

Study of Blood Glucose and Serum Nitric Oxide Levels in Alcoholic and Non-Alcoholic Type-2 Diabetes Mellitus

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Abstract: Introduction: Diabetes mellitus is one of the most common disease encountered in the present health scenario. Hyperglycemia and free radicals cause oxidative damage leading to microvascular complications. The endothelial dysfunction has been attributed to lack of bioavailable Nitric Oxide (NO). Alcohol suppresses induced-NO production by inhibition of iNOS in different cells. Study of effects of alcoholism on blood glucose and serum Nitric Oxide levels in Type-2 Diabetics will be helpful for the physician to give better advice to alcoholics suffering from diabetes mellitus.

AIM: To study blood glucose and serum nitric oxide levels in alcoholic and non-alcoholic type-2 diabetes mellitus.

Materials & Methods: Study was conducted on 60 subjects among them 20 were normal healthy individuals, 20 were Non alcoholic type-2 diabetes mellitus patients and another 20 were Alcoholic type-2 diabetes mellitus patients.

Results: There is a significant increase in blood glucose (fasting & post-prandial) and decrease in serum nitric oxide levels in patients suffering from diabetes mellitus when compared to controls. There is a significant increase in blood glucose (fasting & post-prandial) and decrease in serum nitric oxide levels in patients suffering from diabetes mellitus with alcoholism when compared to non alcoholic diabetes mellitus patients.

Conclusion: Blood glucose levels are increased and serum NO levels are decreased in patients suffering from diabetes mellitus when compared to controls. Blood glucose levels are increased and serum NO levels are decreased in patients suffering from diabetes mellitus with alcoholism when compared to non-alcoholic diabetes mellitus patients suggesting the effect of alcohol in decreasing Nitric oxide levels in Diabetics increasing the risk of vascular complications.

Keywords: Diabetes mellitus, Alcoholism, Glucose, Nitric oxide.

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I. Introduction

Diabetes mellitus is an endocrinal disorder, characterized by polyuria, polyphagia and polydipsia. It is a complex disorder which has an aberrant metabolism of carbohydrates, lipid and proteins due to either relative or absolute deficiency of insulin in secretion or action. These result in hyperglycemia, glycosuria, ketonuria and ketoacidosis (Joslins 2005)⁸. There are various experimental studies suggesting the over-production of reactive oxygen and nitrogen species to be involved in the initiation and development of vascular complications in diabetes. Free radicals are reactive chemical substances, which can cause oxidative injury by attacking the macromolecules like lipids, carbohydrates, proteins and nucleic acids. Under normal circumstances there is a critical balance in generation of oxygen free radicals and antioxidant defence mechanism to protect them from free radical toxicity (Halliwell B et al 2004)⁷. Breach in this balance leads to oxidative stress. Oxidative stress is known to be a component of molecular and cellular tissue mechanism in a wide spectrum of human disease. The oxidative modification of low-density lipoprotein (LDL) in the artery wall is believed to be central to the pathogenesis of atherosclerosis. There are reports in animal experiments to show that alcohol intake further aggravates the generation of free radicals.

Vascular injury in diabetes consequential to hyperglycemia has been associated with oxidative stress. The endothelial dysfunction has been attributed to lack of bioavailable NO due to reduced ability to synthesize NO from L-arginine. So, NO is reduced in the course of Diabetes Mellitus. There are reports in animal experiments to show that alcohol intake further aggravates the generation of free radicals.

Hence the study is undertaken to find out the effect of alcoholism and free radical generation in patients of Diabetes Mellitus by measuring and comparing the levels of Blood Glucose and Serum nitric oxide levels in diabetes mellitus patients with and without alcoholism.

II. Materials And Methods

The study was conducted on 60 subjects in the age group of 35- 60yrs of both genders Among them 20 were normal healthy individuals who formed the control group. Twenty (20) patients suffering from Type-2 Diabetes Mellitus without history of alcoholic intake formed the study group -1. Twenty (20) patients suffering from Type-2 Diabetes Mellitus with Alcoholism formed the study group-2.

Inclusion criteria:

1. Normal healthy individuals as controls.
2. Cases are divided into 2 groups: GROUP 1 comprises of patients suffering from TYPE 2 DIABETES MELLITUS ONLY. GROUP 2 comprises of patients suffering from TYPE 2 DIABETES MELLITUS WITH ALCOHOLISM.
3. Diabetes mellitus patients who are on treatment with and without complications like coronary artery disease, neuropathy and nephropathy are included. History of alcoholism is taken and subjects are divided accordingly.

Exclusion criteria:

Diabetes mellitus patients with severe acute complications and women with pregnancy are excluded.

Collection of blood sample for analysis:

A fasting (12 hours) venous blood sample (5ml) was drawn from the patients and controls into a sterile disposable syringe which was transferred into centrifuge tubes and was allowed to clot for 30 minutes. A post prandial venous blood samples are also collected from same patients .The samples were centrifuged at 3000 rotations per minute for 10 minutes and the serum was separated and collected from the centrifuge tubes and analyzed within one hour as follows. In all samples blood glucose estimation was done by “GOD-POD method”(Tietz 6th edition)¹⁶ and serum nitric oxide estimation is done by “modified copper-cadmium reduction method”(kranti sorte & anjan basak)¹⁵.

