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Effects of N-Acetyl-Cysteine (NAC) Administration on Glucose Homeostasis Parameters in Prediabetic Patients

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Abstract

Background Prediabetes is determined on the bases of glycemic parameters, which are above normal but below diabetic thresholds. Prediabetes is associated with the presence of insulin resistance and β-cells dysfunction. N-acetyl cysteine (NAC), as a safe and inexpensive medication, is commercially accessible since long-time ago. This drug is not found in natural sources, although cysteine is present in some meals like chicken and turkey meats, garlic, yogurt, and eggs. NAC prevents apoptosis and oxygen related genotoxicity in endothelial cells by increasing intracellular levels of glutathione and decreasing mitochondrial membrane depolarization reaction.

Objective To evaluate the effects of NAC administration on glucose homeostasis parameters in prediabetic patients.

Methods This study included, 25 patients treated with dietary control and life style modifications for 12 weeks, 25 patients treated with NAC (600 mg) oral tablets twice daily plus dietary control and life style modifications for 12 weeks. Other 20 in addition to 50 patients to have an idea about the normal values of study parameters and in order to assess how much the drug used in the study were able to normalize the abnormal parameters.

Results NAC demonstrated a significant decrease in the fasting blood sugar, HbA1c, fasting Insulin and insulin resistance at the end of 12 weeks ($P<0.05$) compared with baseline measurements.

Conclusion The results of the study showed that N-acetyl cysteine has an effective effect on glycemic control.

Keywords Prediabetes , N-acetyl cysteine , glycemic control

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List of abbreviations: AGEs = Advanced glycation end products, FPG = Fasting plasma glucose, GSH = Glutathione, HOMA = Homeostatic model assessment, IFG = Impaired fasting glucose, IGT = Impaired glucose tolerance, NAC = N-acetylcysteine, ROS = Reactive oxygen species

Introduction

Prediabetes is determined on the bases of glycemic parameters which are above normal but below diabetes thresholds. It is a high risk state for diabetes with an estimated annual conversion rate of 5-10%; a similar proportion is converting back to normoglycemia [(1)]. Prediabetes is associated with the presence of insulin resistance and β-cell dysfunction. These abnormalities start before glucose changes are detectable [(2)].

The high risk for developing diabetes is related to two states; impaired fasting glucose (IFG) (defined as fasting plasma glucose (FPG) of 5.7-6.9 mmol/L in the absence of impaired glucose tolerance (IGT), and to IGT (defined as post-load plasma glucose of 7.8-11.0 mmol/L based on 2-hour oral glucose tolerance test (OGTT) or

a combination of both [3]. A lower cut-off value for IFG (FPG 5.6-6.9 mmol/L) is employed by the American Diabetes Association. In addition, it has introduced hemoglobin A1c levels of 5.7-6.4% as a parameter of high diabetes risk [4]. Combination of IFG and IGT marks a more advanced disturbance of glycemic homeostasis [5].

The central mechanism that is responsible for risks in prediabetes is endothelial dysfunction due to the elevated formation of reactive oxygen species (ROS) and advanced glycation end products (AGEs) as well as increased lipid peroxidation under hyperglycemic conditions [6].

N-acetyl cysteine (NAC), the acetylated variant of the amino acid L-cysteine, is an excellent source of sulfhydryl (SH) groups, and is converted in the body into metabolites capable of stimulating glutathione (GSH) synthesis, promoting detoxification, and acting directly as free radical scavengers [7]. It is a powerful antioxidant and a potential treatment option for diseases characterized by the generation of free oxygen radicals [8].

The objectives of this study was to evaluate the effects of NAC administration on glucose homeostasis parameters in prediabetic patients.

Methods

Study design

The current study was conducted on 50 prediabetic patients (31 males, 19 females) their ages from 30-65 year were seen in Al-Sader Teaching Hospital. The patients were diagnosed clinically by physician as having prediabetes. Criteria for the diagnosis of prediabetes and diabetes [9] is shown in table 1.

Table 1. Criteria for the diagnosis of prediabetes and diabetes

	Prediabetes	Diabetes
A1C	5.7-6.4%	≥6.5%**
FPG	100-125 mg/dL (5.6-6.9 mmol/L)	≥126 mg/dL (7.0 mmol/L)**
OGTT*	140-199 mg/dL (7.8-11.0 mmol/L)	≥200 mg/dL (11.1 mmol/L)**
RPG		≥200 mg/dL (11.1 mmol/L)***

* 2-hour plasma glucose value after a 75-g OGTT

** Confirm results with repeating testing

*** Diagnotic in patients with established symptoms of hyperglycemia

A1C: Glycated hemoglobin, FPG: Fasting plasma glucose, OGTT: oral glucose tolerance test, RPG: random plasma glucose

To have an idea about the normal values of study parameters and in order to assess how much the drug used in the study were able to normalize the abnormal parameters, other 20 patients was added to 50 patients.

