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RESEARCH ARTICLE
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ROLE OF NEUROPEPTIDE GALANIN IN THE PATHOGENESIS OF TYPE 2 DIABETES MELLITUS

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ABSTRACT

Introduction: In this study, the characteristic milestones of type 2 diabetes mellitus were condensed as two analogical models, insulin resistance-type 2 diabetes mellitus and galanin resistance-type 2 diabetes mellitus. Galanin resistance and insulin resistance should be related to each other. Obesity and BMR along with other factors which are components of syndrome X should have a relation to both insulin resistance and galanin resistance.

Aim & Objective: Aim is to determine the accuracy of circulating galanin for early prediction of Type 2 Diabetes Mellitus. Objective is to assess the comparative serum level of galanin in patients with type-2 diabetes mellitus and healthy controls along with the correlation of serum galanin level with Insulin Resistance & the risk factors of type-2 diabetes mellitus.

Material & Method: This study was conducted in the Department of Pharmacology & Therapeutics at King George's Medical University (KGMU), Lucknow. Patients registered in OPD of the Department of Medicine, King George's Medical University, Lucknow who were diagnosed to be the cases of T2DM were screened based on selection criteria for the study. Controls were screened among healthy men and women between 18-60 years of age who have not been diagnosed with any physical or psychological illness for which they are not on any kind of regular medication. A total of 50 healthy individuals of similar ethnicity from North India were recruited as controls. 50 selected cases as well as controls were subsequently called for sample collection on the next day in a fasting state.

Result: This study shows that the level of serum galanin is significantly higher in Type-2 Diabetes Mellitus patients as compared to healthy controls in the North Indian population. The Body Mass Index, Waist-to-Hip ratio, Systolic Blood Pressure, Diastolic Blood Pressure, Fasting Blood Sugar, Total Cholesterol, Triglycerides, and LDL-C are significantly higher in type-2 diabetic mellitus

patients as compared to healthy controls. The level of HDL-C is higher in healthy controls than in Type-2 Diabetes patients. Fasting Insulin level and Insulin resistance index (HOMA-IR) was found to be significantly higher in Type-2 Diabetes patients as compared to healthy controls. Pancreatic β-cell index (HOMA-B) levels of healthy controls were found to be significantly higher than that of Type-2 Diabetic patients. A strong positive correlation of Galanin was observed with Weight, BMI, Triglyceride levels, Insulin and HOMA-IR levels. A moderate correlation of Galanin was observed with Fasting Blood Sugar and HbA1C. Galanin did not show any correlation with age and LDL levels of the study population.

Conclusion: An optimum cut-off value for serum Galanin above 19.135 pg/ml is predictive of Type-2 Diabetes Mellitus with 86.0% Sensitivity and 78.0% Specificity.

Keywords: Type-2 DM, Galanin, Insulin, Insulin resistance, Galanin resistance

I. INTRODUCTION

Diabetes is one of the first ancient diseases described in historic texts, with an Egyptian manuscript (from c. 1500 BC) mentioning "too great emptying of the urine." [1] Two types of diabetes were identified as separate conditions for the first time by the Indian physicians Sushruta and Charaka in 400–500 BC with one type being associated with young age and another type with being overweight.[1] Galen referred to the disease as “diarrhea of the urine” (diarrheaurinosa) and “the thirsty disease.” [2]

Type 2 DM is the commonest form of diabetes and it is characterized by hyperglycemia, insulin resistance, and relative deficiency of insulin. The prolonged hyperglycemia accompanying diabetes causes tissue damage, which results in degenerative complications in many organs including the kidney, heart, muscles, eye, and many other organs.

There are many neural satiety signals and peptide hormones that were initially detected in the hypothalamus but later found in many peripheral tissues like the adipose tissue and the GI tract. Many of those peptides play essential roles in body energy balance, feeding patterns, and body weight balance.[3]

The Neuropeptide Galanin

Neuropeptides are chemical messengers made of protein-like molecules (peptides) used by neurons to communicate with each other. They bind to G-protein-coupled receptors (GPCR) to modulate neural activity and other organs like the heart, muscles, and gastrointestinal tract.

Neuropeptides are synthesized from large, inactive precursor proteins (prepropeptides) which are cleaved, processed, and packaged into dense core vesicles. Neuropeptides are released by these dense core vesicles after depolarization of the cell. Expression of neuropeptides in the nervous system is diverse. They are often co-released with other neuropeptides and neurotransmitters, yielding a diversity of effects depending on the combination of release.[4]

Galanin is a biologically active neuropeptide encoded by the GAL gene,[5] that is widely expressed in the CNS (Central Nervous System), PNS (Peripheral Nervous System), and GIT (Gastro-Intestinal Tract) of humans as well as other mammals. In 1983 Prof. Victor Mutt and colleagues isolated the Galanin molecule from porcine intestine and understood its structure.[6]

Galanin has been associated with modulation/inhibition of action potentials in neurons. Galanin has been found to have significant roles in diverse biologically functions, including feeding, cognition, nociception, waking and sleep regulation, regulation of blood pressure, regulation of mood, and even as a trophic factor.[7]

There are three cloned galanin subtype receptors: GalR1, 2, 3. Both GalR1 and GalR2 are majorly distributed in the hypothalamus, paraventricular nucleus (PVN), amygdala, hippocampus, brainstem, spinal cord, peripheral nervous system, and other tissues.[8] GalR3 mainly exists in the peripheral nervous system and other tissues.[8]

Animals with galanin metabolic disorder have an increased probability of developing insulin resistance finally progressing to Type-2 diabetes mellitus.[12] During a glucose tolerance test, blood

glucose levels in healthy individuals, patients with Type-2 diabetes, and gestational diabetes cases under fasting states show a positive correlation to galanin secretion.[9, 10, 11, 12]

Glucose transporters maintain glucose homeostasis by undertaking glucose uptake into the skeletal muscle/adipose tissue. Out of 14 known glucose transporters, GLUT4 is the most important. GLUT4 is found densely in the plasma membranes of myocytes and adipocytes which results in enhanced glucose uptake and elimination. Endogenous galanin stimulates levels of glucose transporter 4 (GLUT4) and GLUT4 mRNA expression in type 2 diabetic rats.[12, 13, 14]

Recent studies[16] on type-2 diabetic rats revealed (via quantitative densitometry) that treatment with a galanin antagonist like M53 induced a significant reduction in both GLUT4 protein and GLUT4 mRNA expression levels in skeletal muscle as well as adipose tissue. Also, the ratios of GLUT4 contents in the plasma membrane to total cell membranes were lower in the group treated with galanin antagonist M35 compared with the diabetic controls.

Several studies suggest the relationship between galanin and GLUT4 protein is multifaceted and endogenous galanin not only increases GLUT4 protein concentration along with GLUT mRNA expression but also enhances the translocation of GLUT4 from the intracellular compartment to plasma membranes in the skeletal muscle and adipose tissue cells. This helps in sustaining insulin sensitivity and maintaining glucose homeostasis.[14, 15, 16, 17]

In a recent study it was reported that the coadministration of both galanin and insulin combined resulted in significantly enhanced GLUT4 protein compared to singular treatment with galanin or insulin in skeletal muscle cells of type 2 diabetic rats.[13]

Similarly, the ratios of GLUT4 immunoreaction in plasma membranes to total cell membranes of myocytes were higher in the group which got both insulin and galanin compared to the insulin or galanin group. The inference of results of these studies indicates that both galanin and insulin work cooperatively/synergistically to improve insulin sensitivity and GLUT4 translocation.

Table 3.3: The potential antidepressant and antidiabetic effects of the galanin system.

SPECIES	EXPERIMENTAL MODEL	EFFECT
1.Human	Type-2 diabetes mellitus	Inhibited rise of plasma sugar conc.
2.Human	Healthy human (GTT)	Increased plasma galanin
3.Human	Gestational diabetes mellitus	Increased plasma galanin
4.Mouse	Galanin-KO mouse	Impaired glucose disposal
5.Mouse	GalR1-KO mouse	Impaired glucose disposal
6.Mouse	Overexpressing mice	Increased metabolic rates
7.Rat	Healthy rats (i.p. M35)	Increased insulin resistance
8.Rat	Type-2 diabetic rat (i.p.M35)	Increased insulin resistance
9.Rat	Type-2 diabetic rat (i.c.v. M35)	Increased insulin resistance
10.Rat	I.c.v. galanin	Reduced circulating glucose
11.Rat	Isolated rat pancreatic islets with galanin	Inhibited glucose-stimulated insulin release

M35-A galanin receptor antagonist, KO-knockout, i.p.-Intraperitoneal,
i.c.v.-Intracerebroventricular, GTT- Glucose tolerance test

[Adapted from: Fang P, Min W, Sun Y, Guo L, Shi M, Bo P, Zhang Z. Pharmacology Biochemistry and Behaviour. 2014 May 1;120:82-7.]

In another study related to myocytes and adipocytes of type-2 diabetic rats vs healthy rats, it was observed that galanin administration enhanced glucose infusion rates in euglycemic–hyperinsulinemic clamp tests and 2-deoxy-[3H]-D-glucose contents suggesting that insulin sensitivity of test subjects got enhanced.[14, 15, 16, 17]

Interestingly, although galanin may directly[18] decrease insulin secretion from β-cell (via G(o)2 of the G(i/o) protein family) but this inhibitory effect of galanin doesn't interfere in its beneficial action on insulin sensitivity.

These roles of galanin may be blocked by the injection of M35. In a recent study compound M53 was used on galanin- or GalR1-knockout mice model [19, 20] during glucose tolerance tests and the result was reduced insulin response and insulin-dependent glucose elimination. But the homozygous galanin transgenic C57BL/6J mice model (obese phenotype)[21] showed decreased energy expenditure and a rise in body weight.These studies indicate that galanin is closely associated with the mechanism of insulin resistance and development of diabetic state.

