

Hyperglucagonemia – a Potent Threat which can Worsen the Diabetes Mellitus

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Background: Diabetic ketoacidosis and hyperosmolar hyperglycemic non-ketotic coma (HHNK) are two serious acute complications of diabetes mellitus. DKA consists of the biochemical triad of hyperglycemia, ketonemia and acidemia. In DKA and HHNK dehydration and sodium depletion is seen. Lack of insulin causes hyperglycemia and also inhibits entry of potassium into the cells leading to hyperkalemia. Moreover Hyperglucagonemia also contributes to hyperglycemia and can worsen the diabetic state.

Study Design: This study was retrospective, analytical case control study. Non – probability convenient sampling technique was used.

Materials and Methods: We reviewed the hospital admissions and patients coming to OPD with type 1 & 2 diabetes mellitus as well as diabetic complications like diabetic ketoacidosis (DKA) and hyperosmolar hyperglycemic non-ketotic coma (HHNK). We compared the groups for plasma glucose, plasma osmolality, plasma glucagon, serum electrolytes and arterial blood gases (ABGs) with control group.

Results: Twelve persons were considered as control being non-diabetic with normal oral glucose tolerance. Mean plasma glucose level & mean plasma osmolality level in the patients of uncontrolled type 2 diabetes mellitus, uncontrolled type 1 diabetes mellitus, DKA and HHNK was significantly higher ($p < 0.001$) as compared with control subjects. Mean plasma glucagon level in the patients of uncontrolled type 1 diabetes mellitus and DKA was found significantly higher ($p < 0.001$) as compared with control subjects. Serum potassium level was significantly higher in patients of uncontrolled type 2 diabetes mellitus, uncontrolled type 1 diabetes mellitus, HHNK ($p < 0.001$) and DKA ($p < 0.01$) as compared with control subjects. Arterial pH was significantly lower in patients of DKA ($p < 0.001$), uncontrolled type 1 diabetes mellitus ($p < 0.05$) and HHNK ($p < 0.01$). Arterial PCO_2 was significantly lower in patients of DKA ($p < 0.05$). Plasma bicarbonate levels were found significantly lower in patients of DKA ($p < 0.001$) and HHNK ($p < 0.01$).

Discussion: The present study showed that in type 1 DM hyperglucagonemia was more marked leading to excessive ketone bodies production and resulting in DKA. The ketoacids formed during DKA are strong acids that fully dissociate at physiological pH. So ketonuria lead to excretion of positively charged cations (Na^+ , K^+ , NH_4^+). Moreover, the hydrogen ions were titrated by plasma bicarbonate ions, resulting in metabolic acidosis and retention of anions lead to increase in the plasma anion gap in DKA. The degree of hyperosmolality and hyperglycemia was more marked in patients with HHNK as compared with DKA. The osmotic effects of glycosuria resulted in impairment reabsorption of $NaCl$ and H_2O and ultimately hyponatremia. Whereas Serum potassium level was found to be significantly higher in uncontrolled type 1 diabetes mellitus, uncontrolled type 2 diabetes mellitus, DKA and HHNK. These observations were according to the results of previous studies.

Conclusions: Hyperglucagonemia causes marked hyperglycemia under conditions of relative insulin deficiency and can worsen the diabetic state like development of DKA when insulin deficiency becomes absolute as in type 1 diabetes mellitus.

Key words: Diabetes mellitus (DM), diabetic ketoacidosis (DKA), hyperosmolar hyperglycemic non-ketotic coma (HHNK), arterial blood gases (ABGs), plasma osmolality.

Diabetes mellitus, the important health problems, is classified as Type 1 Diabetes Mellitus and type 2 diabetes mellitus, malnutrition related diabetes mellitus and other types of diabetes associated with certain conditions and syndromes. People with diabetes mellitus have insulin hyposecretion and hyperglucagonemia. Glucagon is a potent stimulus of glucose production but the magnitude of its effect is dependent on insulin concentration. In patients with type 1 DM, lack of postprandial suppression of Glucagon in the presence of absolute insulin deficiency impairs glucose tolerance. Diabetes mellitus is associated with acute complications like diabetic ketoacidosis (DKA), which is life threatening if untreated and chronic complications like hyperosmolar hyperglycemic non-ketotic coma (HHNK) which is insidious in onset and progressive in course¹.