Estimation Of Serum Nitric Oxide By A Modified Copper-Cadmium Reduction Method

Principle

In aqueous solution, nitric oxide rapidly degrades to nitrate and nitrite. Colorimetric determination of nitrite using Greiss reagent is straight forward and sensitive, but does not measure nitrate, causing a possible underestimation of nitric oxide.

This method employs granular cadmium metal for chemical reduction of nitrate to nitrite prior to quantitation of nitrite using Greiss reagent. In acid solution, nitrite is converted to nitrous acid (HNO₂) which diazotizes sulfanilamide.

This sulfanilamide-diazonium salt is then reacted with N-(1-Naphthyl)ethylenediamine (NED) to produce a chromophore which is measured at 540 nm.

Normal reference value: Serum Nitric oxide:-11.5-76.4 μmol/L (males) 10.1-65.6 μmol/L (females)

III. Results

Blood glucose and serum Nitric oxide levels were measured in control group and study group -1 and study group -2. All the values are expressed as Mean ± SD . Mean values of both parameters are compared between control group and study group-1 (Table-1). Mean values of both parameters are compared between the two study groups 1 and 2 (Table-2). Statistical analysis done by student t test and the statistical significance is expressed as p value ≤0.05. The statistical analysis is done by SSPS statistical system. Comparison of control group with group 1 of cases(diabetes mellitus only)

Table-1

S.No	INVESTIGATION		CONTROL (n=20)	GROUP 1 (n=20)
1	FASTING BLOOD SUGAR	MEAN	88.6	206.55
		SD	6.52	42.38
		T-test	12.3019	
		P-value	0.0001	
2	POST PRANDIAL BLOOD SUGAR	MEAN	126.75	284.70
		SD	11.18	104.06
		T-test	6.7493	
		P-value	0.0001	
3	SERUM NITRIC OXIDE	MEAN	45.3	26.15
		SD	9.211	10.106

	T-test	6.2655
	P-value	0.0001

Comparison of 2 groups of cases(Type II diabetes and Type II diabetes mellitus with alcoholism)

Table-2

S.No	INVESTIGATION	GROUP 1	GROUP 2
1	FASTING BLOOD SUGAR	MEAN	206.55
		SD	42.38
		T-test	0.6170
		P-value	0.5409
2	POST PRANDIAL BLOOD GLUCOSE	MEAN	284.7
		SD	40.06
		T-test	0.6736
		P-value	0.5046
3	SERUM NITRIC OXIDE	MEAN	26.15
		SD	10.106
		T-test	4.4124
		P-value	0.0001

Table 1:(Statistical values of controls and Type 2DM) shows the comparative data of fasting and post prandial blood glucose in control and cases with type 2 Diabetes. The means and S.D of fasting and post prandial blood glucose in control is 88.6 ± 6.52 , 126.75 ± 11.18 as compared to type 2 DM 206.55 ± 42.38 , 284.70 ± 40.06 p value is 0.0001 which is highly significant. (P-value <0.05 is significant)

Table 1:(Statistical values of controls and Type 2DM) shows the comparative data of serum nitric oxide in control and cases with type 2 Diabetes 45.3 ± 9.211 and 26.15 ± 10.106 . P value is <0.005 which is highly significant in all the above parameter.

Table 2:(Statistical values of Type 2 DM and Type 2DM with chronic alcoholism) shows the comparative data of fasting and post prandial blood glucose in 2 groups of cases . The means and S.D of fasting and post prandial blood glucose in Type 2 DM is 206.55 ± 42.38 , 284.7 ± 40.06 as compared to type 2 DM with chronic alcoholism 220.60 ± 45.69 , 303.80 ± 43.47 p value is >0.005 in both parameters which is highly insignificant. (P-value <0.05 is significant)

Table 2:(Statistical values of Type 2 DM and Type 2DM with chronic alcoholism) shows the comparative data of serum nitric oxide in Group1 and Group2 of cases 26.15 ± 10.106 and 15.3 ± 4.354 . P value is <0.005 which is highly significant in the above parameter.

IV. Discussion

Fasting and Post-Prandial Blood Sugar:

In our study we have found elevation of blood glucose concentration in patients suffering from type 2 diabetes mellitus compared to controls. Hyperglycemia is primarily due to reduced glucose uptake by tissues and its increased production via gluconeogenesis and glycogenolysis(U. Satyanarayana et al 2010)¹⁴.