B. Clinical Characteristics of Galanin –

a. Appetite - Injecting galanin either into the lateral ventricle or directly into the hypothalamus produces the urge to feed (particularly fats)[22]Galanin inhibits the secretion of insulin from pancreatic β-cells.[22]

b. Addiction - Galanin has been found to play a role in addiction regulation in cases of alcohol, nicotine, and opiates.[23]

c. Alzheimer's Disease – In brain tissue of advanced cases of Alzheimer's disease overgrown galanin-containing fibers have been found in the remaining healthy cholinergic neurons.[23]

d. Cognitive performance -Galanin affects cognition and has been shown to weaken learning and cognition performances.[22]

e. Depression - galanin might play a role in the regulation of depression since both of the neurotransmitters involved in depression (Noradrenaline and Serotonin), are co-expressed and modulated by galanin.[24] GAL2 receptor stimulation causes a decrease in depression-like behaviour whereas GAL1 and GAL3 receptor stimulation increase depression-like behaviour.[24] The mechanism involved in this process is an increase in glucocorticoid secretion brought by galanin via stimulation of the HPA axis.[24]

f. Epilepsy - Galanin may act as an anticonvulsant since it is capable of increasing the seizure threshold by acting as an inhibitor[22] of glutamate inside the hippocampus.

g. Pain and Neuro-protection - Galanin has been shown to attenuate[25] pain in high doses. It reduces neuropathic pain after administration in the spinal cord.[26] Galanin is also believed to be effective in reducing spinal hyperexcitability.Sensory neurons release galanin when they are damaged.Higher concentrations of galanin promote neurogenesis and are believed to be neuroprotective.[22]

h. Parental Behaviour – In a study related to the assessment of parental behaviour of male mice towards pups it was observed that galanin-expressing neurons[27] (located in the medial preoptic area of the hypothalamus in the brain) are responsible for the regulation of aggressive behaviour of the male mice towards pups.

If we summarize the influence of galanin signaling cascades on GLUT4, it is reasonable to assume that the high circulating levels of galanin observed in diabetic subjects are a compensatory response to the failure in this signaling conduction. This is a possible mechanism to account for the discrepancy between high galanin levels and low glucose handling.

Overall, galanin is an important hormone to elevate insulin sensitivity and to repress morbidity of type 2 diabetes mellitus in humans and animals. Numerous animal experiments indicated a suppressive effect of galanin on insulin release from the pancreatic islets in a dose-dependent manner.[28]But this inhibitive effect of galanin on insulin secretion does not interfere with its beneficial role in elevating the insulin sensitivity of subjects.

On the basis of above cited literature, it is clear that galanin may be a marker for the prediction of type 2 diabetes mellitus. No studies, to date, have examined the association between serum galanin level and insulin resistance in type 2 diabetes mellitus patients in the Indian population.

So, in the present study, we want to explore whether serum galanin levels associated with the risk of type 2 diabetes mellitus could potentially be useful for the prediction, prevention, and early treatment of the disease. We will investigate whether serum galanin levels in diabetics/healthy help in improving the prediction of type 2 diabetes beyond clinical characteristics.

Many clinical and animal-based studies have reported that galanin attenuates insulin resistance i.e. improves insulin sensitivity and also enhances glucose clearance from adipose tissue, heart, and skeletal muscle via glucose transporter type 4 receptors. But besides these, there are still many other predisposing risk factors accounting for type 2 diabetes mellitus which are yet to be identified and recognized. Recent pieces of evidence and studies have indicated that defects in galanin function may contribute to the development of type-2 diabetes mellitus.

In this study, the characteristic milestones of type 2 diabetes mellitus were condensed as two analogical models, insulin resistance-type 2 diabetes mellitus and galanin resistance-type 2 diabetes mellitus. Galanin resistance and insulin resistance should be related to each other. Obesity and BMR along with other factors which are components of syndrome X should have a relation to both insulin resistance and galanin resistance.

Conceptualizing the etiopathogenesis of type 2 diabetes mellitus as a disorder of galanin resistance may create a new concept to understand the depths of our knowledge of the pathogenesis of type 2 diabetes mellitus. It might eventually help us to discover novel preventive and curative interventions against type-2 diabetes mellitus.

II. MATERIAL AND METHOD

Study Design-This study was an age-sex matched prospective case-control study initiated after obtaining ethical clearance **(Reference code: 101st ECM II B-Thesis/P27)** from the institutional ethics committee of King Georges Medical University, Lucknow.

Study Site-This study was conducted in the Department of Pharmacology & Therapeutics at King George's Medical University (KGMU), Lucknow

Study Subjects- Recruitment of cases and controls and their sampling was done at the OPD (Out-Patient Department) of Medicine at King George's Medical University, Lucknow

Cases – Patients registered in OPD of the Department of Medicine, King George's Medical University, Lucknow who were diagnosed to be the cases of T2DM as per the International Diabetes Federation (IDF) criteria - 2021, were screened based on selection criteria for the study and enrolled only after obtaining full written informed consent (Annexure-I). They were subsequently called for sample collection on the next day in a fasting state. Cases were selected based on the following criteria.

Inclusion Criteria

1. Patients of age between 18-60 years
2. Newly diagnosed patients with Type-2 Diabetes Mellitus as defined by the International Diabetes Federation (IDF) guidelines 2021. (Refer to Appendix II).
3. Written informed consent.

Exclusion Criteria

1. Patient not willing to give informed consent.
2. Patients of Type I Diabetes Mellitus.
3. Patients with a history of an angina attack, heart failure, or myocardial infarction.
4. Patients with a history of cerebrovascular accidents.
5. Subjects having liver & renal disease.
6. A subject having an inflammatory disease.
7. A subject having a genetic disorder.
8. A subject having HIV disease.
9. Subjects having any type of cancer.
10. Subjects having bacterial and viral infections.

11. Patient on other medication (hypoglycemic, steroids, hypolipidemic, etc.) multidrug antihypertensive therapy.
12. Patients suffering from major medical/surgical illnesses.
13. Hypersensitivity to any of the used drugs.
14. Patient with an autoimmune disease like multiple sclerosis (MS), lupus (systemic lupus erythematosus, SLE), rheumatoid arthritis (RA), or other conditions)
15. Pregnant and lactating female

Controls-
Controls were screened among healthy men and women between 18-60 years of age who have not been diagnosed with any physical or psychological illness for which they are not on any kind of regular medication.A total of 50 healthy individuals of similar ethnicity from North India were recruited after obtaining written informed consent.

Methods:
Patients presenting in the Medicine out-patient department (OPD), KGMU, Lucknow, who were diagnosed to be the cases of type-2 diabetes mellitus, were screened on the selection criteria.
Written informed consent was taken from the patients before inclusion in the study.
Diagnosis of type-2 diabetes was confirmed as per the International Diabetes Federation (IDF) guidelines 2021. Details of the patients were recorded in the Case Record Form.
The following parameters were assessed at baseline -
1. Weight
2. Height
3. BMI
4. Waist-to-Hip Ratio
5. SBP
6. DBP
7. Fasting Plasma Glucose
8. HbA1C
9. Total Cholesterol
10. Triglycerides
11. HDL-C
12. LDL-C
13. Fasting Insulin
14. HOMA-IR
15. HOMA-B
16. Galanin

2.1. Anthropometric Measurements:
Height, weight, waist circumference, hip circumference, and blood pressure were measured as per the WHO STEP wise Approach to Surveillance (STEPS) Manual, WHO 2008. The STEPS Manual provides a complete overview, guidelines, and supporting materials for countries wishing to undertake NCD risk factor surveys using the WHO STEP wise approach to noncommunicable disease (NCD) risk factor surveillance

2.1.A. Height: Steps given below were followed to take the height of a participant.
Procedure:
1. Ask the participant to remove their:footwear&headgear
2. Ask the participant to stand on the board facing you.
3. Ask the participant to stand with:feet together, heels against the back board, &knees straight.
4. Ask the participant to look straight ahead and not tilt their head up.
5. Make sure the eyes are at the same level as the ears.

6. Move the measuring arm gently down onto the head of the participant and ask the participant to breathe in and stand tall.
7. Read the height in centimeters at the exact point.
8. Ask the participant to step away from the measuring board.
9. Record the height measurement in centimeters in the patient's record form.

2.1.B. Weight: Steps given below were followed to take the weight of a participant. A portable digital weighing scale was used.
Procedure:
1. Ask the participant to remove their footwear (shoes, slippers, sandals, etc) and socks.
2. Ask the participant to step onto the scale with one foot on each side of the scale.
3. Ask the participant to:stand still, face forward, place arms on the side andwait until asked to step off. Weight in recorded in kilograms

2.2. Sample Collection:
Patients were called after overnight fasting and five milliliters of venous blood was drawn from the antecubital vein by a standard venipuncture method and divided into three parts.
One part (1.5 ml.) was kept into a fluoride vial for fasting glucose estimation, the second part (1.5 ml) was kept in a K3 EDTA vial and the third part (2 ml.) was kept in a plain vial.
The patient's name, ID, age, sex, and date of collection were noted on each vial. Fasting plasma glucose, HbA1c, and lipid profile estimation were done on the same day of sample collection.

2.3. Separation of Serum:
Whole blood was collected in a plain vial.
Samples were kept for one hour at room temperature to allow clotting.
Centrifuged for 10 minutes at 1000 g.
Using the clean pipette technique serum was collected in an Eppendorf tube labeled with a tracking number and "serum".
Serum samples were kept at –20 °C temperature immediately.