Patients

This study included, 25 patients treated with dietary control and life style modifications for 12 weeks, 25 patients treated with NAC (600 mg) oral tablets twice daily plus dietary control and life style modifications for 12 weeks.

Sample collection and preparation

After 12 hours fasting, blood samples were collected from all patients and healthy subjects by venipuncture (10 mL), before starting drug treatment (as zero time) then after 12 weeks of treatment to follow up the changes in the studied parameters. Blood samples were divided into two tubes, one heparinized tube (1 mL of whole blood used for HbA1c determination) and the other part was collected in plane tube, then centrifuged at 3000 rpm for 10 min at 4 °C. After centrifugation and isolation of cellular fraction; the obtained plasma fraction was divided into

two parts in Eppendorf tubes and stored frozen until analysis performed.

Measurement of glycemic control

Fasting blood glucose Level (FBS)

Serum glucose level was evaluated using a ready-made kit for this purpose, according to the method of [10], which is based on enzymatic oxidation of glucose to form glucuronic acid and hydrogen peroxide, and the reaction of the later with phenol and formation of quinonimine was followed spectrophotometrically at 505 nm. Results were expressed as mg/dL, based on comparison with a standard glucose solution treated with same method.

Glycated Hemoglobin (HbA1c)

The Bio-Rad VARIANTT Mhemoglobin A1C program is intended for the determination of HbA1c in human whole blood using the principles of ion exchange high performance liquid chromatography (HPLC) for the automatic and accurate separation of hemoglobin A1c (HbA1c). It is fully automated assay using HPLC technology to deliver precise and accurate HbA1c results. Program offers a simple preparation followed by automatic sampling, and an analysis time of three minutes per sample. Preceding analysis, a simple preparation of the sample is required to hemolyze and remove labile A1C. Samples are first diluted with hemolytic reagent and then incubated at 18-28Co for a minimum of 30 min. The VARIANTS II dual-piston pumps deliver programmed buffer gradients of increasing ionic strength to the analytical cartridge. Prepared samples are automatically injected into analytical cartridge where the hemoglobin is separated based on their ionic interaction with the material. The separated hemoglobin then passes through the flow cell of the filter photometer, where changes in the absorbance at 415 nm are measured. A chromatogram of the changes in the absorbance is plotted versus the retention time. This chromatogram helps in result interpretation [11].

Serum Insulin levels

The Demeditec insulin ELISA (enzyme-linked immunosorbent assay) is a solid phase ELISA based on sandwich principle. The microtiter wells are coated with a monoclonal antibody directed towards a unique antigenic site on the insulin molecule. An aliquot of patient sample containing endogenous insulin is incubated in the coated well with enzyme conjugate, which is anti-insulin antibody conjugated with Biotin. After incubation the unbound conjugate is washed off. During the second incubation step Streptavidin –Peroxidase –Enzyme complex binds to the biotin-anti-insulin-antibody. The mount of horseradish peroxidase (HRP) complex is proportional to the concentration of insulin in the sample. Having added the substrate solution, the intensity of the color developed is proportional to the concentration of Insulin in the patient sample. The result expressed as uU/mL.

Insulin resistance

The homeostatic model assessment (HOMA) model is simple and accessible measurement method for the evaluation of insulin sensitivity and consider as a model of interactions between glucose and insulin dynamics that is then used to predict fasting steady-state glucose and insulin concentrations for a wide range of possible combinations of insulin resistance and β-cell function. Both the original HOMA and the updated HOMA2 assume a feedback loop between the liver and β-cell [12,13] glucose concentrations are regulated by insulin-dependent HGP, whereas insulin levels depend on the pancreatic β-cell response to glucose concentrations. Thus, deficient β-cell function reflects a diminished response of β-cell to glucose-stimulated insulin secretion. Likewise, insulin resistance is reflected by diminished suppressive effect of insulin on glucose production. HOMA describes this glucose insulin homeostasis by a set of empirically derived nonlinear equations. The model predicts fasting steady state levels of plasma glucose and insulin for any given combination of pancreatic β-cell function and insulin sensitivity [14]. The approximating

equation for IR has been simplified and uses a fasting plasma sample in which glucose (fasting plasma glucose; FPG) and insulin (fasting plasma insulin; FPI) are measured, together with a constant. The product of FPG×FPI is an index of IR.

