VOLUME - 11, ISSUE - 10, OCTOBER - 2022 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Biochemistry

EVALUATION OF INCRETIN HORMONE LEVEL IN IMPAIRED GLUCOSE REGULATION

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ABSTRACT **Background:** A metabolic state known as impaired glucose regulation exists between diabetes and healthy glucose homeostasis.Both the glucose-dependent Insulinotropic Peptide (GIP) and the glucagon-like peptide-1 (GLP-1) are naturally occurring incretin hormones that are released by the intestinal mucosa's L- and K-cells in response to gastrointestinal glucose absorption. **Aim:** By analysing the connection between the incretin hormone and the prediabetic subtypes of impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and combined IFG/IGT, it may be determined if a low incretin hormone response may be a predictor of adult-onset prediabetes. **Method & Material:** Present study is a cross-sectional study,was conducted in the Department of Biochemistry,J.L.N. Medical college,Ajmer(Raj.).186 subjects were enrolled in three groups, 62 with DM2,62 prediabetes state and 62 norml subjects of first-degree relatives of diabetic group. After clinical examination, blood samples were taken to measure fasting blood glucose, HbA1c, lipids, insulin, and GLP-1,GIP concentrations. **Result:** The average blood levels of Incretin Hormone(GLP-1&GIP) in all groups were lower than the normal range. **Conclusion:** This study found Patients with type 2 diabetes and IGT showed a more pronounced decline in the plasma levels of incretin hormones following an oral glucose load.

KEYWORDS : Incretin,Glucagone like pepdite-1(GLP-1), Impaired fasting glucose (IFG),), Impaired glucose tolerance (IGT), and combined IFG/IGT, Impaired glucose regulation,Type 2 DM,Prediabetes

INTRODUCTION

According to World Health Organization (WHO) predictions, India will have the greatest rise in diabetes cases. According to the International Diabetes Federation (IDF), India now has around 40.9 million diabetics, with that figure expected to increase to 69.9 million by the year 2025. The first faults causing fasting and post-prandial glucose dysregulation in people with impaired fasting glucose (IFG) and impaired glucose tolerance need to be clarified in order to acquire a deeper understanding of the pathophysiology of type 2 diabetes (IGT).**Impaired glucose regulation** is a metabolic state that exists between diabetes and healthy glucose homeostasis. It is recognised as a danger condition between normal glucose tolerance and type 2 diabetes mellitus. Pre-diabetes is indicated by impaired glucose tolerance and impaired fasting glucose levels. A test for oral glucose tolerance can detect IGT. IFG and IGT are both conditions that might raise the chance of developing Type 2 diabetes. [1]. The greatest risk factor for Type 2 diabetes is pre-diabetes [2]. With an annual conversion rate of 8–10%, pre-diabetes has a significant risk of evolving into diabetes. [3]. After eating, there is an increase in the creation of certain gut peptides that act on distant target sites to aid in the efficient intake and storage of energy. These particular enteroendocrine cells, which are located in the epithelium of the stomach, small intestine, and large intestine, create these peptide hormones, which are released during a fast at low baseline levels. The liver, muscles, adipose tissue, and islets of Langerhans in the pancreas all have active neural circuits that are influenced by gut hormones. [5].

When glucose is orally ingested, it elicits a much greater insulin response (two- to threefold) than if glucose is intravenously injected to give the same blood glucose level. This phenomenon is called the **incretin effect** and is due to the secretion of glucagon like peptide-1(**GLP-1**) and glucose-dependent insulinotropic peptide (**GIP**)which increases the glucose-induced insulin secretion[3].**GLP-1** is a gastrointestinal hormone secreted from the L cells of the distal part of the small intestine. It is derived from a large proglucagon (i.e., glucagon precursor) that also encodes for glucagon.**GIP** is a 42-chain amino acid peptide secreted by the lymphocyte K cells, which are located within the intestinal epithelium of the proximal duodenum and are regulated predominantly by fat consumption. Receptors for GLP-1 and GIP can be found in a number of organs, including brain, duodenum, kidneys, liver, lungs, pancreas and stomach.[6]
Numerous studies have demonstrated alterations in incretin hormone levels in IFG, IGT, and Type 2 diabetes mellitus; however, the data demonstrating the relationship between these parameters' levels and impaired glucose control are not entirely understood, necessitating more study. As a result, the current study will be conducted to measure the levels of the incretin hormone (GLP-1 & GIP) in impaired glucose regulation (IFG+IGT) and newly diagnosed type 2 diabetes mellitus, as well as to evaluate the relationship between the incretin hormone and the prediabetic state and explore whether incretine may be a predictor of prediabetes in adults. It will also evaluate the sensitivity, reliability, and high diagnostic accuracy of these markers between healthy people and those with impaired glucose regulation.

