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ORIGINAL ARTICLE

Update on Early Detection and Management of Diabetes: Comparison of Fasting Serum Glucagon among Diabetics and Non-Diabetics

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Abstract

Background: Diabetes is a well- known disease that is spreading globally and causing many other complications like nephropathy, eye disorders, foot disease and other heart related diseases. It also has a high mortality rate. Glucagon is the principal hyperglycemic hormone, and acts as a counterbalancing hormone to insulin. Its level increases in early stages of diabetes, however with the progression of disease it gradually decreases in diabetic patients.

Aim: The aim of this study was to compare the glucagon levels in diabetics and non-diabetics.

Materials and Methods: This was a cross-sectional study in which the data and blood samples of diabetics and non-diabetics with or without family history of diabetes were collected during December 2022 to May 2023. Then by SDS Page analysis glucagon levels were estimated in the blood samples of participants and results were analyzed by SPSS statistical version 25.0.

Results: In Group A, 25 (83.33%) had high levels of fasting serum glucagon while in group B only 18 (60%) had raised serum glucagon levels with the p value of 0.001. High glucagon level was observed in non-diabetics with family history of diabetes, as maximum number of participants 60% (n=18) with high level glucagon fall in this group. The p value is 0.001 shows the association between glucagon and family history of nondiabetic is significant.

Conclusion: Fasting serum glucagon could be an early sign of insulin resistance in non-diabetics and diabetics. Therefore, in clinical management of glucagon level must be accounted and managed accordingly.

Key Words: *Glucagon; Diabetes; Family history; Non-diabetics*

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Introduction

Diabetes is comprised of a group of multifactorial, genetically heterogeneous metabolic disorders all of which result in glucose imbalance secondary to absolute or relative insulin deficiency or both [1]. In diabetics, blood glucose levels remain elevated either due to lack of insulin production or due to impaired insulin uptake and metabolism. Global burden of diabetes in increasing day by day as number has been doubled in last two decades [2] affecting more young adults and middle-aged population. Prediabetes is condition where serum glucose levels lies above normal range but below the diabetic threshold and every year up to 10 percent prediabetics are being diabetics [3].

As per reports of World Health Organization (WHO), 19.9 million people in Pakistan (10 percent of population) is diabetic, out of them 15.4 million is diagnosed while rest is still undiagnosed (Switzerland and Organization 2016) [4]. Currently, Pakistan is seventh largest country with diabetes, and would be fourth largest nation by 2030. Diabetes not only impairs glycemic control and if affects the whole physiological functions including immune system, periodontal disease, retinopathy, nephropathy, somatic and autonomic neuropathy, cardiovascular diseases and vasculopathy.

Frequent urination, excessive thirst, intense hunger, unexpected weight loss, increased fatigue, irritation, and hazy vision are among the seven symptoms of diabetes listed by the American Diabetes Association (ADA) [5]. The most preferred test for diabetes is fasting plasma glucose (FPG). Normal persons that have FPG ≥100 mg/dl but <126 mg/dl are referred as Impaired fasting glycaemia (IFG) or Impaired glucose tolerance (IGT), and are called pre-diabetics showing risk level in progressing diabetes [6].

Glucagon is a hormone released by alpha cells in the pancreatic islet of Langerhans that regulates glucose distribution [7]. Through relative equality of glucose input and outflow, bi-hormonal control of glucoregulation via a push-pull mechanism keeps the glucose concentration of extracellular fluid within limited limits regardless of glucose flux rates. Diabetes mellitus occur not because of only partial or complete deficiency of insulin but also due to glucagon imbalance. Both glucagon and insulin play a part in glucose homeostasis, being reciprocally released in response to glycemic oscillations; insulin dominate in the fed state, promoting glucose uptake by its target organs whereas glucagon move hepatic glucose in the fasting state to ensure the maintain the normal levels of glucose [8].

The protective mechanisms against hypoglycemia include suppression of insulin secretion and rise of counterregulatory hormones, especially glucagon. Glucagon levels have gotten just insufficient consideration; however, the accessible data recommends an improved reaction. Glucagon fundamentally follows up on the liver to start glycogenolysis and gluconeogenesis, bringing about a quick expansion in an endogenous creation of glucose [9]. With longer excitement, glucagon activity at the liver outcomes in a glucose-sparing activation of free unsaturated fat oxidation and the development of ketones. It is clear that glucagon secretion plays an important role in the pathogenesis of type 2 diabetes (T2DM), which is characterized by absolute or relative hyperglucagonemia in the fasting state as well as postprandially. The significant degrees of glucagon have been displayed to contribute

critically to diabetic hyperglycemia [10]. This present work is warranted to assess levels of glucagon in diabetics and compare them with non-diabetics.