2.4. Biochemical Estimations:
2.4A. Fasting Plasma Glucose: Plasma Glucose was determined in the morning fasting blood sample of the subject using 'SELECTRA' auto-analyzer (PRO XL) and related kit (Catalog No.: GLUS0240PS). Fasting was defined as no caloric intake for at least 8 hours. Fasting plasma glucose ≥126 mg/dL was considered as diagnostic for T2DM.

2.4B. Estimation of HbA1c: It was determined in the subject's whole blood sample collected in an EDTA vial using 'BIO-RAD' D-10^{TM} high-performance liquid chromatography (HPLC) and related kit (Catalog No. 220-0201). HbA1c ≥ 6.5 % was considered as diagnostic for T2DM.

2.4C. Serum Lipid Profile: Total cholesterol, triglyceride, and HDL was determined in the serum of the fasting blood sample using 'SELECTRA' auto-analyzer (PRO XL) and related kits.

KIT	**Catalog No.**
Cholesterol	CHOS0240PS
Triglycerides	TRGS0240PS
HDL-CHOLESTEROL	HDL0380PS

Table 4.1: Kit and catalog number

LDL was calculated by the theFriedewald equation:
[LDL-cholesterol] = [total cholesterol] − [HDL-cholesterol] − [triacylglycerol]/5

2.4D. Fasting Insulin- Forquantitative measurementof insulinin the serawe used the ELISA kit of 'Human Insulin, INS GENLISA ELISA KRISHGEN Biosystems', INDIA, Catalog No. KBH0010. The assay procedure followed was as per the instructions given in the enclosure leaflet provided along with the kit.

Assay Procedure-

Prior to assay allow the reagents to stand at room temperature. Gently mix all reagents before use.

1. Coated strips were placed into the holder.
2. 50 µLof Insulin was added to the standard well.
3. 40 µL sample was added to the respective sample wells.
4. 10 µL Biotinylated INS Antibody was pipetted into sample wells.
5. 50 µL Streptavidin- HRP conjugate was pipetted into respective sample wells and also the standard wells.
6. It was mixed well. The plate was covered with a sealer and was incubated for 60 minutes at 37^0C.
7. The plate was aspirated and washed 4 times with dilute Wash Buffer (1X) and the residual buffer was blotted by firmly tapping the plate upside down on absorbent paper. Any liquid from the bottom outside of the microtiter wells was wiped off as any residue can interfere with the reading step.
8. 50 µL substrate A was added followed by 50 µL substrate B in all the wells
9. The plate was incubated at 37ºC for 10 minutes.
10. 50 µL of Stop solution was added in all wells.
11. Absorbance at 450 nm was noted within 10- 15 minutes with a microplate after the addition of Stop solution.

2.4E. HOMA-IR and HOMA-B: These parameters were assessed indirectly from the values of fasting plasma glucose and fasting insulin by the following formulae.

HOMA-IR: fasting insulin (µIU/ml) × fasting glucose (mg/dl)/405

HOMA-B: 360 × fasting insulin (µIU/ml)/fasting glucose (mg/dl) − 63

2.5F. Galanin: Forquantitative measurementof galaninin the serawe used the ELISA kit of 'Human Galanin, GAL GENLISA ELISA KRISHGEN Biosystems', INDIA, Catalog No. KBH1332. The assay procedure followed was as per the instructions given in the enclosure leaflet provided along with the kit.

Assay procedure:

1. 50µLof the standard was added to the standard well.
2. 40 µLof the sample was added to the respective sample wells.
3. 10 µL Biotinylated GAL Antibody waspipetted to the respective sample wells.
4. 50 µL Streptavidin-HRP Conjugate was pipetted to respective sample wells and the standard wells.
5. It was mixed properly. The plate was covered with a sealer and incubated for 60 min at 37°C.
6. Plate was washed 4 times with diluted Wash Buffer (1x**)** and blot residual buffer by firmly tapping the plate upside down on absorbent paper.
7. 50 ul Substrate A was pipetted followed by 50 ul Substrate B in the wells.
8. Plate was incubated at 37 °C for 10 min.
9. 50 µL of Stop solution was pipetted in all wells.
10. The absorbance was read at 450 nm with a microplate within 10-15 min after addition of stop solution.

Sample size –
There was no published data available on the level of circulating galanin in Type-2 DM cases in India. Thus, considering it a pilot study, a sample size of 50 was allotted to both cases and controls.
Group-A: Diabetes Mellitus Type-2, n=50 (CASE GROUP)
Group-B: Healthy Volunteers, n=50 (CONTROL GROUP)

Statistical Analysis –
All the results are presented as Mean ± SD or %. Clinical and laboratory parameters of both cases and controls were compared with the unpaired Student's t-test.
The correlation between data values was compared using Spearman's Correlation Analysis. The relationship between galanin and other parameters was determined using Univariate linear regression analysis. Significant variables with P value ≤ 0.10 in the univariate regression analysis were used in multivariate linear regression by the backward selection method to determine the contribution of various variables to the serum level of galanin.

III. RESULTS

The present study was conducted in the Department of Pharmacology, King George's Medical University U.P., Lucknow to determine the accuracy of circulating galanin for early prediction of Type 2 Diabetes Mellitus and to find the correlation between serum galanin with insulin resistance and other risk factors of type 2 diabetes mellitus. Table 1 shows the distribution of study population:

SN	Group	Description	No. of cases	Percentage
1-	Cases	Type 2 diabetes mellitus patients	50	50.0
2-	Controls	Normal Healthy subjects	50	50.0
			100	100.0

Table 3.1: Groupwise Distribution of Study Population

The present study comprised of 50 (50.0%) diagnosed patients of type 2 diabetes mellitus fulfilling the inclusion criteria of the study and 50 normal healthy subjects who served as controls in the present study.

SN	Characteristics	Cases (n=50)		Controls (n=50)		Total (N=100)	
1-	Mean age ± SD (Range)	47.62 ± 9.69 (23-60)		44.10 ± 9.05 (25-60)		45.86 ± 9.49 (23-60)	
	Student t-test	't'=1.877; p=0.063					
2-	Gender	**No.**	**%**	**No.**	**%**	**No.**	**%**
	Female	19	38.0	18	36.0	37	37.0
	Male	31	62.0	32	64.0	63	63.0
	Male: Female	1.63		1.78		1.70	
	Chi-square test	χ^2=0.043; p=0.836					

Table 3.2: Comparison of Demographic Profile of Cases (Diabetic patients) and Controls (Normal Healthy subjects)

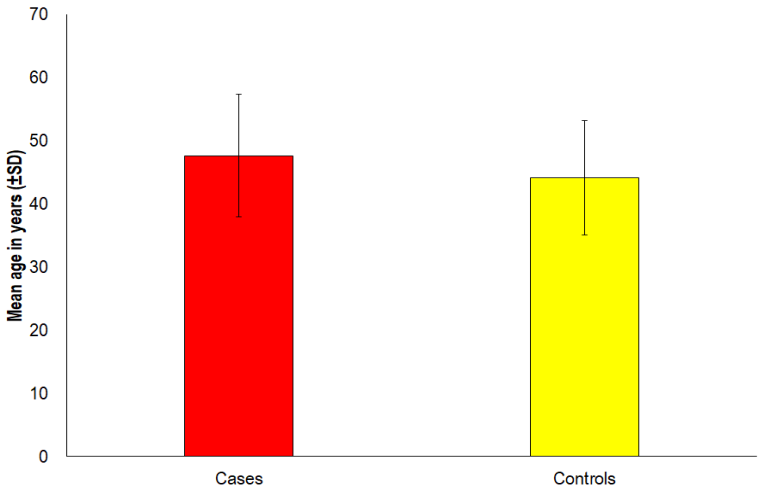


Fig 3.1: Comparison of Age of Cases and Controls

The age of overall subjects enrolled in the study ranged between 23 and 60 years, mean age was 45.86±9.49 years. Though Cases (47.62±9.69 years) were older as compared to Controls (44.10±9.05 years) yet this difference was not found to be significant statistically.

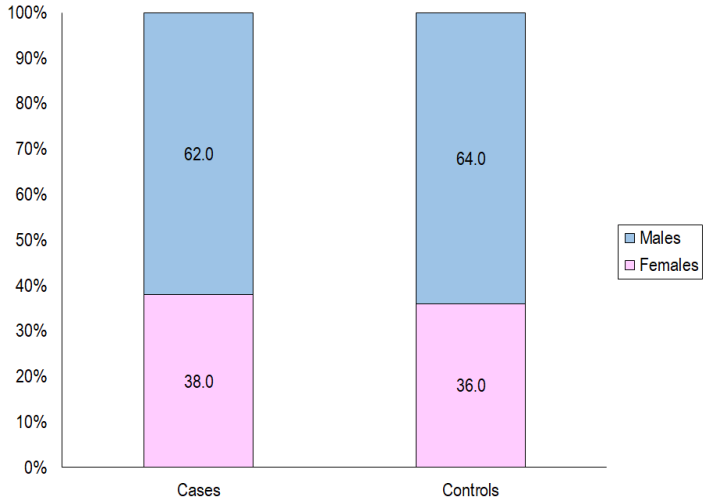


Fig 3.2: Comparison of Gender Distribution of Cases and Controls

The majority of overall (63%) as well as Cases and Controls (62% & 64%) were males, rest of the subjects were females. The proportion of females was slightly higher among Cases as compared to Controls (38% vs. 36%) but this difference was not found to be significant statistically.