MATERIALS AND METHODS

This study is a cross-sectional study, was conducted in the Department of Biochemistry, J.L.N.Medical college and Associated group of Hospitals, Ajmer(Raj.).124 cases of prediabetes,Diabetes Mellitus attending Medical OPD of J.L.N. Hospital were enrolled in three groups,62 newly diagnosed DM2 subjects (one year or less) were put into the first group.62 with fasting plasma glucose (FPG) concentration between 100 and 126 mg/dl confirmed twice in repeated measurements or those with impaired glucose tolerance (IGT)defined by ADA criteria (Two-hour plasma glucose 140–200 mg/dL during a75 gr oral anhydrous glucose tolerance test)were considered as prediabetes group 2.For control group 62 Offspring of patients with DM2 who were more than twenty years old were invited and checked for their FPG and those who were normoglycemic (FPG <100 mg/dl)

were entered in 3rd group. This study was approved by institutional ethical committee. All the participants were informed about the aims of study and written consent were obtained from all of them.

Inclusion Criteria for study group

Age group between 20-50 year of both sex diagnosed as DM2,pre-daibetec and healthy individuals.

Exclusion Criteria for study group

- Patient with history of using oral hypoglycemic agents or insulin.
- Patient with history of medications that affect blood lipids or insulin levels, supplements and appetite altering drugs.
- Case of Heart failure
- Hepatic and Renal Failure cases
- Acute and Chronic Inflammatory dieases .

Venus Blood samples were taken after at least 12 hours of fasting in all the participants. A standard 75g glucose OGTT was conducted for each subject after an overnight fasting (longer than twelve hours). Blood samples were collected only at the 0 and 2 hours following OGTT, as most of the subjects were unwilling to accept the blood collections at 15, 30 and 60 min during their OGTTs. The blood used for the GLP-1 determinations was collected in tubes without any aprotinin, DPP-IV inhibitor or anticoagulant. After centrifugation at 4 ℃, all serum samples were stored at -80 ℃ till they were analyzed.Plasma glucose was measured by the glucose-peroxidase colorimetric enzymatic method with a sensitivity of 5 mg/dl. HbA1c Serum Cholesterol and Triglyceride and High density lipoprotein cholesterol(HDL-C) levels were measured with AU-680 (Backman) fully autoanalyzer,fasting serum Insulin level were measured with electrochemiluminescence immunoassay(ECLIA) using commertial kits (Roche, German), with sensitivity of 0.75 iu/ml (normal range:0.7-25 iu/ml). To calculate insulin resistance, HOMA-IR was used based on the formula of glucose × insulin/405 and values higher than 2.1 was considered insulin resistance[14].

A standard approach for the collection of blood samples was employed, including collection of blood samples, to get precise data on GLP-1& GIP concentrations. Place a full blood sample in a centrifuge for 20 minutes at a speed of around 1000 g for 2 hours at room temperature or overnight at 2 to 8 °C. Take the supernatant and do the test right away.

Serum GLP-1 and GIP was determined using the Elisa kit from Wuhan Fine Biotech Co., Ltd,china.These tests were done with long immunological reaction method (incubation 4 to 20 hours) to achieve maximum sensitivity of 0.188 ng / ml for GLP-1 and 28.15pg/ml for GIP. Intra assay and inter assay CV of the kit to measure GLP-1 was <8% and <10% respectively. Intra assay and inter assay CV of the kit to measure GIP was <8% and <10% respectively.

Statistical analysis

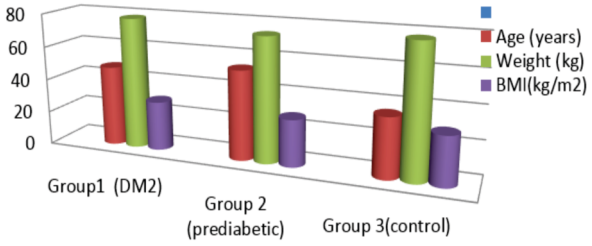
All data were analyzed with SPSS-13 version. Descriptive statistics such as mean,median and standard deviation were used to describe the statistics. ANOVA was used to compare the groups for quantitative and chi-square test was used for qualitative variables. To assess the relationship between the variables simple and multivariate regression analysis were used.

RESULTS

186 eligible patients were assigned in 3 groups as follows: 62 subjects with diabetes mellitus type 2 in 1st group, 62 patients with IFG or IGT in 2nd group, and 62 participants with normoglycemic status in 3rd group.The mean age of the first group was 48.7 ± 12.6 years and in the second group was 52.6 ±13.2 years. Although no significant difference was found between diabetic and prediabetic patients for their age, normoglycemic subjects in the third group were significantly younger(31.4 ± 10.3, p < 0.0001). There were no significant differences within three groups by sex.Clinical and laboratory characteristics of the participants have been illustrated in Table 1.

