are diabetic as per current study data. BMI wise it was observed that most of the diabetic patients are overweight. Current study data shows that blood sugar fasting is good control in those patients who are using modern biotechnological medicinal products i.e., semaglutide and fix-dose combination (degludec+ liraglutide). Similar control of diabetes depicted in singh et al study(Singh, Singh, & Misra, 2022). Whereas blood sugar random control as almost same in modern biotechnological medicinal product user and traditional rDNA user. Data shows that weekly acting semaglutide is very effective for reducing HbA1c. Modern biotechnological products not only effective for management of diabetes but also shows additional benefit in weight reduction and other diabetes related complications. Present data reveals that modern rDNA product are beneficial in overweight, obese and extreme obese diabetic patients as compared to traditional human insulin.

Conclusion

- Our data demonstrate that biotechnological medicinal products are very useful for management of type 2 diabetes in twin city of Pakistan.
- Clinical data shows that most the patient fall within age of 45 to 65 years of an age. Blood sugar fasting, HbA1c and body weight is better control by using modern biotechnological medicinal products as compare to traditional rDNA human insulin.
- Our results support and add to the existing literature of different countries which shows very similar results.
- Current study shows that traditional rDNA insulin is effective for management of diabetes but it increase the body weight which leads to insulin resistance with the passage of time. As compare to traditional rDNA insulin, modern biotechnological medicinal products are very good options for management of chronic diseases like diabetes mellitus due to additional benefits. The additional benefit of newly developed weekly acting rDNA semaglutide is weight reduction and no chances of hypoglycemia, which is major side effect of traditional human insulin.
- American Diabetes Association/International Diabetes Federation/Pakistan Endocrinology Society guidelines were followed for management of type 2 diabetes in most of the prescription of the patients.

Conflict of interest

None

Future Perspective

- This study is the first study to access the effect of different biotechnological medicinal products like; i. traditional rDNA human insulin ii. Modern rDNA glucagon like peptide (semaglutide) iii. Modern fix-dose combination (Degludec a long acting insulin + Glucagone like peptide i.e., Liraglutide) in type-2 Diabetes mellitus patients visiting general family clinics, hospitals and the specialized diabetic clinics in twin city of Pakistan (Rawalpindi & Islamabad).
- In future new innovation in route of administration, biotechnological product is under study and somewhere under approval like semaglutide weekly acting glucagon like peptide in oral dosage form is under process of approval in Pakistan. This dosage form of semaglutide is freely available in USA and many developed countries.
- Modern rDNA products are under process in patches dosage form for management of diabetes.

Study Limitation

This study had some limitations. First one was a few patients were not comfortable to injectable dosage form. Secondly, both traditional and modern biotechnological medicinal products are expensive than pharmaceuticals. Thirdly, time frame for completion of this research was a minor limiting factor. Fourth, there was a lack of properly organized data and further prospective studies are needed to explore such type of comparative study on large population.

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Department of Biotechnology QUAID-I-AZAM UNIVERSITY, ISLAMABAD

Clinical implication and comparative analysis of different biotechnological medicinal products in treatment of diabetes mellitus in Pakistan, Prospective study

PATIENT DATA RECORD SHEET

Patient weight (kg): _____ Patient height (cm): _____ BMI: _____ (Obesity)

Section One: Patient Information	
Nationality:	
Do you smoke? □Yes □No **Do you drink alcohol?** □Yes □No	
What You like to smoke □ Cigarette □Shisha □ Alcohol	
Age: _____	**Gender:** □ Male □ Female
Education: □ Illiterate □ Up to Primary level □ Secondary level (Matriculation) □ High School/College (Intermediate) □ Graduation □ Postgraduate □ Diploma □ Religious	**Working Status:** □ Jobless □ Housewife /stay at home □ Student □ Businessman □ Private Job □ Government Job □ Retired
Marital Status: □ Single □ Married □ Divorced □ Widowed	**Number of Children:** □ None □ 1 □ 2-3 □ 4-5 □ 6 and above
Are you dependent on your family for living and routine expenses □ Yes □ No	**Who bear expenses for your treatment** □ Yourself □ Family □ Employer □ Government □ Your insurance company
Household (Monthly) income of respondent (PKR) □ Dependent on family □ 30001 -45000 □ PKR below 15000 □ Above 45000 □ PKR 15000 - 30000 □ Don't want to disclose / Refused / Didn't know	

Section Two: Family History	
Does any of your family member [Parents/Brother/Sister] has following disease(s)?	
Disease	**Yes / No**
Hypertension	
Diabetes Mellitus	
Kidney Disease/ Renal Failure	
Heart Disease	
Hepatitis	
Others	
2. Family History of Diabetes mellitus	□ First-degree relatives (Parents - Siblings - Children) □ Second-degree relatives (grandparents - aunts / uncles & their children) □ No family History □ Don't Know

Section Three: Medical History

1. **Since how long you are suffering from Diabetes mellitus – II (years)** _____
2. **Late complications due to diabetes mellitus:**
   - □ Diabetic nephropathy
   - □ Diabetic neuropathy
   - □ Diabetic retinopathy
   - □ Coronary Heart Disease
   - □ Hypertension
   - □ Others: _____

Section Four: Current medication used by patient for Diabetes mellitus

- □ Oral hypoglycemic agents (OHA) **ONLY** _____ (Number of OHA): 1 2 3 4
- □ Insulin **ONLY**
- □ Insulin + Oral hypoglycemic agents.

Section Five: Current medication used by the patient

1. **Oral hypoglycemic agent(s)**

Drug (Brand and Generic Name)	**Dose**	**Frequency**

2. **Insulin / Parenteral Hypoglycemic agent(s)**

i. rDNA Human Insulin 70/30
ii. rDNA fix doze combination (Degludec+Liraglutide)
iii. rDNA Semaglutide weekly acting

3. **Medicine for other disease(s)**

Section Six: Lab Tests

Time points	**Prior to the treatment**	**Drug 1**	**Drug 2**	**Drug 3**
1.	**Initial parameters**			
	Blood Glucose: Fasting (< 126mg/dl) Random (< 180mg/dl) HbA1C (<6.5%) Episode of Hypoglycemia (<70mg/dl) Change in Body Weight			
2.	**After 15 days**			
	Blood Glucose: Fasting (< 126mg/dl) Random (< 180mg/dl) Episode of Hypoglycemia (<70mg/dl) Change in Body Weight			
3.	**After 30 days**			
	Blood Glucose: Fasting (< 126mg/dl) Random (< 180mg/dl) Episode of Hypoglycemia (<70mg/dl) Change in Body Weight			
4.	**After 60 days**			
	Blood Glucose: Fasting (< 126mg/dl) Random (< 180mg/dl) Episode of Hypoglycemia (<70mg/dl) Change in Body Weight			
5.	**After 90 days**			
	Blood Glucose: Fasting (< 126mg/dl) Random (< 180mg/dl) HbA1C (<6.5%) Episode of Hypoglycemia (<70mg/dl) Change in Body Weight			