HOMA-IR = (glucose × insulin)/405.

Insulin concentration is reported in uU/mL and glucose in mg/dL. The constant of 405 is a normalizing factor, i.e. normal FPI of 5 uU/mL × the normal FPG of 81 mg/dL typical of a 'normal' healthy individual = 405. Therefore, for an individual with "normal" insulin sensitivity, HOMA-IR =1.

Statistical analysis

Paired Student's t test was used to compare values obtained before and after treatment administration within each group while independent sample t tests were used for between all patients and healthy subjects. Multiple comparisons were also carried out by using Analysis of variance (ANOVA) with least significant difference (LSD) post-hoc testing to compare changes in variables between groups before and after the 12 weeks' treatment period. Data are presented as mean ± Standard deviation (SD). For all statistical analyses, P<0.05 was considered statistically significant using a two-tailed test. Statistical analysis of data was performed using the Statistical Package for Social Sciences software version 16.0 [15].

Results

Comparison of patients with prediabetic and healthy subjects with respect to different parameters

In healthy group, the mean±SD for FBS, HbA1c, fasting serum insulin, insulin resistance were 83.42±9.73, 5.12±0.06, 11.14±1.23 and 2.84 ±0.71 respectively.

In patients group the mean±SD for FBS, HbA1c, fasting serum insulin, insulin resistance were 119.42±5.56, 6.14±0.09, 13.54±1.89 and 3.99±0.6 respectively. Unpaired t-test was used to compare the baseline characters between the healthy and prediabetic patients group, revealed that were significant differences in FBS, HbA1c, fasting serum insulin and insulin resistance (p<0.001) levels between both groups as shown in table 2.

Table 2. Comparison of different parameters of prediabetic patients to that of healthy subjects at baseline

Parameters	Patients		Healthy		P value
	Mean	±SD	Mean	±SD	
FBS (mg/dL)	119.42	5.56	83.42	9.73	0.001
HbA1c %	6.14	0.09	5.12	0.06	0.001
Fasting Insulin (?)	13.54	1.89	11.14	1.23	0.001
In. resistance	3.99	0.6	2.84	0.71	0.001

Effect of study treatment on (FBS, HbA1c, fasting insulin and insulin resistance)

Study treatment demonstrated a significant decrease in the FBS, HbA1c, fasting insulin and insulin resistance at the end of 12 weeks (P<0.05) compared with baseline measurements. In comparing with control group, the reductions in FBS, HbA1c, fasting insulin and in insulin resistance was significantly at week 12 of the study (P<0.05) as shown in table 3 and figure 1.

Table 3. Effect of study treatment on (Fasting blood glucose, HbA1c, fasting insulin and insulin resistance) after 12 weeks' treatments in study group and multiple comparison of the change from baseline

Parameters	Groups	Control		N-acetyl cysteine (NAC)	
		Mean	±SD	mean	±SD
FBS	Baseline	118.12	5.42	121.19	5.32
	12 weeks	107.25*	4.97	106.13*	5.14
	ΔF.B.S	-10.87	1.22	-15.06a	1.87
HbA1c	Baseline	6.22	0.084	6.19	0.092
	12 weeks	6.1*	0.081	5.63*	0.093
	ΔHbA1c	-0.09	0.022	-0.56a	0.019
Fast. In	Baseline	12.44	1.17	12.35	1.31
	12 weeks	11.09*	1.13	9.91*	0.97
	ΔFast.In	-1.35	0.14	-2.44a	0.16
In. resis	Baseline	3.61	0.34	3.69	0.34
	12 weeks	2.94*	0.33	2.59**	0.31
	Δ In. resis	-0.67	0.07	-1.1a	0.03

Fast. In: Fasting Insulin, In. resis: Insulin resistance

*=statistically significant (P<0.05) difference after 12 weeks compared with the baseline by using paired t-test

**= statistically highly significant (P<0.001) difference after 12 weeks compared with the baseline by using paired t-test

a= statistically significant (P<0.05) difference after 12 weeks compared with control group using ANOVA post hoc test or unpaired t-test