SN	Parameter	Cases (n=50)		Controls (n=50)		Student t-test	
		Mean	SD	Mean	SD	't'	'p'
1-	Weight (kg)	83.98	8.56	67.76	8.18	9.688	<0.001
2-	BMI (kg/m^2)	32.32	2.85	24.22	2.24	15.793	<0.001
3-	Waist hip ratio	1.02	0.15	0.94	0.10	3.245	0.002
4-	Systolic BP (mm Hg)	142.32	11.93	131.28	8.35	5.362	<0.001
5-	Diastolic BP (mm Hg)	89.20	6.68	83.16	4.93	5.145	<0.001

Table 3.3: Between Group Comparison of Anthropometric and Hemodynamic Parameters

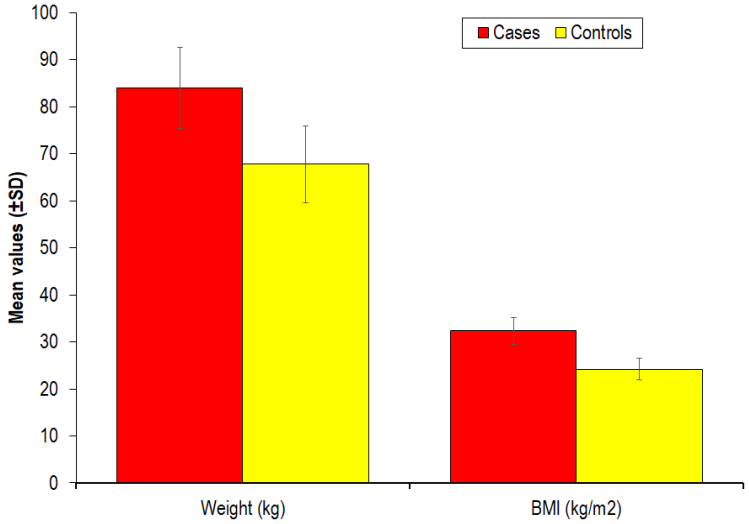


Fig 3.3: Comparison of Weight and BMI of Cases and Controls

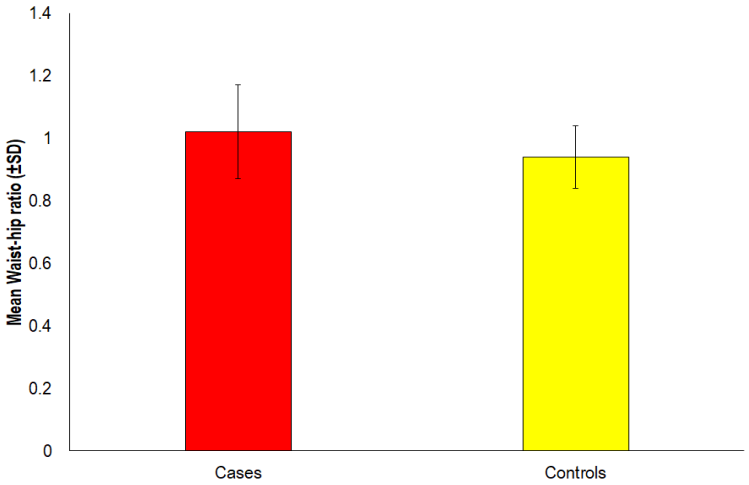


Fig 3.4: Comparison of Waist-Hip Ratio of Cases and Controls

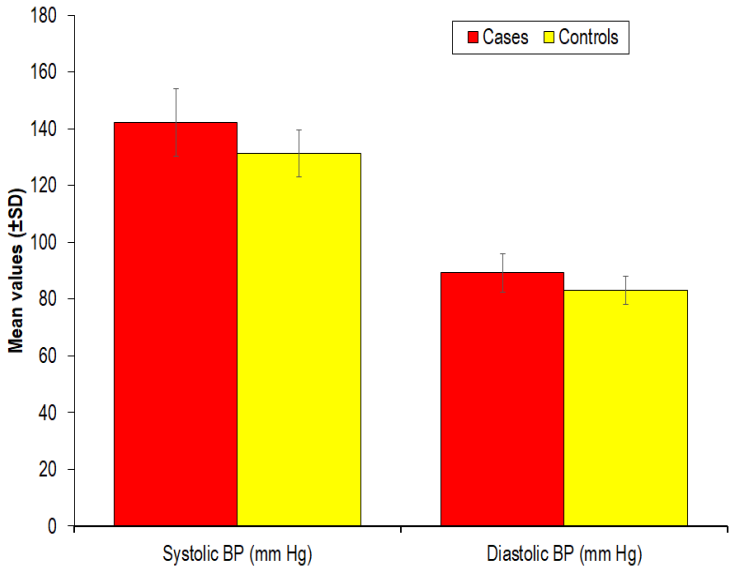


Fig 3.5: Comparison of Systolic and Diastolic BP of Cases and Controls

Type 2 diabetes mellitus cases as compared to controls had significantly higher body weight (83.98 ± 8.56 vs. 67.76 ± 8.18 kg), BMI (32.32 ± 2.85 vs. 24.22 ± 2.24 kg/m^2), Waist hip ratio (1.02 ± 0.15 vs. 0.94 ± 0.10), Systolic BP (142.32 ± 11.93 mm Hg) and Diastolic BP (89.20 ± 6.68 vs. 83.16 ± 4.93 mm Hg).

SN	Parameter	Cases (n=50)		Controls (n=50)		Student t-test	
		Mean	SD	Mean	SD	't'	'p'
1-	Blood sugar (mg/dl)	171.10	16.59	92.56	6.10	31.426	<0.001
2-	HBA_{1c}(%)	8.15	0.71	5.22	0.28	26.971	<0.001
3-	Total cholesterol (mg/dl)	186.00	26.41	165.74	14.05	4.789	<0.001
4-	Triglyceride (mg/dl)	210.34	44.38	112.66	27.26	13.262	<0.001
5-	S. HDL (mg/dl)	45.22	5.72	57.96	6.14	-10.735	<0.001
6-	S. LDL (mg/dl)	98.68	24.69	85.18	12.03	3.476	0.001

Table 3.4: Between Group Comparison of Glycemic and Lipid Parameters

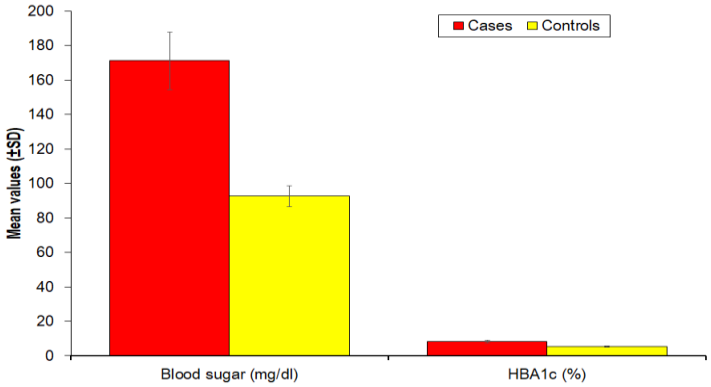


Fig 3.6: Comparison of Glycemic Parameters of Cases and Controls

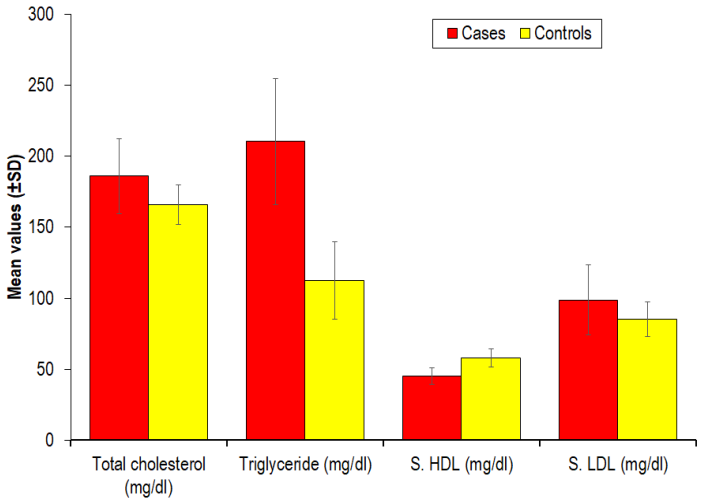


Fig 3.7: Comparison of Lipid Levels of Cases and Controls

The above table shows that type 2 diabetes mellitus Cases as compared to Controls had significantly higher glycemic levels i.e. Fasting blood sugar (171.10 ± 16.59 vs. 92.56 ± 6.10 mg/dl), HbA_{1c} (8.15 ± 0.71 vs. 5.22 ± 0.28%). On comparing the lipid levels of Cases and Controls, Controls had significantly higher HDL levels (57.96 ± 6.14 vs. 45.22 ± 5.72 mg/dl) but Cases had significantly

higher Total cholesterol (186.00 ± 26.41 vs. 165.74 ± 14.05 mg/dl), Triglyceride (210.34 ± 44.38 vs. 112.66 ± 27.26 mg/dl) and LDL (98.68 ± 24.69 vs. 85.18 ± 12.03 mg/dl).

