

EFFECT OF METFORMIN ON URINARY VMA LEVELS IN TYPE II DIABETIC SUBJECTS

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ABSTRACT

The purpose of this study was to observe the effect of metformin on 24–hours urinary VMA levels in newly diagnosed untreated type 2 diabetic subjects. The study consisted of four weeks for each participant with weekly follow up visits. Samples were collected at 0800–0900 hours after over night fast. Study was conducted in the Department of Pharmacology & Therapeutics, BMSI, JPMC, Karachi. Total duration of study was six months. Fifteen newly diagnosed untreated type 2 diabetics, with fasting plasma glucose levels ≥ 126 mg/dl on two occasions and/or postprandial glucose levels ≥ 200 mg/dl were enrolled in the study. Patients with concurrent illness or diabetic complications were excluded. Metformin was started from 500 mg/day and titrated at weekly intervals according to glycaemic control and the subjects tolerance to the drug. A 24 –hour urinary VMA was assessed at day – 0 (before metformin therapy) and day – 28 (4 weeks after metformin therapy) by using VMA reagent kit of Biosystems Spain on Spectronic –21 spectrophotometer USA. Metformin caused highly significant ($P < 0.001$) reduction in mean fasting plasma glucose from 233.33 ± 15.62 mg /dl on day-0 to 151.53 ± 6.02 mg/dl on day – 28, and a significant ($P < 0.01$) decrease in 24 – hour urinary VMA levels from $5.18 + 0.50$ mg / 24 hours on day-0 to $3.32 + 0.28$ mg / 24 hours on day-28. Our results indicate that metformin causes highly significant reduction in fasting plasma glucose and a significant decrease in 24 – hour urinary VMA levels in newly diagnosed untreated type 2 diabetic subjects.

INTRODUCTION

The system that keeps blood glucose levels near normal ranges involves the combined action of hepatic auto regulation, insulin, glucagon and epinephrine¹. Insulin resistance in type 2 diabetics can be triggered by target tissue defects in insulin's action or circulating hormonal and other antagonists². Epinephrine plays an important role in antagonizing several metabolic functions of insulin³. Elevation in catecholamines may be responsible for initiation or maintenance of decompensated metabolic diabetic state and pharmacological modulation of catecholamine secretion may result in improved control of diabetes in man⁴.

In this study we intended to observe the effect of metformin therapy on 24 – urinary VMA levels (a metabolic end product of catecholamines, excreted in urine) in type 2 diabetic subjects.

SUBJECTS AND METHODS

This study was conducted in the Department of Pharmacology and Therapeutics, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Center, Karachi. Fifteen type II diabetics were enrolled in the study from diabetic clinic Medical

Unit III, Jinnah Postgraduate Medical Center, Karachi.

Selection Criteria:

Newly diagnosed untreated type 2 diabetic subjects of either sex with their ages ranging between 35 – 55 years, with fasting plasma glucose levels ≥ 126 mg/dl on two occasions and /or postprandial blood glucose levels ≥ 200 mg/dl⁵, were enrolled in the study. People with type1 diabetes mellitus, type 2 diabetic persons who had received any type of antidiabetic treatment in the past, persons taking drugs known to affect serum catecholamines or 24 – hour urinary VMA levels, patients with severe diabetic complications, pregnant and lactating mothers and patients with any concurrent medical illness were excluded from study.

After explaining the limitations, an informed consent was obtained from all study participants before enrollment. The study period consisted of 4 weeks for each participant with weekly follow up visits. Dose of metformin was started from 500 mg/day and was titrated at weekly interval according to person's glycaemic status and tolerance to the drug. At each weekly visit fasting plasma glu-

glucose levels were recorded and subjects were inquired about drug compliance, side effects of the drug and symptoms related to hypoglycaemia and hyperglycaemia. They were also motivated to keep their nutritional habits, physical activity and general life style as constant as possible throughout the study period. All studies commenced between 0800 – 0900 hours after, 12 hour over night fast. A 24 – hour urine was collected for the assessment of VMA levels on day – 0 before starting metformin therapy and at day-28 after 4 weeks of metformin therapy.

Collection of Samples and Analytical Methods:

Blood sample was drawn from each study participant after an over night fast by venepuncture using plastic disposable syringe under aseptic conditions. Blood glucose levels were determined by using glucose reagent Merck Germany on Vita Lab Selectra-2, blood Chemistry auto-analyzer Merck Germany.

A 24 – hour urine was collected in a clean jar. After measuring total volume of the urine 5ml was separated in a test tube and the rest was discarded. pH of urinary sample was adjusted between 1 and 2 with concentrated HCL. Urinary samples were analyzed for 24 –hour VMA by using reagent kit of Biosystems Spain on Spectronic – 21 Spectrophotometer, USA.

Statistical Analysis:

The statistical significance of difference between the mean values of observations was calculated by student's "t" test.

P- value

The degree of probability was computed by comparing the calculated value of "t" with tabulated

Table 1: Mean fasting glucose & 24 – hour urinary VMA level at day– 0 & day – 28 of treatment with Metformin in type 2 diabetics (N=15).

Parameter	Day – 0	Day – 28	P – Value
Glucose mg / dl	233.33 ± 15.62	151.53 ± 6.02	< 0.001**
Urinary VMA mg / 24 hours	5. 18 ± 0. 50	3. 32 ± 0.28	< 0.01*

Each value represents mean of total observations; ± Indicates standard error of mean. * Significant. ** Highly significant.

Table 2: Mean fasting glucose & 24 – hour urinary VMA level at day– 0 & day – 28 of treatment with Metformin in type 2 diabetics (N=15).