Methodology

This was an experimental cross-sectional study was carried out at department of medicine Central Park Teaching Hospital in collaboration with Diabetes Institute Pakistan (DIP) from December 2022 to May 2023. Non-random convenient sampling technique was employed to recruit the study participants in which participants with the age range of 18 to 50 years of age were recruited and were as; 30 diabetics with and without family history (Group A; diabetics, n=30) and 30 non-diabetic participants with family history of diabetes were enrolled (Group B; non-diabetics, n=30).

Diabetic patients and family members of diabetic patient (Non-diabetics) were recruited from Medical OPD of medicine department and from DIP. Family history was defined as a patient having diabetes in first degree relative (parents and siblings). Those patients who had type 1 diabetes and diabetic complications including fundal changes and vasculopathies were excluded from the study.

After the approval of Institutional Review Board (IRB), ethical letter from Central Park Medical College and Teaching Hospital was obtained and individuals fulfilling the inclusion criteria were recruited for this study. They were explained the purpose and detailed methodology of this study, those who agreed to participate were enrolled and signed the consent form in Urdu language. After this some medical history was recorded from them on a study proforma and they were requested to donate their 3ml blood for assessment of fasting serum glucagon levels.

Collection and storage of sample

Participants were requested to donate 3 ml of venous blood that was withdrawn under aseptic conditions by trained phlebotomist at the time of enrollment. Serum was separated by centrifuging the blood samples at 4000 rpm for 5 minutes and then stored at -80^{o}C in aliquots until further use. 5ul of sample buffer was added to 5ul samples and mix by flicking the tube. The sample was centrifuged at 4,000 rpm for 1 minute at 4°C.

Statistical Analysis

The study data was analyzed using SPSS version 25.0. The outcome of study included quantitative (age, duration of diabetes, Glucagon levels) and qualitative factors (gender, BMI and family history of diabetes), which were analyzed using Chi square test to find association. The association of glucagon levels was initially assessed between non-diabetics & diabetic participants, and family history of diabetes in diabetics and non-diabetics. A p value less than 0.05 was considered as significant.

Results

A total of 60 participants were recruited for this study, among them 36.67% (n=22) were males and 63.33% (n=28) were females. Most of the participants n=42 (60%) were from the age group 18 to 40 years while 40 % were from above 40 years of age group.

The maximum number of participants n=45 (64.3%) had weight ranging from 46-75 kg. Data shows that the most of the persons n=41 (58.6%) were in range of 153-170 cm (1.53 to 1.7m) of height. The majority of respondents n=45 (64.3%) were overweight and while only

2.9% were underweight. **Group A** (Diabetics with & without family history group) shows the mean age as 53.0±12.7 years, height 1.60±0.2 meters, weight 77.1±8.8 kg and BMI as 29.7±4.0 kg/m^2. **Group B** (Non-diabetics with family history of diabetes) has the mean age of 47.0±13.5, height 5.5±0.2, weight 77.4±14.6 and BMI as 28.2±5.0.

SDS analysis of serum patients shows glucagon levels. In Figure 1 verticals lanes are labeled as 1-10. Lane 1 is ladder, lanes labeled 2-5 indicates our diabetics individuals, lanes 5-10 shows our non-diabetics group. Lane 2 and 3 and 5 shows normal concentration of glucagon whereas lane 4 shows increase concentration of glucagon. Line 6-7 shows normal bands. Lanes 1 and 2 depict normal concentration whereas in lane 10 there is thick band that shows increase concentration of glucagon (Figure 1).

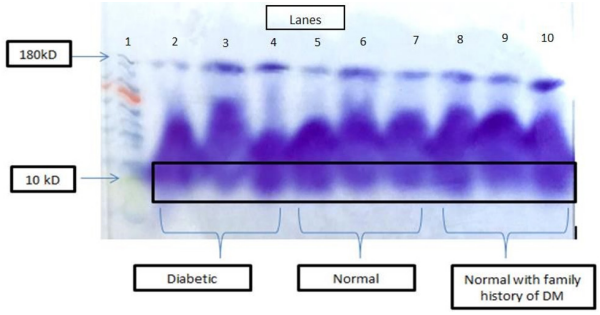

Figure 1) *SDS page analysis of study groups (Group A: Diabetics and Group B: Non-diabetics).*

Table 1 shows that Glucagon levels are measured among study groups and reported as normal and high values of glucagon. The p value was 0.001 which shows that there is association between these variables by appliance of chi-square correlation. A total of 30 participants were in Group B out of which 12 participants showed normal values and 18 showed high value of glucagon. On the other hand, 30 were in Group A in which 5 showed normal level of glucagon and 25 were those who showed high levels.