Serum nitric oxide (NO):

We have observed significantly low in diabetes as compared to controls along with differences in other biochemical parameters. Free radical NO has emerged as a fundamental signalling molecule regulating virtually every critical cellular function and is a potent mediator of cellular damage in many conditions. Vascular injury in diabetes consequential to hyperglycemia has been associated with oxidative stress that leads to depletion of intracellular glutathione with an augmented plasma extracellular superoxide dismutase which intervenes lipid peroxidation and diabetic complications. NO is synthesized as a byproduct of conversion of its physiological precursor l-arginine to l-citrulline. This reaction is catalyzed by a family of enzymes known as NOS(NITRIC OXIDE SYNTHASE). NO is produced from the substrate l-arginine via eNOS (endothelial Nitric Oxide Synthase). Increased asymmetric dimethylarginine levels cause eNOS uncoupling a mechanism which leads to decreased NO bioavailability. The endothelial dysfunction has been attributed to lack of bioavailable NO due to reduced ability to synthesize NO from l-arginine. So,NO is reduced in the course of vascular disease eg..diabetes.(Paolo Tessari et al 2010)¹¹

Fasting and Post-Prandial Blood Sugar:

In our study we have found an increase in fasting and post-prandial blood glucose in diabetic alcoholics(as our group 2 of cases are suffering from Type 2 DM with chronic alcoholism) compared to patients suffering from type 2 diabetes mellitus only. The mechanisms underlying the increasing hyperglycemia in chronically drinking diabetics are still unknown. The most common form of diabetes, type2 diabetes, is associated with both insufficient insulin secretion and insulin resistance. Previous studies have

indicated that alcohol consumption increases the insulin resistance which in turn leads to chronic hyperglycemia. Along with insulin resistance, chronically drinking diabetics may show worse compliance with their dietary and pharmacological treatment regimens, which also may result in uncontrolled blood sugar levels. (Nicholas V. Emanuele et al 1998)¹⁰.

Serum Nitric Oxide:

In our study we have found decrease in serum nitric oxide levels in chronically drinking diabetics when compared to Type 2 DM only. Ethanol (EtOH) in alcoholic beverages is consumed by a large number of individuals and its elimination is primarily by oxidation. The role of nitric oxide (NO) in ethanol's effects is important since NO is one of the most prominent biological factors in mammals. NO is constantly formed endogenously from L-arginine. Dose and length of ethanol exposure, and cell type are the main factors affecting ethanol effects on NO production.

Either acute or chronic ethanol ingestion affects inducible NO synthase (iNOS) activity, however it seems that ethanol suppresses induced-NO production by inhibition of iNOS in different cells. On the other hand, it is clear that acute low doses of ethanol increase both the release of NO and endothelial NOS (eNOS) expression, and augment endothelium-mediated vasodilatation, whereas higher doses impair endothelial functions. Ethanol selectively affects neuronal NOS (nNOS) activity in different brain cells, which may relate to various behavioural interactions. Therefore, there is an excellent chance for ethanol and NO to react with each other. Effects of ethanol on NO production and NOS activity may be important to ethanol modification of cell or organ function. Nitrosated compounds (alkyl nitrites) are often found as the interaction products, which might be one of the minor pathways of ethanol metabolism. NO also inhibits ethanol metabolizing enzymes. Furthermore, NO is involved in ethanol induced liver damage and has a role in fetal development during ethanol exposure in pregnancy. The mechanisms underlying these effects are only partially understood. Hence, the current discussion of the interaction of ethanol and NO is presented. The effects of ethanol on the endothelium are complicated. For example, the endothelium responds to increases in flow by releasing vasodilator mediators, most notably endothelium derived relaxing factor, identified as NO [Cockcroft JR 2005]⁴. There are favorable effects on endothelial function with low-dose ethanol exposure but the induction of endothelial dysfunction with higher doses (Puddey IB et al 2001)¹².

In vitro, acute exposure to ethanol increases NO production and eNOS expression in endothelial cells derived from systemic vessels [Acevedo CG et al 2001]¹ [Venkov CD et al 1999]¹³. Further more eNOS activity is regulated not only at the level of expression [Davis ME et al 2001]⁵, but also post-translationally by mechanisms including protein-protein interactions [Forsterman U et al 1991]⁶ and phosphorylation [Bauer PM et al 2003]³. Indeed acute low-dose ethanol (10–50 mmol/liter) directly activates Ca²⁺-activated K⁺ channels in cultured human umbilical vein endothelial cells, leading to an increase of endothelial proliferation and production of NO. However, higher dose ethanol (100 and 150 mmol/liter) significantly reduced NO synthesis [Kuhlmann CR et al 2004]⁹.

In vivo, animal studies have shown that acute low doses of ethanol increase the release of NO and augment endothelium-mediated vasodilatation, whereas higher doses impair endothelial functions [Altura BM et al 1982]².

V. Conclusion

By studying all the above parameters in normal healthy individuals, type 2 diabetes mellitus and type 2 diabetes mellitus with alcoholism we have come to a conclusion that the blood glucose levels are increased and serum NO levels are decreased in patients suffering from type 2 diabetes mellitus when compared with controls, blood glucose levels are increased and serum nitric oxide levels are decreased with statistical significance in chronic alcoholics with diabetes mellitus when compared to non-alcoholic diabetes mellitus patients suggesting the effect of alcohol in decreasing Nitric oxide levels in Diabetics increasing the risk of vascular complications.

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