S. No	Fasting plasma Glucose (mg/dl)			24- hour urinay VMA mg/24 hours		
	Day - 0	Day - 28	Diff:	Day - 0	Day - 28	Diff:
1.	210	147	63	6.87	3.14	3.73
2.	236	181	55	4.69	3.20	1.49
3.	165	129	36	4.54	3.47	1.07
4.	183	126	57	5.96	3.26	2.70
5.	218	141	77	5.00	4.38	0.62
6.	217	146	71	2.79	2.32	0.47
7.	213	131	82	4.98	2.25	2.73
8.	221	177	44	5.81	5.04	0.77
9.	162	110	52	8.72	5.12	3.60
10.	367	171	196	9.09	4.96	4.13
11.	303	147	156	5.37	2.20	3.17
12.	202	182	20	3.00	2.18	0.82
13.	315	173	142	3.36	2.91	0.45
14.	302	176	126	4.46	3.18	1.28
15.	186	136	50	3.00	2.14	0.86
Mean	233.33	151.53	81.8	5.18	3.32	1.86
SEM	15.62	6.02	12.94	0.50	0.28	0.34
P value	<0.001**			<0.01*		

* Significant ** Highly Significant.

values in the table of "t". The difference in the mean values of two sets of observation was regarded statistically significant if the p – values were equal to or less than 0.05 and non – significant if the p – value was more than 0.05. It was highly significant if the p –value was less than 0.001.

RESULTS

In 15 type 2 diabetic persons treated with metformin for 4 weeks mean fasting blood glucose levels reduced from 233.33 ± 15.62 mg /dl on day - 0 to 151.53 ± 6.02 mg /dl on day - 28. The reduction in mean fasting plasma glucose was highly significant ($p < 0.001$), when evaluated statistically. Mean 24 - hour urinary VMA levels decreased from 5.18 ± 0.50 mg / 24 hours on day - 0 to 3.32 ± 0.28 mg / 24 hours on day - 28. The reduction in 24 - hour urinary VMA level was significant ($p < 0.01$) on statistical analysis. Results are shown in table 1 & 2 and figure 1.

DISCUSSION

The human body functions in a delicate state of equilibrium that is constantly being challenged from within and from without⁶. When person is confronted with stressful situation, that requires increased activity, the neural signals trigger the release of epinephrine and norepinephrine from adrenal medulla¹. Released catecholamines have profound effects on carbohydrate and lipid metabolism⁴. Epinephrine inhibits insulin secretion through α_2 receptor effect⁷. Although β - adrenergic stimulation can increase insulin secretion, epinephrine and nor epinephrine predominantly stimulate α receptors, therefore inhibit insulin secretion⁸. It has recently been reported that mutation of β_3 - adrenergic receptors result in early onset of type 2 diabetes mellitus, abdominal obesity and resistance to insulin⁹. Catecholamines also increase other anti-insulin hormones particularly glucagon and growth hormone, consequently causing deterioration of carbohydrate and lipid metabolism in types 2 diabetes mellitus⁴.

Epinephrine plays a pivotal role in regulating glucose metabolism both in splanchnic and peripheral tissues. It inhibits splanchnic glucose uptake and enhances hepatic glucose production by both glycogenolysis and gluconeogenesis⁷. Epinephrine activates glycogen phosphorylase and inactivates glycogen synthase, thus stimulating conversion of glycogen into glucose in liver¹. Skeletal muscle is also an important site of epinephrine mediated insulin resistance. It inhibits insulin-mediated glycogenesis in human skeletal muscle because of increase in glucose-6-phosphate, an inhibitor of hexokinase activity⁷. Epinephrine also opposes the effects of insulin by altering the activity and number of glucose transporters in skeletal muscles, with insulin stimulates the expression of GLUT4, while epinephrine reduces the expression of GLUT4 in skeletal muscle³. Finally epinephrine stimulates glucagon release, reinforcing its effects of mobilising fuels and inhibiting fuel storage¹.

In this study we intended to evaluate the effect of metformin therapy on 24 - urinary VMA levels (a metabolic product of catecholamines excreted in urine) in type 2 diabetic subjects. As in our socio-economic and environmental conditions, stress factor is prominent and catecholamines being well known, stress hormones may play a role in type 2 diabetic persons. Interestingly we found a significant decrease in 24 - hour urinary VMA levels after 4 weeks of metformin therapy.

Metformin acts primarily by improving peripheral insulin sensitivity by inhibiting gastrointestinal glucose absorption and most importantly by decreasing hepatic glucose output^{10,11}. The currently proposed mechanism of action of metformin is; 1. direct stimulation of glycolysis in tissues with increased glucose removal from blood 2. decreased hepatic gluconeogenesis 3. slowing of glucose absorption from GIT 4. reduction of plasma glucagon levels¹². Metformin offers an advantage to obese persons with type 2 diabetes mellitus, that it facilitates weight loss and is associated with decreased triglycerides, LDL - cholesterol and total cholesterol levels.^{10,13} Obesity is an extremely important environmental factor in type 2 diabetics.¹⁴ It is virtually always associated with insulin resistance, which is arguably the earliest detectable and dominant metabolic defect in type 2 diabetes mellitus.¹⁵ Higher rates of insulin resistance are found in type 2 diabetic subjects with hypertriglyceridaemia and low HDL - cholesterol levels.^{16,17}

The over all improvement in insulin sensitivity and decrease in anti-insulin hormones i-e glucagon, produced by metformin as its therapeutic effects may have a suppressive effect on circulating catecholamines that is reflected by a decrease in 24 - hour urinary VMA levels in type 2 diabetic persons.

It is **concluded** that the present data of our study suggests that metformin therapy causes a significant reduction in 24 - hour urinary VMA levels in newly diagnosed untreated type II diabetic subjects.

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