Diabetic ketoacidosis (DKA) is the hallmark of type 1 diabetes mellitus and may result due to previously undiagnosed diabetes, interruption of insulin therapy or stress of intercurrent illness. DKA is a state of uncontrolled catabolism associated with insulin deficiency and excess of its hormonal antagonist glucagon. Decreased insulin increases hepatic glucose production and decreases peripheral glucose uptake leading to hyperglycemia. Whereas increased level of glucagon leads to increase lipolysis and elevated circulatory free fatty acid (FFA) level. Elevated FFA level leads to increase formation of acetyl CoA and ultimately increase formation of ketone bodies. Accumulation of ketone bodies produces metabolic acidosis. Respiratory compensation for the acidosis leads to hyperventilation. Acidosis leads to nausea and vomiting leading to loss of fluids and electrolytes and ultimately dehydration.²

The second acute hyperglycemic complication of DM is hyperosmolar hyperglycemic non-ketotic coma (HHNK). HHNK was described for the first time by Dreschfeld in 1886³. In non-ketotic hyperosmolar diabetic (HHNK) state, a metabolic emergency, hyperglycemia develops without significant ketosis. The degree of insulin deficiency is less severe and sufficient to inhibit hepatic ketogenesis, whereas glucose production is unrestrained, in contrast to DKA, HHNK is characterized by severe hyperglycemia (blood glucose > 33.3 mmol/L), absence of ketosis and hyperosmolality (>350 mOsm/kg water)⁴. Dehydration and altered sensorium are common features between DKA and HHNK. The partial or relative deficiency of insulin causes hyperglycemia and at the same time residual insulin secreted in these patients prevents ketoacidosis by decreasing glucagon levels. Hyperglucagonemia is the hallmark of both type 1 diabetes mellitus and type 2 diabetes mellitus and apart from its hyperglycemic effect it has lipolytic activity.⁵

Aims and Objectives

Main objective of the present study was to find out the plasma glucagon level in various grades of severity of diabetes mellitus and to find out the relationship between the degree of hyperglucagonemia and extent of metabolic derangements seen in type hyperosmolar hyperglycemic non-ketotic coma.

Patients and Methods

This study was retrospective, analytical case control study. Non – probability convenient sampling technique was used. This study was conducted at Military Hospital, Rawalpindi with 50 cases, from August 2003 to September 2004. Patients were divided into five groups. Control (n=20), type 1 diabetes mellitus (n=8), type 2 diabetes mellitus (n=12), diabetic ketoacidosis (n=7), hyperosmolar hyperglycemic non-ketotic coma (n=3). Control group comprised of normal healthy non-diabetic subjects. Diagnosis of type 1 and type 2 diabetes mellitus was established according to WHO

diagnostic criteria. Those patients were selected who, in spite of getting appropriate treatment, were found to be hyperglycemic. Patients with DKA presented with acetone breath, ketonuria, loss of consciousness and acidosis. Patients with HHNK presented with hyperosmolality, marked hyperglycemia and altered sensorium. Standard protocol was devised for blood collection and storage. Arterial blood was taken in heparinized disposable plastic syringe. Plasma was separated from venous blood after refrigerated centrifugation at 4—8°C and stored at - 80°C temperature for estimation of plasma glucagon level. Plasma glucose was estimated by enzymatic colorimetric method using glucose Oxidase enzyme to oxidize glucose.^{6,7} Plasma glucagon level was estimated by Radioimmunoassay (RIA) technique⁸. Plasma osmolality was determined by Freezing point depression method.⁹ Serum electrolytes were estimated by Ion selective electrode method.¹⁰

Partial pressure of carbon dioxide (PCO₂) and Partial pressure of oxygen (PO₂) was determined by Ion selective electrode method¹¹. Bicarbonate ion concentration was calculated by using the measured parameters pH and PCO₂ in the Henderson — Hasselbalch equation.¹¹.

$$\text{HCO}_3^- = (\alpha \text{ PCO}_2) \text{ antilog} (\text{pH} - \text{pKa})$$

Statistical Analysis

All statistical calculations were done with computer software programme “Statistical Package for Social Sciences (SPSS)” for windows, version 10.00. Data was subsequently examined by One — Way ANOVA test. Results are expressed as mean ± s.e.m. Significance levels (p- value < 0.05) were considered statistically significant.