Table -1 Anthroprometric Parameters of DM2,Prediabetic and control healthy subjects

	Group1 (DM2) [N: 62]	Group 2 (prediabetic) [N: 62]	Group 3(control) [N: 62]	P-value
Age (years)	48.7 ± 12.6	52.6 ± 13.2	31.4 ± 10.3	0.00011
Weight (kg)	78.2 ± 18.2	73.7 ± 13.2	77.3 ± 14.1	0.521
BMI(kg/m2)	29.2 ± 5.4	27.8 ± 4.5	28.8 ± 4.3	0.548



In Table 2, there was significant difference for each parameter among the groups (P<0.05). Multiple comparisons results between the groups were as follows: [1]. FPG: NGT and isolated IGT groups < isolated IFG group < IFG+IGT group < NDDM group (P<0.005); [2]. 2hPG: NGT and isolated IFG groups < isolated IGT group < IFG+IGT group < NDDM group (P<0.005); [3]. Fasting insulin (FINS) concentrations: NGT group < isolated IFG, isolated IGT, IFG+IGT and NDDM groups (P<0.005); [4]. 2-hour insulin (2hINS) concentrations during the OGTT: NGT group < isolated IFG group < isolated IGT, IFG+IGT and NDDM groups (P<0.005); [5]. Total FGLP-1 levels: NGT, isolated IFG and isolated IGT groups > NDDM group (P<0.005), while isolated IGT group > IFG+IGT group > NDDM group (P<0.005); [6]. Total 2hGLP-1 levels: NGT, isolated IFG and IGT groups > IFG+IGT and NDDM groups (P<0.005); [7]. GLP-1: NGT and isolated IFG groups > IFG+IGT and NDDM groups (P<0.005), while NGT group > isolated IGT group > IFG+IGT and NDDM groups (P<0.005).

Table 2. Comparisons of plasma glucose, serum insulin, total GLP-1 concentrations (at each time point) and ΔGLP-1 in different hyperglycemic conditions.

	NGT	Isolated IFG	Isolated IGT	IFG+IGT	NDDM	Overall p value
FPG (mmol/l)	4.6± 0.4	6.0± 0.3	4.8± 0.5	6.2± 0.36	7.4± 2.3	0.005
FINS (mU/l)	7.1± 2.9	9.0± 4.33	9.1± 4.2	11.0± 6.2	11.2± 9.4	0.005
Total FGLP-1(ng/ml)	23.9± 11.2	23.1± 11.7	26.4± 8.1	20.1± 11.3	10.6± 8.1	0.005
2hPG (mmol/l)	5.4± 1.3	5.8± 1.3	8.4± 0.8	9.2± 0.9	14.9± 4.3	0.005
2hINS(mU/l)	32.6± 26.2	44.5±38.1	72.1±40.1	68.8±45.2	69.42 ±60.3	0.005
Total 2hGLP-1(ng/ml)	35.3± 15.0	34.1±15.3	35.6±13.2	22.4±16.2	16.49 ±14.1	0.005
ΔGLP-1(ng/ml)	15.8± 10.0	10.2±9.2	9.7±10.5	2.7±6.1	4.0±6.4	0.005

Although the total FGLP-1 levels were not significantly different among NGT, isolated IFG and IGT groups, they reduced obviously in the IFG+IGT and NDDM groups (P<0.005), especially in the NDDM group. After the 75g

glucose load, the total 2hGLP-1 concentrations were increased in all groups. No statistical significance was found among the NGT, iso-lated IFG and IGT groups (P>0.005).

Lab.variable	Mean ±SD median (group 1 DM2, n:62)	Mean ±SD median (group 2 PDM, n:62)	Mean ± SD median (group 3 control, n:62)	P - value
GLP-1 (ng/ml)	4.1 ± 1.2 3.9	10.2 ± 3.4 10.3	15.8 ± 46.1 15.9	0.1
GIP (ng/ml)	1391 ± 46.1 1362	1135 ± 46.1 1177	1989 ± 46.1 1978	0.05
Tota Cholestero l (mg/dl)	186.3 ±31.4 198	161.6 ± 45.6 166	138.6 ±20.1 133	0.1
TG (mg/ dl)	215.9 ±91.7 222	153.4 ± 82 153.5	86 ± 44.5 86	<0.000 1
HDL (mg/ dl)	35.2 ± 6.4 32	40.1 ± 7.9 41	47.72 ± 3.9 47.5	<0.000 1
LDL (mg/ dl)	141.4 ± 35.5 144	94.2 ±38.7 97.5	71.6 ± 17.2 77	0.2

However, the 2hGLP-1 levels in the IFG+IGT and NDDM groups were lower than that in the previous three groups (P<0.005). There was no significant disparity of ΔGLP-1 between the NGT and isolated IFG groups (P>0.005). But compared to them, the GLP-1 responses to the OGTT decreased significantly in the isolated IGT, IFG+IGT and NDDM groups (P<0.005). Moreover, there was no significant difference for GLP-1 in the IFG+IGT and NDDM groups (P>0.005), however, it was manifestly lower than that in isolated IGT group (P<0.005).