SN	Parameter	Cases (n=50)		Controls (n=50)		Student t-test	
		Mean	SD	Mean	SD	't'	'p'
1-	Fasting insulin (µIU/ml)	23.02	5.69	14.24	4.56	8.506	<0.001
2-	HOMA-IR	9.71	2.61	3.24	1.04	16.258	<0.001
3-	HOMA-B	78.64	22.92	182.17	75.09	-9.325	<0.001
4-	Galanin	31.46	12.37	15.53	5.04	8.433	<0.001

Table 3.5: Between Group Comparison of Insulin resistance parameters and inflammatory markers

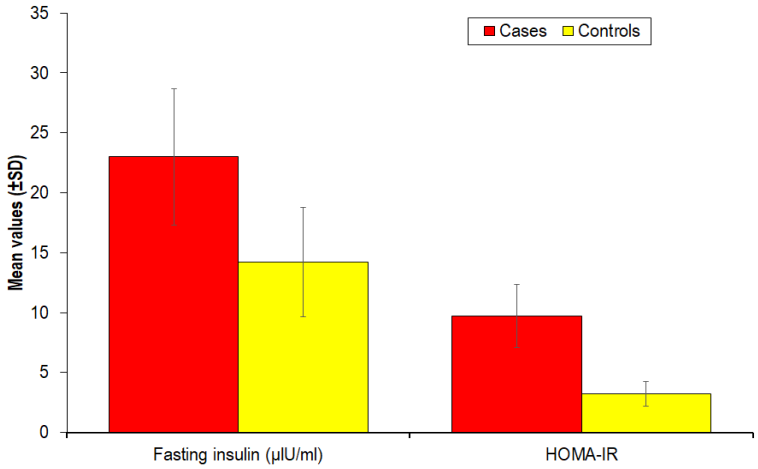


Fig 3.8: Comparison of Fasting Insulin & HOMA-IR of Cases and Controls

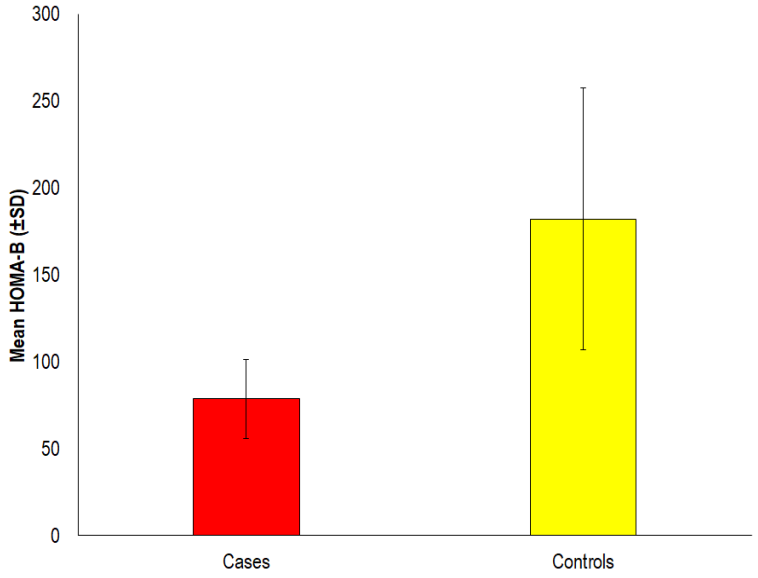


Fig 3.9: Comparison of HOMA-B levels of Cases and Controls

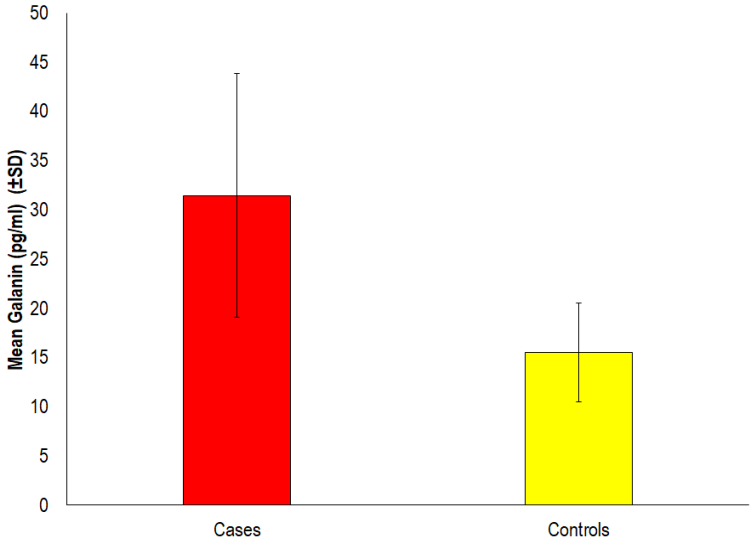


Fig 3.10: Comparison of Serum Galanin levels of Cases and Controls

Type 2 diabetes mellitus cases had significantly higher Fasting insulin (23.02 ± 5.69 vs. 14.24 ± 4.56 μIU/ml), HOMA-IR (9.71 ± 2.61). HOMA-B levels of Controls were found to be significantly higher than that of type 2 DM Cases (182.17 ± 75.09 vs. 78.64 ± 22.92).
Serum Galanin levels of type 2 DM Cases (31.46 ± 12.37 pg/ml) were found to be significantly higher than Controls (15.53 ± 5.04 pg/ml).

Parameters	Correlation coefficient ('r')	'p' value
Age	0.136	0.178
Weight	0.730	<0.001
Body mass Index	0.823	<0.001
Waist hip ratio	0.490	<0.001
Systolic BP	0.360	<0.001
Diastolic BP	0.338	0.001
Glucose	0.674	<0.001
HbA_{1C}	0.635	<0.001
Total Cholesterol	0.419	<0.001
Triglycerides	0.779	<0.001
HDL Cholesterol	-0.417	<0.001
LDL Cholesterol	0.189	0.060
Insulin	0.849	<0.001
HOMA-IR	0.881	<0.001
HOMA-B	-0.276	0.005

Table 3.6: Correlation of Galanin with different parameters (Pearson's Correlation)