TABLE 1
Glucagon Levels Among Study Groups based on SDS Analysis

Samples	Glucagon levels	
	Normal	High
Group A (Diabetics)	5	25
Group B (Non-Diabetics)	12	18
Total	29	41

High glucagon level was observed in non-diabetics with family history of diabetes, as maximum number of participants 60% (n=18) with high level glucagon fall in this group. The p value is 0.001 shows the association between glucagon and family history of nondiabetic is significant. In group A, diabetic individuals who also have diabetes in their family, as 33.3% (n=10) participants among high level of glucagon falls in this group. The p value that is 0.38 shows no significant association of glucagon with family history among diabetic patients.

Discussion

Diabetes Mellitus is a widespread issue that is spreading throughout the world. It is not a single disease, but a combination of disorders with related symptoms, indications, and problems but distinct etiologies. Hyperglycemia due to impaired secretion of insulin and insulin secretion is stimulated together with hyperglycemia by the incretin peptides glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) [11]. These peptides are released from the intestine in response to nutrient ingestion. Patients with T1DM also have impaired -cell function, which is indicated by abnormal fasting and postprandial glucagon secretion. Recent research indicates that in T1DM patients, this abnormal glucagon output contributes more and more to hyperglycemia, insulin-induced hypoglycemia, and exercise associated hypoglycemia [12].

In this study samples were initially analyzed for physical parameters like age, weight, height, BMI, history of diabetics and non-diabetics with relation to family history of all individuals. Four groups of people were taken for this study such as, diabetic individuals with no family history in group 1, diabetics with family history in Group 2 non-diabetics with family history of diabetes were place in Group 3 and non-diabetics with no family history were in Group 4 [13]. Firstly, we studied the demographic characteristics (age, weight, height and BMI) of participants. We assessed the association of glucagon with study groups and found that there was an association present between them because the p value is 0.01. The average BMI of diabetic subjects was greater than control and shows positive significant ($p<0.01$) when statistical rules were applied, which means that higher the BMI higher the chances of having diabetes and complications [14]. Then we analyzed the association of glucagon with the duration of diabetes there is also significant association between them. After that we analyze the study group for glucagon associate with family history we find p value 0.38 and it shows that there is no association of glucagon the family history.

These results are in accordance with Ranjan et al. that worldwide presence of hypertension and diabetes mellitus exist in all age groups but it is more likely to occur in individuals with higher BMI [15]. Previously, increased BMI increases the risk of developing diabetes type 2. In our study that described the association of diabetes with family history and many other studies reveals similar conclusion with our studies. Family history risk, categories of diabetes have a significant, independent, and graded association with the prevalence of this disease. Although obesity, central fat distribution, and a family history of diabetes were significantly associated with the increased prevalence of IGT or NIDDM, they explained only a minor proportion of the variance in 2-h glucose values.

In a study done in Japan, family history of diabetes was associated with the incident risk of diabetes, and these associations were independent of other risk factors, such as obesity, insulin resistance, and lifestyle factors [16].

The results of our study shows that there is no significant association between glucagon level and family history of diabetes because glucagon levels didn't rise in normal individuals with family history and glucagon levels rise in diabetes that shows no association [17]. As there is rare data found on this type on relation of glucagon and family history of diabetes hence more information is needed in this this regards to confirm the results of our study. This study was limited due to smaller sample size, lack of glucagon quantification and other confounders including fasting serum insulin and index of HOMA-IR was not taken into account due to lack of funding therefore a new study with larger number of patients is warranted.

Conclusion

The results of our study showed that there is no significant association found in relation glucagon with family history of diabetes although fasting serum glucagon could be an early sign of insulin resistance in non-diabetics and diabetics. Therefore, in clinical management of glucagon level must be accounted and managed accordingly. This is a novel research work done in Pakistan and it gives the future prospects to other researchers to work on glucagon in association with family history of diabetes with can be a significant predictor of diabetes in the future.

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