DISCUSSION

Prediabetic individuals (IFG and IGT) have a higher risk of type 2 diabetes [7,8,9]. Based on fasting and two-hour glucose levels, the classifications of IFG and IGT describe categories of glucose tolerance that may have various etiologies, metabolic profiles, and prognostic significance. The incretin hormones glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) account for a substantial part of the insulin secreted after ingestion of glucose (4, 5, 6). It has been shown that the incretin effect is impaired in type 2 diabetes and obesity (5, 7, 8)

Zhang et al. [29] found a reduced fasting GLP-1 when comparing IFG/IGT with i-IGT.When comparing IFG/IGT with both NGT, i-IFG and i-IGT, they found a reduced 2-hour GLP-1 and a reduced ΔGLP-1.Potential Mechanisms for the Eventually Reduced GLP-1 Response in Prediabetes. Rask et al. [36] have found a reduced GLP-1 secretion in response to a mixed meal in nondiabetic men with insulin resistance. This suggests an association between insulin resistance and GLP-1 secretion.

It is thought that incretin effect determines about 50–70% of the postprandial insulin response. There is evidence that incretins' action is disturbed in type 2 diabetes mellitus (T2DM) – a condition which is levels and a reduced response towards both incretins– GLP-1 and GIP.(10).According to Toft-Nielsen et al. (2001), individuals with type 2 diabetes mellitus did not have substantially lower fasting GLP-1 levels than those in the normal glucose tolerance (NGT) group.

The area under the curve (AUC), the postprandial GLP-1 levels, and the GLP-1 increments were all considerably lower in the 4-hour mixed meal tolerance tests as compared to the NGT group. And those levels were between the two groups for persons with impaired glucose tolerance (IGT) (the NGT and T2DM groups).

Laakso M et al. (2008) discovered that all three groups with impaired glucose tolerance saw decreased secretion of GLP-1 but not GIP. GLP-1 levels were reduced in both IFG and IGT participants, and since these states have different early insulin responses to glucose, it can be inferred that this abnormality is unlikely to be the cause of the glucose intolerance. However, reduced incretin effect and reduced GLP-1 levels have previously been reported in type 2 diabetic participants as well as in those with IGT.

According to Fang Zhang et al. (2012), those with IFG+IGT and NDDM exhibited considerable GLP-1 secretion impairment and were experiencing more severe hyperglycemia than people with pure IFG or IGT. Additionally, isolated IGT participants had lower levels of 2hGLP-1 (after oral glucose stimulation), and those levels were considerably lower in those with IFG+IGT and NDDM. Additionally, while comparing IFG/IGT with i-IGT, a lower fasting GLP-1 was discovered. They discovered a reduced 2-hour GLP-1 and a reduced GLP-1 when comparing IFG/IGT with both NGT, i-IFG, and i-IGT.

D.Nathanson et al. (2010) interpret that impaired GLP-1 secretion is associated with IGT and Type-2 diabetes mellitus. **Agus Lastya et al. (2014)** shows that Both FGLP-1 and 1hGLP-1 levels were lower in subjects with Type-2 diabetes mellitus than in subjects with NGT. Low level of GLP-1 was an important risk factor of Type-2 diabetes mellitus. **A.K. Singh et al. (2015)**suggests that the Incretin(mainly GLP-1) level progressively decreases with increasing duration of dysglycemia, meta-analysis of various studies suggests no significant changes in GLP-1 level. Nevertheless, the final conclusion can only be derived from prospective or longitudinal studies, Incretin activity, which stimulates the absorption of glucose by muscle tissue and the liver while concomitantly lowering glucagon release by the islet cells, leads in reduced endogenous synthesis of glucose from hepatic sources. Together, GLP-1 and GIP have been demonstrated to fully explain the incretin effect and are believed to be physiologically active substances that contribute to the glucose-dependent release of insulin. It is widely believed that T2D patients and, to a lesser extent, those with prediabetes have insufficient Icretin secretion .

CONCLUSION

This study found Patients with type 2 diabetes and IGT showed a more pronounced decline in the plasma levels of incretin hormones following an oral glucose load.Longitudinal prospective studies are needed to assess whether a reduced Incretin response is a predictor of diabetes,prediabetes.

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