Results

Mean plasma glucose level in patients of uncontrolled type 2 diabetes mellitus, uncontrolled type 1 diabetes mellitus, DKA and HHNK was significantly higher (p < 0.001) as compared with control subjects (table 1) Mean plasma glucagon level in the patients of uncontrolled type I diabetes mellitus and DKA was found significantly

Table 1: Comparison of plasma osmolality, plasma glucose and plasma glucagon levels of uncontrolled type 2 diabetes mellitus, uncontrolled type 1 diabetes mellitus, diabetic ketoacidosis and hyperosmolar hyperglycemic non-ketotic diabetes with control group. The number of patients is given in parenthesis. The values are mean ± s.e.m. 1 & type 2 diabetes mellitus, diabetic ketoacidosis and

| Group | Plasma glucose (mmol/L) | Plasma osmolality (mOsmol/Kg of water) | Plasma glucagon (pg / ml) |
|---|-------------------------|--|---------------------------|
| Control | 5.4 ± 0.1 (20) | 283 ± 0.8 (20) | 59.1 ± 4.04 (20) |
| Uncontrolled type 2 diabetes mellitus | 15.7 ± 1.4*** (12) | 299 ± 1.9*** (12) | 70.83 ± 13.3 (12) |
| Uncontrolled type 1 diabetes mellitus | 15.8 ± 0.9*** (8) | 299 ± 3.4*** (8) | 89.4 ± 5.32*** (8) |
| Diabetic ketoacidosis | 18.6 ± 0.4*** (7) | 322.6 ± 3.8*** (07) | 156.1 ± 32.8*** (07) |
| Hyperosmolar hyperglycemic non-ketotic diabetes | 39.7 ± 0.3*** (3) | 355 ± 2.89A*** (03) | 70.3 ± 3.93 (03) |

***p < 0.001 as compared with normal control subjects (very highly significant).

Table 2: Comparison of serum sodium and serum potassium levels of uncontrolled type 2 diabetes mellitus, uncontrolled type 1 diabetes mellitus, diabetic ketoacidosis and hyperosmolar hyperglycemic non-ketotic diabetes with control group. The number of patients is given in parenthesis. The values are mean \pm s.e.m.

| Group | Serum sodium (mmol/L) | Serum potassium mmol/L) | pH |
|---|------------------------|-------------------------|--------------------|
| Control | 138.1 \pm 0.3 (20) | 3.90 \pm 0.02 (20) | 7.42 + 0.004 (20) |
| Uncontrolled type 2 diabetes mellitus | 138 \pm 0.3 (12) | 4.30 \pm 2.2*** (12) | 7.43 + 0.01 (12) |
| Uncontrolled type 1 diabetes mellitus | 132.9 \pm 1.4*** (8) | 4.50 \pm 0.2*** (8) | 7.38 + 0.02* (8) |
| Diabetic ketoacidosis | 136.6 \pm 0.7* (7) | 4.13 \pm 0.13** (7) | 7.34 + 0.02*** (7) |
| Hyperosmolar hyperglycemic non-ketotic diabetes | 135 \pm 0.6*** (3) | 4.60 \pm 0.3*** (3) | 7.38 + 0.02** (3) |

*p < 0.05 as compared with normal control subjects (significant).