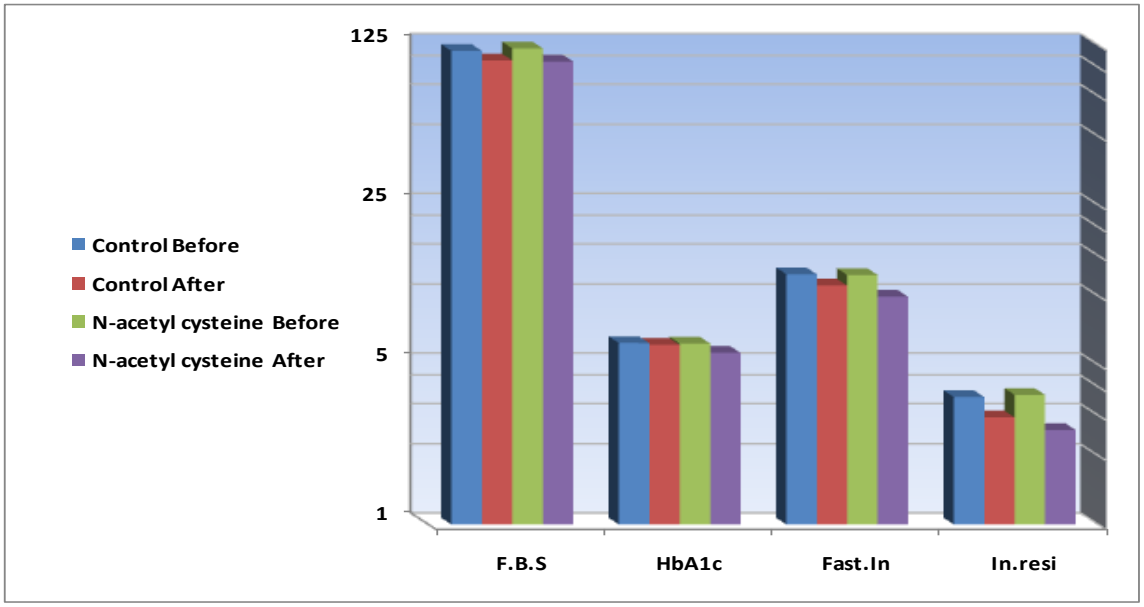

Figure 1. F.B.S, HbA1c, Fast. In and In. resi before and after 12 week of the study treatment

Discussion

Study treatment demonstrated a significant decrease in the FBS, HbA1c, fasting insulin and insulin resistance at the end of 12 weeks ($P<0.05$) compared with baseline measurements.

The NAC is the acetylated precursor of both amino acid L-cysteine and reduced glutathione. It has been shown to have proven activity on insulin secretion in pancreatic cells, as well as on the regulation of the insulin receptor in human erythrocytes [16]. The peak plasma level of NAC is attained 1 hour after an oral dose and it disappears from the plasma after 12 hours. The biological activity of NAC is attributed to its sulfhydryl group, which enhances glutathione-S-transferees activity aiding in the protection of all cells and membranes The activity of NAC on insulin secretion in pancreatic-cells, as well as on the regulation of the insulin receptor in human erythrocytes [17]. It increases the cellularlevels of reduced glutathione (GSH), an antioxidant, which has been shown to influence insulin receptor activity in vivo. Diabetics frequently experience glutathione deficiency, and NAC was shown that it improves endothelial cell function in such patients [18]. According to an intraperitoneal glucose tolerance test in mice, treatment with NAC retained glucose-stimulated insulin secretion and moderately decreased blood glucose levels. Vitamins C and E were not effective when used alone but slightly effective when used in combination with NAC [19]. Further, it is suggested that NAC might protect against oxidant-related upregulation of endothelial adhesion molecules and slow down the progression of vascular damage in non-insulin dependent diabetes [20]. The antioxidant status is poor in non-insulin dependent diabetes mellitus. NAC, as an effective radical scavenger could be a valuable adjunct treatment in diabetic patients, but further studies are required to confirm these findings. Supplementation with antioxidants as a promising complementary treatment can exert beneficial effects in diabetes [20].

According to the results presented in this study it is easy to conclude that the administration of NAC could improve glycemic control with consequent beneficial effects on oxidative stress in prediabetic patients, may be through mechanisms of up regulating peripheral tissue responses to the available insulin at receptor levels in association with potent antioxidant effects.

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Author Contribution

Dawood: performed the laboratory work. Dr. Abu Raghif: study design and statistics, Dr. Yaseen: doses calculation and final revision of manuscript.

Conflict of interest

Authors declare no conflict of interest.

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