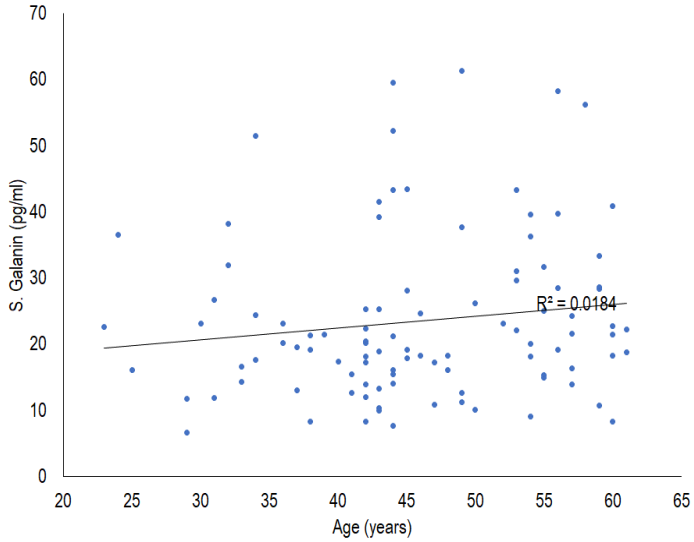


Fig 3.11: Correlation of Serum Galanin and Age of Study Population

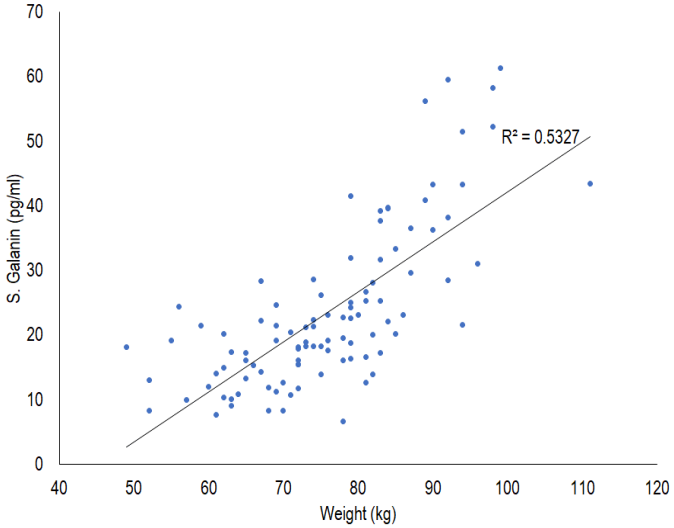


Fig 3.12: Correlation of Serum Galanin and Weight of Study Population

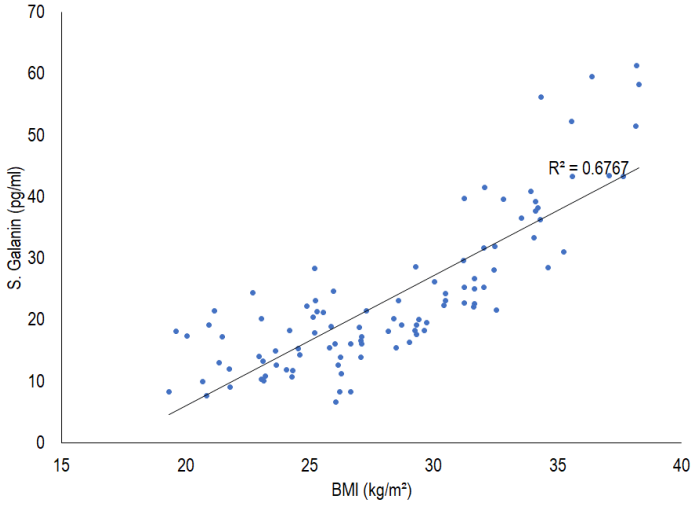


Fig 3.13: Correlation of Serum Galanin and BMI of Study Population

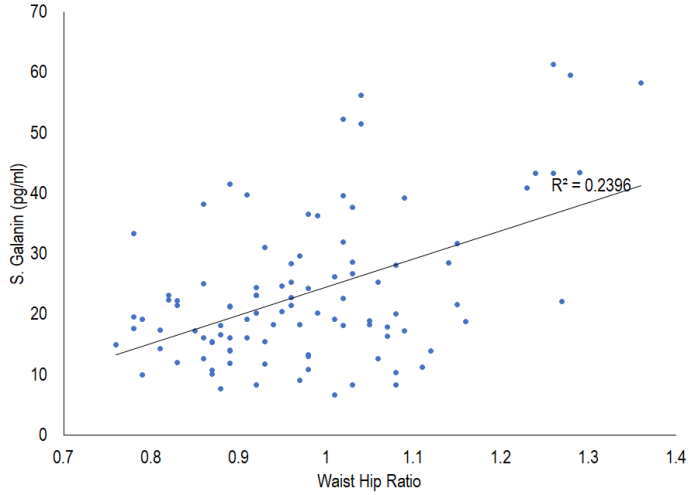


Fig 3.14: Correlation of Serum Galanin and Waist Hip Ratio of Study Population

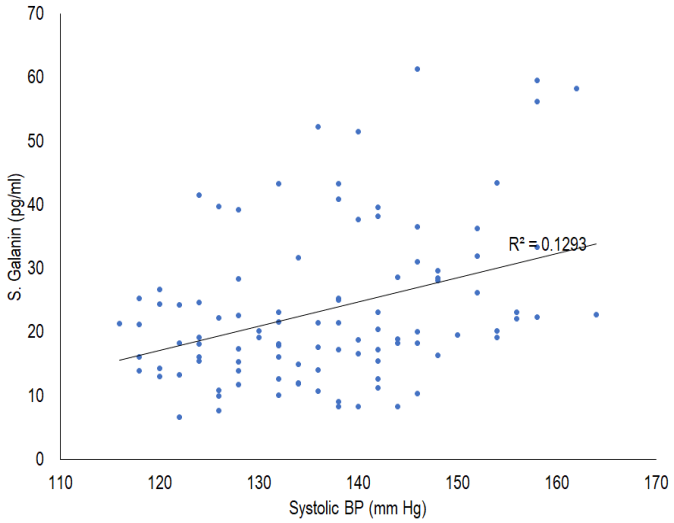


Fig 3.15: Correlation of Serum Galanin and Systolic BP of Study Population

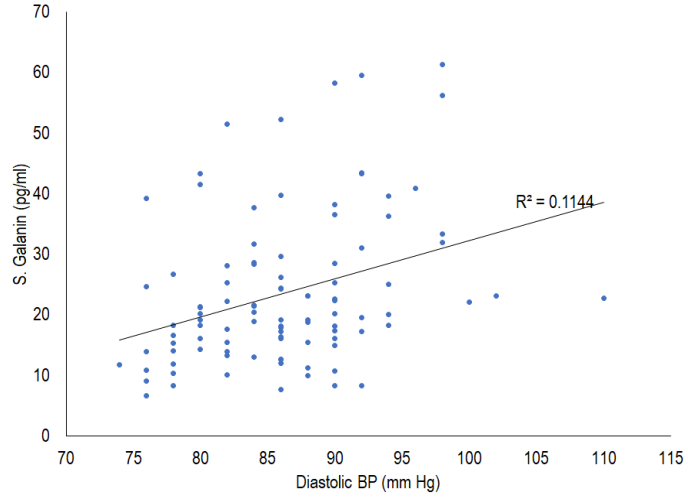


Fig 3.16: Correlation of Serum Galanin and Diastolic BP of Study Population

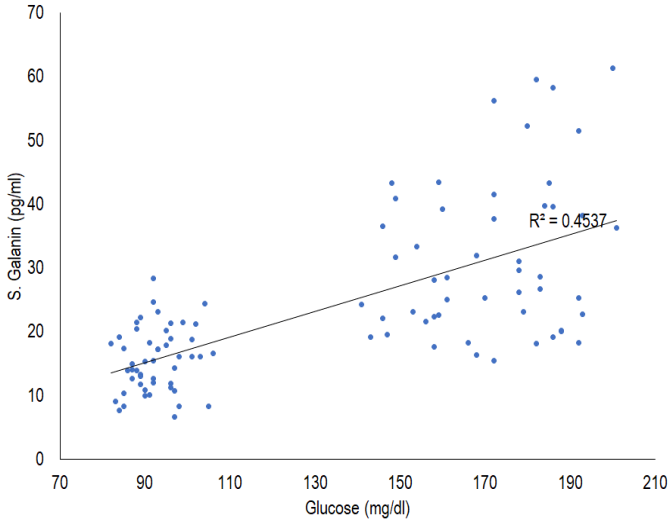


Fig 3.17: Correlation of Serum Galanin and Serum Glucose of Study Population

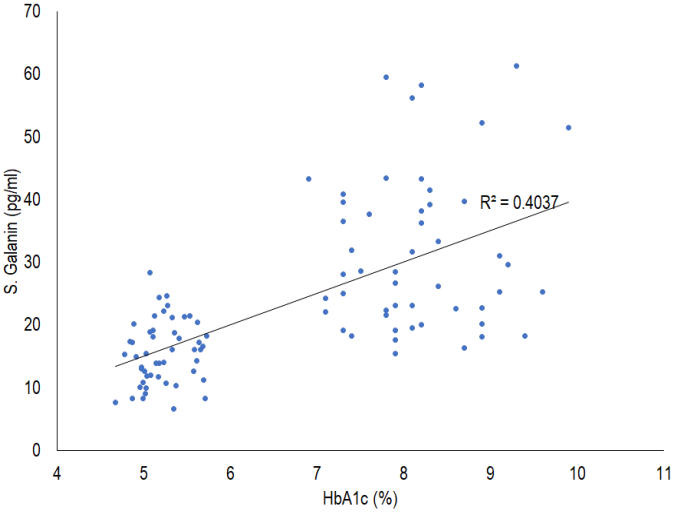


Fig 3.18: Correlation of Serum Galanin and HbA_{1c} of Study Population

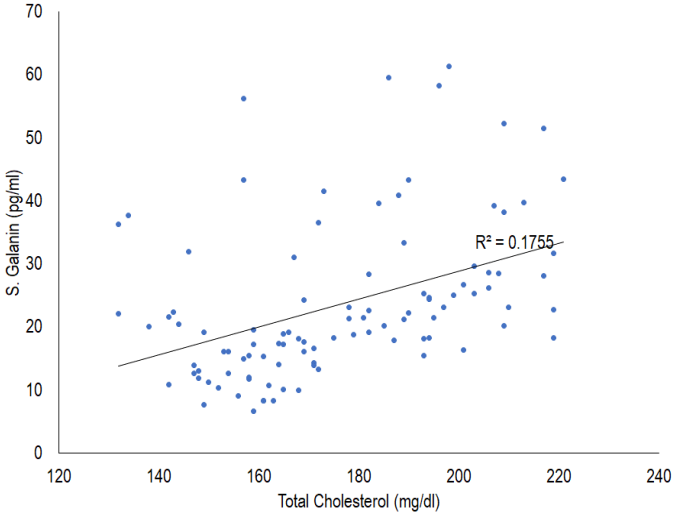


Fig 3.19: Correlation of Serum Galanin and Total Cholesterol of Study Population

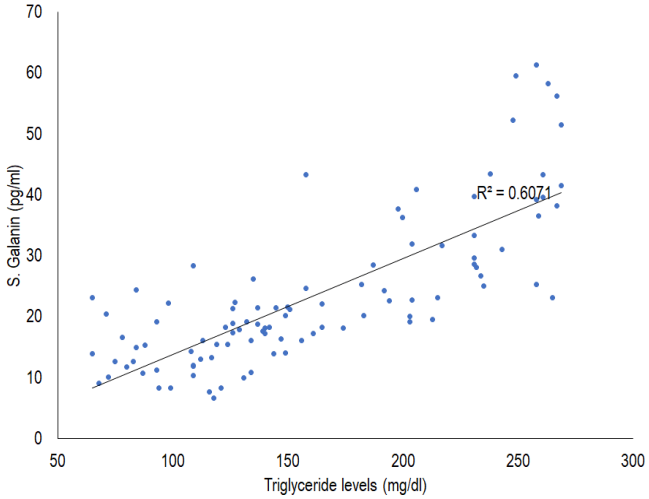


Fig 3.20: Correlation of Serum Galanin and Triglycerides of Study Population

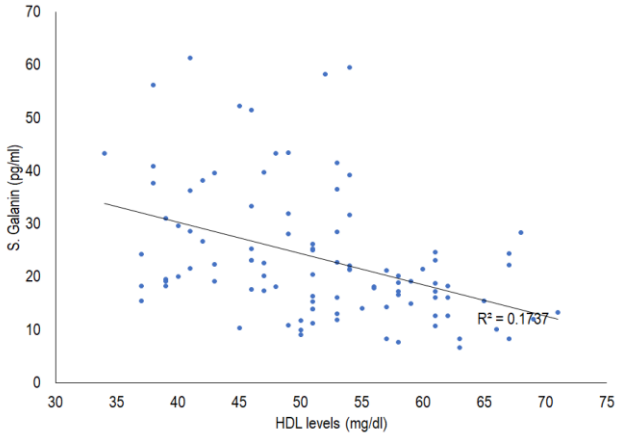


Fig 3.21: Correlation of Serum Galanin and HDL levels of Study Population

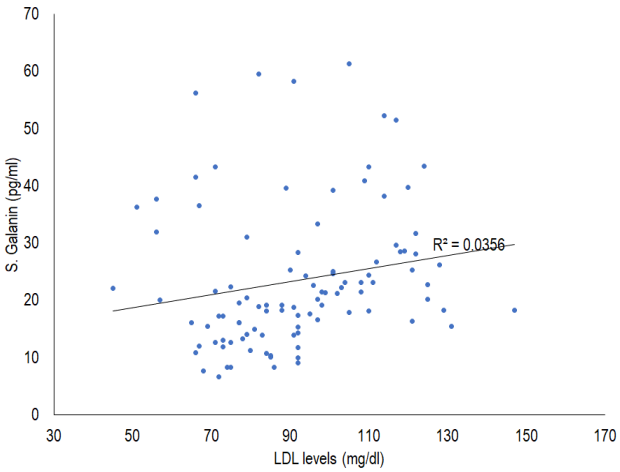


Fig 3.22: Correlation of Serum Galanin and LDL levels of Study Population

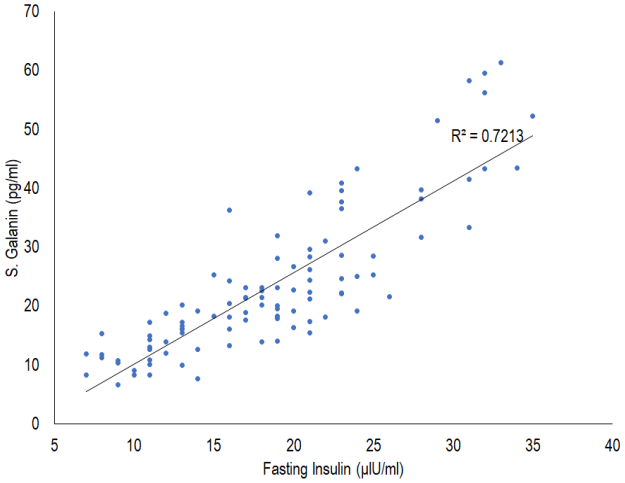


Fig 3.23: Correlation of Serum Galanin and Fasting Insulin of Study Population

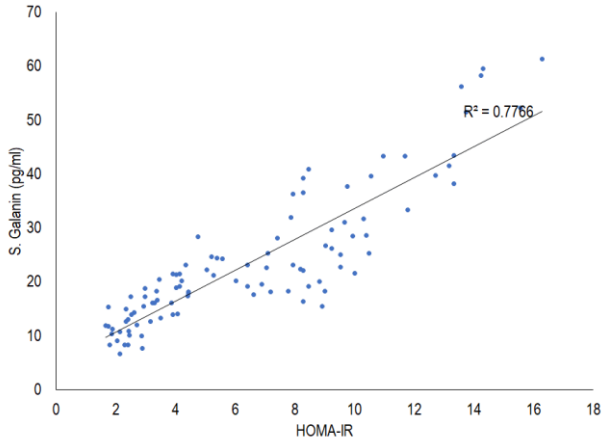


Fig 3.24: Correlation of Serum Galanin and HOMA-IR of Study Population

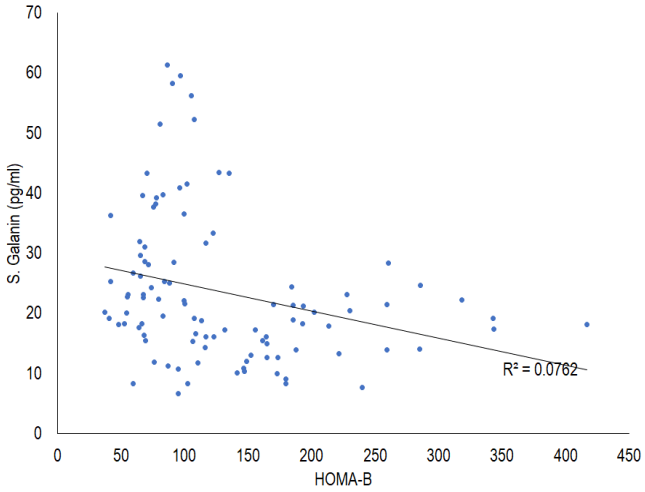


Fig 3.25: Correlation of Serum Galanin and HOMA-B of Study Population

Galanin did not show any correlation with age and LDL levels of the study population, the rest of the above parameters showed significant association with Galanin. A strong correlation of Galanin was observed with Weight, BMI, Triglyceride levels, Insulin, and HOMA-IR levels (r=0.7 to 0.9). A moderate correlation of Galanin was observed with Glucose and HbA_{1c} (r=0.5 to 0.7), the rest of the parameters showed Mild to Weak (HOMA-B).

Parameters	Unstandardized coefficient B (95% CI)	β	't'	'p'
AGE	0.176 (-0.082 to 0.435)	15.405	1.355	0.178
WEIGHT	0.773 (0.628 to 0.918)	-35.150	10.570	<0.001
BMI	2.114 (1.821 to 2.407)	-36.266	14.321	<0.001
HEIGHT	-0.829 (-1.244 to -0.414)	159.367	-3.964	<0.001
WHR	46.697 (30.021 to 63.372)	22.208	5.557	<0.001
SBP	0.381 (0.183 to 0.579)	28.632	3.815	<0.001
DBP	0.634 (0.280 to 0.988)	-31.154	3.557	0.001
Glucose	0.201 (0.157 to 0.245)	-2.991	9.022	<0.001
HbA1C	5.004 (3.785 to 6.224)	-9.958	8.146	<0.001