**p < 0.01 as compared with normal control subjects (highly significant).

***p < 0.001 as compared with normal control subjects (very highly significant).

Table 3: Comparison of Plasma HCO₃, partial pressure of carbon dioxide and partial pressure of oxygen in the arterial blood of uncontrolled type 2 diabetes mellitus, Uncontrolled type 1 diabetes mellitus, diabetic ketoacidosis and hyperosmolar hyperglycemic non-ketotic diabetes with control group. The number of patients is given in parenthesis. The values are mean \pm s.e.m.

| Group | Plasma HCO ₃ (mmol/L) | PCO ₂ (mm Hg) | PO ₂ (mm Hg) |
|---|----------------------------------|--------------------------|-------------------------|
| Control | 26.7 \pm 0.2 (20) | 38.6 \pm 0.823 (20) | 84.6 \pm 2.4 (20) |
| Uncontrolled type 2 diabetes mellitus | 27.0 \pm 0.4 (12) | 38.3 \pm 3.4 (12) | 88.1 \pm 9.7 (12) |
| Uncontrolled type 1 diabetes mellitus | 26.0 \pm 2.2 (8) | 37.1 \pm 1.786 (8) | 77.6 \pm 4.1 (8) |
| Diabetic ketoacidosis | 18.4 \pm 2.5*** (7) | 25.9 \pm 10.04* (7) | 87.4 \pm 17.9 (7) |
| Hyperosmolar hyperglycemic non-ketotic diabetes | 19.0 \pm 0.3** (3) | 29.2 \pm 0.7 (3) | 85.7 \pm 3.0 (3) |

*p < 0.05 as compared with normal control subjects (significant).

**p < 0.01 as compared with normal control subjects (highly significant).

***p < 0.001 as compared with normal control subjects (very highly significant).

higher (p < 0.001) as compared with control subjects, whereas it was increased in uncontrolled type 2 diabetes mellitus and HHNK but the value was statistically non significant (table 1). Mean plasma osmolality in the patients of uncontrolled type 2 diabetes mellitus, uncontrolled type I diabetes mellitus, DKA and HHNK was significantly higher (p < 0.001) as compared with control subjects (table 1).

There was no significant statistical difference in serum sodium level in patients of uncontrolled type 2 diabetes mellitus, whereas it was lower in patients of uncontrolled type 1 diabetes mellitus, HHNK (p < 0.001) and DKA (p < 0.05) (table 2). Serum potassium level was significantly higher in patients of type 2 diabetes mellitus, type 1 diabetes mellitus, HHNK (p < 0.001) and DKA (p < 0.01) as compared with control subjects (table 2).

Arterial PCO₂ was significantly lower in patients of DKA (p < 0.05) as compared with control subjects, whereas no significant statistical difference was observed in patients of type 2 diabetes mellitus, type I diabetes mellitus and HHNK (table 3). No significant difference in PO₂ was seen among patients of uncontrolled type 2 diabetes mellitus, uncontrolled type 1 diabetes mellitus, DKA, HHNK and control subjects. Plasma bicarbonate level was found significantly lower in patients of DKA (p < 0.001) and HHNK (p < 0.01) as compared with control subjects, whereas no significant statistical difference was observed in patients of type 2 diabetes mellitus and type I diabetes mellitus.

Discussion

Hyperglycemia: The increased concentration of plasma glucose (hyperglycemia) due to increased production and

decreased peripheral utilization of glucose is the specific sign of diabetes mellitus and its hyperglycemic emergencies. Plasma glucose level was markedly high in HHNK as compared to DKA which results in more marked hyperosmolality in HHNK. The degree of hyperglycemia was more marked in patients with HHNK as compared with DKA. Similar observations had been noted previously for plasma glucose level in diabetes mellitus.¹²