TC	0.221 (0.125 to 0.317)	-15.398	4.564	<0.001
TG	0.157 (0.132 to 0.182)	-1.859	12.305	<0.001
HDLC	-0.591 (-0.849 to -0.332)	53.962	-4.539	<0.001
LDLC	0.114 (-0.005 to 0.232)	13.044	1.901	0.060
Insulin	1.548 (1.355 to 1.741)	-5.350	15.925	<0.001
HOMA-IR	2.857 (2.550 to 3.164)	4.982	18.458	<0.001
HOMA-B	-0.045 (-0.076 to -0.014)	29.350	-2.844	0.005

Table 3.7: Univariate Linear Regression analysis for Serum Galanin levels

The above table shows on Univariate analysis Age and LDL levels did not show any significant association with Galanin levels. Multivariate analysis was planned to study the factors independently associated with Galanin levels.

	B (95% CI)			β	't'	'p'
	B	Upper bound	Lower bound			
(Constant)	-72.075	-191.987	47.837		-1.195	0.235
AGE	-0.042	-0.152	0.068	-0.032	-0.764	0.447
WEIGHT	-0.426	-1.187	0.335	-0.403	-1.114	0.268
BMI	2.600	0.550	4.650	1.012	2.523	0.014
Height	0.301	-0.414	1.016	0.135	0.837	0.405
WHR	1.486	-8.158	11.130	0.016	0.306	0.760
SBP	0.014	-0.099	0.128	0.014	0.252	0.802
DBP	0.022	-0.181	0.224	0.012	0.215	0.830
Glucose	-0.031	-0.151	0.089	-0.104	-0.515	0.608
HbA1C	-3.172	-5.405	-0.939	-0.403	-2.825	0.006
TC	-0.419	-3.858	3.021	-0.793	-0.242	0.809
TG	0.117	-0.569	0.802	0.579	0.338	0.736
HDLC	0.501	-2.957	3.959	0.354	0.288	0.774
LDLC	0.431	-3.002	3.864	0.715	0.250	0.803
Insulin	-1.193	-2.121	-0.264	-0.654	-2.554	0.012
HOMAIR	4.660	2.575	6.745	1.437	4.446	<0.001
HOMAB	0.064	0.027	0.101	0.394	3.461	0.001

Table 3.8: Multivariate Linear regression for Serum Galanin levels

On a linear regression model, where S. Galanin was considered to be dependent on independent variables Age, Weight, BMI, Height, WHR, Systolic & Diastolic BP, Fasting glucose, HbA_{1c}, Total cholesterol, triglycerides, HDL, LDL, Insulin, HOMA-IR, and HOMA-B. Only BMI, HbA_{1c}, Insulin, HOMA-IR, and HOMA-B showed significant association with Galanin levels. Further, another model keeping only variable significantly associated with Galanin levels was proposed.

	B (95% CI)			β	't'	'p'
	B	Upper bound	Lower bound			
(Constant)	-15.607	-30.581	-0.633		-2.069	0.041
BMI	1.583	1.082	2.084	0.616	6.277	<0.001
HbA1C	-3.540	-5.036	-2.044	-0.449	-4.698	<0.001
Insulin	-1.039	-1.828	-0.250	-0.570	-2.614	0.010
HOMA-IR	4.545	2.907	6.182	1.402	5.511	<0.001
HOMA-B	0.061	0.026	0.095	0.373	3.491	0.001

Table 3.9: Multivariate Linear regression for Serum Galanin levels after excluding non-significant parameters

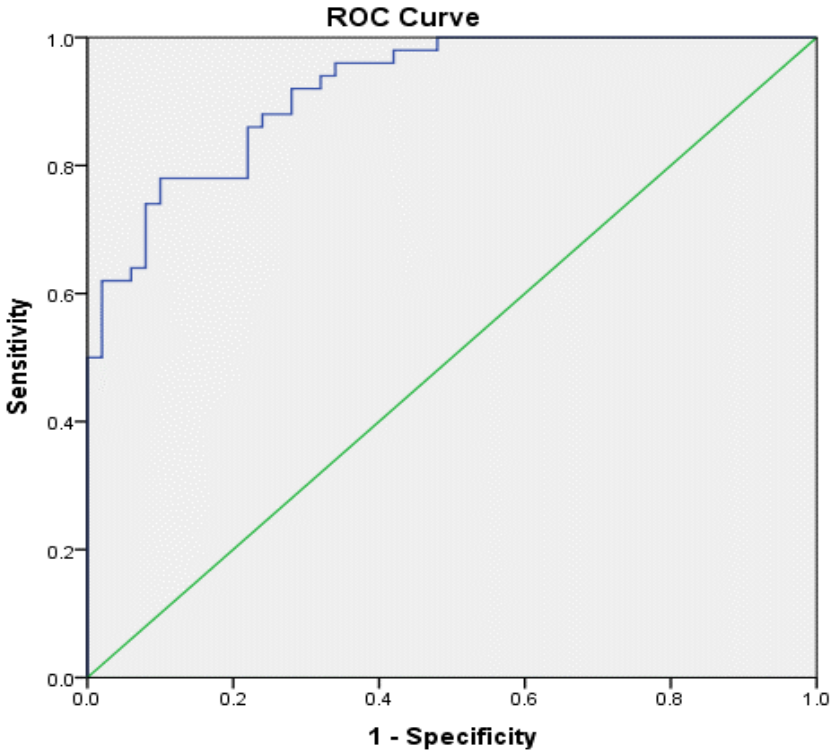
R^2 = 0.933 (p<0.001)

Serum Galanin level was found to be independently associated with BMI, HbA1c, Insulin, HOMA-IR, and HOMA-B levels. The above model was found to be 93.3% reliable (R^2=0.933).