Hyperglucagonemia: The present study was undertaken to examine the plasma glucagon level in various grades of severity of diabetes mellitus. The results show that in type 1 DM hyperglucagonemia was marked leading to excessive hepatic formation of ketone bodies and resulting in DKA. This observation was according to the results of previous studies.¹³ Numerous *in vitro* and some *in vivo* studies have demonstrated a potent role for glucagon in the stimulation of ketogenesis. However, some of these studies have used very high glucagon concentrations, and their physiological significance has been questioned. In a recent study a lipolytic effect of glucagon was demonstrated.¹⁴ Another human study demonstrated modest increase in ketogenesis when plasma glucagon was increased in insulin — deficient subjects. The increased activity of hormone sensitive lipase causes a breakdown of triglyceride into glycerol and FFA. Glycerol is a substrate for gluconeogenesis and FFA serve as precursors of the keto acids in DKA. In the liver FFAs are oxidized to ketone bodies, a process predominantly stimulated by raised level of glucagon in DKA.¹⁵ In addition to increased production of ketone bodies, there is evidence that clearance of ketones is decreased in patients with DKA.¹⁶ Methodological advancements have since allowed us to more accurately assess the effect of hyperglucagonemia, as in present study, although the conclusion remains similar.

Hyperosmolality: Plasma osmolality was significantly raised in patients with various degrees of severity of diabetes mellitus due to hyperglycemia, particularly in patients with DKA and HHNK. The degree of hyperosmolality was more marked in patients with HHNK as compared with DKA. As patients with severe DKA also showed hyperosmolality due to fluid losses caused by osmotic diuresis or impaired fluid intake due to nausea and vomiting. Hyperosmolality in HHNK patients was due to more prolonged osmotic diuresis and inadequate fluid intake seen in chronic debilitated elderly patients.^{17,18} These results are consistent with the previous studies carried out on diabetic patients with various degrees of severity. Serum osmolality is the most important determinant of mental status.¹⁹

Electrolyte Disturbances: Serum sodium level was significantly decreased in patients with uncontrolled type 1 diabetes mellitus, DKA and HHNK. The development of dehydration and sodium depletion in DKA and HHNK is the result of increased urinary output and electrolyte losses.²⁰ The osmotic effects of glycosuria result in impairment of NaCl and H₂O reabsorption in the proximal tubule and loop of Henle. Serum potassium level was found significantly

higher in uncontrolled type I diabetes mellitus, uncontrolled type 2 diabetes mellitus, DKA and HHNK. During DKA and HHNK, intracellular dehydration occurs due to hyperglycemia and increased plasma osmolality. This intracellular dehydration is associated with a shift of potassium out of cells into the extracellular space. Potassium shifts are further enhanced by the presence of acidosis and the breakdown of intracellular protein secondary to insulin deficiency. Moreover, insulin deficiency prevents re-entry of K⁺ into the cells. Osmotic diuresis and ketonuria leads to increase K⁺ loss in urine. This pattern of electrolyte disturbances is remarkably similar to that previously observed in people with diabetes mellitus.²¹

ACIDEMIA: There is evidence that in severe diabetes mellitus the rate of ketone bodies utilization also declines making the ketosis worse because insulin is said to increase ketone uptake in muscle. Ketosis results in fall in arterial blood pH which is more marked in diabetic ketoacidosis.

The ketoacids formed during DKA (β -Hydroxy butyric acid and acetoacetic acid) are strong acids that fully dissociate at physiological pH. So ketonuria leads to excretion of positively charged cations (Na⁺, K⁺, NH₄⁺).

The hydrogen ions are titrated by plasma bicarbonate, resulting in metabolic acidosis and retention of anions leads to increase in the plasma anion gap.¹⁴ Most of the hydrogen ions liberated from acetoacetate and β -hydroxybutyrate are buffered but severe metabolic acidosis still develops. Accumulation of lactate in the blood also complicates diabetic ketoacidosis.²² To find the extent of acidosis serum bicarbonate level was also assayed in the diabetic patients and control subjects. Plasma bicarbonate level was significantly decreased in patients with DKA and HHNK. This pattern of acid – base disturbances is remarkably similar to that previously observed in people with diabetes mellitus and the findings of previous studies also agree with the present study.²³

Conclusion

Present study demonstrates that the lack of suppression of glucagon causes marked hyperglycemia under conditions of relative insulin deficiency and can worsen the diabetic state like development of DKA when insulin deficiency becomes absolute as in type 1 diabetes mellitus. Other findings regarding plasma glucose, plasma osmolality, serum electrolytes and arterial blood gases are remarkably similar to those previously observed in people with various types of diabetes mellitus and associated complications like DKA and HHNK.

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