	B (95% CI)	Wald	OR (95% CI	'p'
Galanin	0.323 (0.184-0.462)	20.923	1.381 (1.203 to 1.586)	<0.001

Table 3.10: Binary Logistic regression analysis for Galanin for the odds of diabetes

ROC Analysis for Galanin



Area under curve	SE	'p'	95% CI (Lower bound – Upper bound)	
0.920	0.025	<0.001	0.870	0.969

Serum Galanin was used to predict Type 2 diabetes mellitus. The area under the curve was 0.920 (indicating a predictive value of 92.0%). Based on the direction, higher values showing positive outcome (type 2 DM), the cut-off for S. Galanin >19.135 was found to be 86.0% Sensitive and 78.0% Specific.

This study shows that the level of serum galanin is significantly higher in Type-2 Diabetes Mellitus patients as compared to healthy controls in the North Indian population.

- The Body Mass Index, Waist-to-Hip ratio, Systolic Blood Pressure, Diastolic Blood Pressure, Fasting Blood Sugar, Total Cholesterol, Triglycerides, and LDL-C are significantly higher in type-2 diabetic mellitus patients as compared to healthy controls.
- The level of HDL-C is higher in healthy controls than in Type-2 Diabetes patients.
- Fasting Insulin level and Insulin resistance index (HOMA-IR) was found to be significantly higher in Type-2 Diabetes patients as compared to healthy controls.
- Pancreatic β-cell index (HOMA-B) levels of healthy controls were found to be significantly higher than that of Type-2 Diabetic patients.
- A strong positive correlation of Galanin was observed with Weight, BMI, Triglyceride levels, Insulin and HOMA-IR levels.
- A moderate correlation of Galanin was observed with Fasting Blood Sugar and HbA1C.
- Galanin did not show any correlation with age and LDL levels of the study population.
- Serum Galanin level was found to be independently associated with BMI, HbA1c, Insulin, HOMA-IR, and HOMA-B levels.

An optimum cut-off value for serum Galanin above 19.135 pg/ml is predictive of Type-2 Diabetes Mellitus with 86.0% Sensitivity and 78.0% Specificity.

IV. DISCUSSION

Galanin is a neuropeptide with multiple roles in the physiology of different organ systems. Although research around galanin is still nascent, the role of galaninergic system has been found in diverse functions inside body systems.

Fang et al. 2012-The galaninergic system has been shown to play key roles in many biologically diverse functions including nociception, sleep regulation, learning and memory, inflammation, depression, feeding, pituitary hormone release, thermoregulation, osmotic regulation and water intake, reproduction andmany others like different areas of metabolism in our body.[29]

Zhang et al 2017 showed that at 1 hour and 2 hours after dinner, serum galanin, insulin and glucose levels were significantly higher in patients with IGT than in controls with NGT. Additionally, the body weights of patients with IGT was higher than those of the controls. Furthermore, a negative correlation was found between galanin levels and 1-hour glucose concentrations (r=–0.580; p=0.048) in patients with IGT.

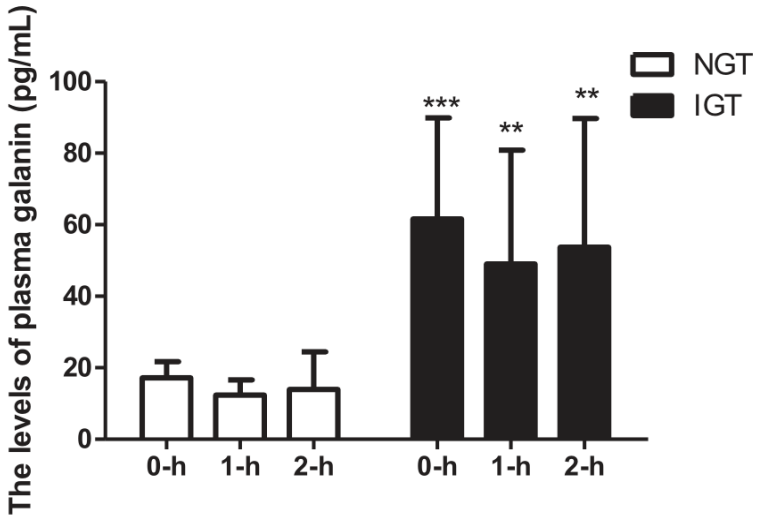


Fig 4.0: Plasma galanin level in subjects with IGT and NGT during a glucose

tolerance test.
Data are expressed as mean ± SD. ***p<0.001 IGT vs. NGT; **p<0.01 IGT vs. NGT.[87]
These results showed that serum galanin levels were higher in patients with IGT than in controls with NGT, irrespective of age, sex, and body mass index, and a negative correlation was found between serum galanin levels and 1-hour glucose levels in patients with IGT. Thus, the increased galanin levels may be taken as a potential surrogate marker to assess glucose tolerance in clinical practice.[30]
Alotibi et al 2019 found that between two study groups (MetS cases and Healthy controls) there was a significant positive correlation of serum galanin with fasting blood glucose, glycosylated hemoglobin, homeostasis model assessment-insulin resistance, and triglycerides.[31]
Sandoval-Alzate, H. F. et al. 2016 found that between two groups (obese non-diabetic and healthy lean) serum galanin levels were positively correlated with BMI, HOMA–IR, total fat, visceral fat, triglycerides, total cholesterol, and Leptin. Obese non-diabetic men had higher serum galanin levels (both in fasting state as well as oral GTT) compared with lean non-diabetic men.[32]
ACAR et al. 2018 while comparing healthy and obese children for serum galanin and leptin found that levels of both galanin and leptin were significantly higher in obese children. He found galanin levels were positively correlated with fasting glucose, insulin, HOMA-IR, and triglycerides.[33]
Fang et al. 2013 found that Galanin plays a crucial role in inhibiting insulin secretion from pancreatic β cells to prevent hyperinsulinemia, which is a characteristic of type 2 diabetes mellitus.[34]
Fang et al. 2016 reported that Galanin homeostasis is closely related to insulin resistance and both of these are ultimately regulated by blood glucose.
In this study, there were two analogical conceptual models, obesity-hyper-insulin-insulin resistance-T2DM and obesity-hyper-galanin-galanin resistance-T2DM. Insulin resistance and galanin resistance are correlative with each other. Conceptualizing the role of galanin in the etiopathogenesis of type-2 diabetes mellitus as a disorder of galanin resistance may bring a new concept about the prevention and treatment of type 2 diabetes mellitus.[35]

Anne Abot et al. 2018 showed the effect of oral galanin administration on glucose homeostasis involving the enteric nervous system. They discovered that the neuropeptide galanin is expressed in our small intestine where it reduces duodenal contraction by increasing NO (Nitric Oxide) release from neurons of the enteric nervous system. This modification of hypothalamic nitric oxide release favors glucose uptake in metabolic tissues such as skeletal muscle, adipose tissue, and liver. Galanin showed enhanced insulin sensitivity in diabetic mice after chronic oral gavage. It also showed improvements in fasting glucose, glucose tolerance, and insulin.[36]
In our experiment, we analyzed the relation between galanin and various markers related to diabetes like BMI, WHR, TC, TG, LDL-C, HDL-C, FPG, FPI, HOMA-IR, HOMA-B and HbA1C. A strong positive correlation of Galanin was observed with Weight, BMI, Triglyceride levels, Insulin, and HOMA-IR levels. Fasting insulin levels and serum galanin levels, both were significantly higher in diabetic patients as compared to healthy controls.
Type-2 diabetes mellitus is characterized by hyperglycemia, hyperinsulinemia, increased plasma galanin levels with decreased galanin receptor activities.
Now, just as the discrepancy between high insulin level and low glucose handling is called insulin resistance, the discrepancy between high galanin level and low glucose handling may be called galanin resistance.However, more research with a larger sample size is needed to consolidate the galanin-diabetes relationship. Since most of the research on galanin was done on animals, human research is in the nascent phase. Drugs acting as novel galanin receptor agonist/antagonist candidates are showing huge potential to evolve as future anti-diabetic medications.
Despite many factors resulting in cardiomyocyte injury and dysfunction in diabetes, insulin resistance is still a critical etiology of diabetic cardiomyopathy. Preclinical and clinical studies have revealed an intriguing role for galanin in the pathogenesis of insulin resistance and diabetic heart disease. A significant change in plasma galanin levels occurred in patients suffering from type 2 diabetes or cardiomyocyte injury. In turn, galanin may also distinctly mitigate hyperglycemia and

insulin resistance in diabetes as well as increase glucose metabolism and mitochondrial biogenesis in cardiac muscle[37,38].

Limitations of the study: There are a few limitations of our study. It was conducted only on the population residing in the northern part of India. It is a time-bound single-center study conducted on a small sample size. However, additional studies with larger sample sizes and with different ethnic population groups are needed to further validate our study findings.

DECLARATIONS:

Conflicts of interest: There is no any conflict of interest associated with this study

Consent to participate: There is consent to participate.

Consent for publication: There is consent for the publication of this paper.

Authors' contributions: Author equally contributed the work.

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