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Contributory role of adenosine deaminase in metabolic syndrome

[1]Nwankwo A.A.; [2]Osim E.E. and [2]Bisong S. A.

Department of Physiology, [1]College of Medicine & Health Sciences, Abia State University. Uturu, [2]College of Medical Science, University of Calabar, Calabar, Nigeria.

Summary: Adenosine deaminase (ADA) is an enzyme of purine metabolism commonly associated with severe combined immunodeficiency disease and believed to modulate bioactivity of insulin. Its contributory role in patients with metabolic syndrome (having features such as obesity, insulin resistance, fasting hyperglycaemia, lipid abnormalities and hypertension) in South Eastern Nigeria was studied. Body mass index (BMI), fasting blood glucose (FBG), Glycated haemoglobin (GHbA1c), total cholesterol, HDL-cholesterol, LDL-cholesterol (usually impaired in metabolic syndrome) and total serum ADA activity were measured in different groups of patients with metabolic syndrome (test subjects) and apparently healthy subjects (controls). The test subjects comprised six subgroups made up of the following; obese diabetic (N=25), obese non-diabetic (N=25), Non-obese diabetic (N=25), patients with hypercholesterolaemia (N=25), LDL-cholesterolaemia (N=25) and HDL-cholesterolaemia (N=25). The results showed that the mean values of all the parameters studied (BMI, FBG, GHbA1c, total cholesterol, HDL-cholesterol and LDL-cholesterol) were higher in the test subjects than their controls. BMI did not correlate significantly with FBG, GHbA1c, and ADA in the test and control subjects respectively. The mean serum ADA activity in the test subjects of obese diabetic, obese non-diabetic and non-obese diabetic subjects was higher than in controls ($p< 0.001$). ADA activity was also higher in the test subjects of hypercholesterolaemia, HDL-cholesterolaemia and LDL-cholesterolaemia than in control ($p< 0.001$). ADA activity also correlated positively with hypercholesterolemia ($r = 0.640$; $p<0.001$), HDL-cholesterolaemia ($r = 0.646$; $p<0.001$) and LDL-cholesterolaemia ($r = 0.932$; $p<0.001$), with the highest correlation in the LDL-cholesterolaemia. In conclusion, ADA activity is increased significantly in all parameters of metabolic syndrome studied and showed a significant correlation with all the three groups of dyslipidaemic subjects studied. ADA could therefore be used in daily routine laboratory assessment of most metabolic diseases especially in obese and diabetic patients.

Keywords*:* Diabetes Mellitus, Adenosine deaminase, Immunity, Metabolic Syndrome

**Address for correspondence: azunkwo@yahoo.com, +2348030814753*

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INTRODUCTION

Metabolic syndrome is a term used for describing a cluster of cardiovascular risk factors comprising obesity, glucose intolerance/type 2 diabetes mellitus, dyslipidaemia and hypertension (Hauner, 2002), and it is an important coronary heart disease risk factor. (Yong-Woo et al, 2003).

Metabolic syndrome has insulin resistance as the common factor which is determined by physiological (aging, physical fitness), pathological (obesity), and genetic factors. Others are ethnicity, socioeconomic and lifestyle characteristics (Colagiuri, 2002). The metabolic compensatory response to insulin resistance is hyperinsulinaemia, the primary purpose being to maintain normal glucose tolerance. The prevalence rate of the metabolic syndrome is staggering. About 22.8% and 22.6% of U.S. men and women respectively were affected in a study in 1999 (Greenland et al, 1999) and in Nigeria, the prevalence of type 2 diabetes was about 2.7% according to the study of Ofoegbu et al (2004).

Several metabolic pathways have been reported to be deregulated in the metabolic syndrome especially the immune system (Nieman et al, 1999). Lymphocyte numbers and proliferation responses were reported to be altered in obese subjects and genetically obese animals (Tanaka et al, 1998). Cytokine balance was also changed in diet induced obese mice (Mito and Hiyosin, 2002). Although Mito et al (2000) reported that decreased proliferation of T-cell lines by insulin was the mechanism and factor responsible for impaired immunity in obesity and metabolic syndromes, the pathophysiologic process of this mechanism has not been well elucidated.

ADA is a polymorphic enzyme that irreversibly deaminates adenosine to inosine, contributing to the regulation of intracellular and extra cellular concentrations of adenosine (Brady, 1942). Adenosine on the other hand functions to increase

glucose uptake in the tissues and also inhibits proliferation of T-cells and cytokine synthesis. Thus, if ADA activity is increased, insulin insensitivity/resistance, cellular proliferation, inflammation, T-cells etc which are associated with the metabolic syndrome also increase (Green, 1987). As a marker for assessment of cell-mediated immunity in man (Sadasivudu et al, 1982) adenosine deaminase modulates cell growth (Lelieuve et al, 2000) and carbohydrate metabolic pathways through adenosine modulation. This modulation includes concomitant down-ward and up-ward regulation of adenosine A1 and A 2 receptors respectively. Recent studies have indicated that defective signalling from the insulin receptors is an important component of the insulin resistance associated with obesity in both animal models and humans (Folli et al, 1992). Here we carried out a study to demonstrate that ADA activity is very central to metabolic syndrome and could be used in its assessment.

MATERIALS AND METHODS

Seven groups of subjects (comprising six test groups and one control group) each consisting of twenty-five subjects were recruited as follows:
Group I: Obese and Non-insulin dependent diabetes mellitus (NIDDM) patients
Group II: Obese, Non- diabetics
Group III: Non-Obese, NIDDM patients
Group 1V: Hypercholesterolemia.
Group V: LDL-hypercholeslerolemia
Group V1: HDL Hypercholeslerolemia
Group V11: Apparently healthy people (control) without record of diabetes or obesity.

The subjects for this study were recruited from the diabetic clinic of the University of Nigeria teaching hospital, Enugu in Enugu State, Nigeria. Approval by the ethical committee of the hospital was sought and received. Informed consent was also sought and obtained from the patients for the study. The subjects were mainly type II diabetic and obese patients, males and females between the ages of 45-70years. The study was done in accordance with the Helsinki Declaration of Human studies.

Demographic data and medical history of each subject were obtained and recorded. Blood pressure (systolic/diastolic mmHg) was measured from each subject. Height and body weight were measured on a calibrated ruler and weighing balance respectively. Body mass Index (BMI) was calculated in kg/m^2.Blood samples were obtained from each subject after 9 to 12-hours fasting in about 80% of the subjects.

Fasting blood glucose (FBG) determination was carried out by the Glucose Oxidase method of Trinder and Barham (1972). Glycated Heamoglobin (GHbA1c) was determined by the chemical procedure of Flukinger and Winterhalter (1976). Adenosine deaminase activity was determined by the method of Martinek (1963), modified by Giusti and Galants (2000). Total cholesterol determination was done by the enzymatic commercial kit (Biosystem Cat. No. 11506, U.S.A). LDL- Cholesterol and HDL-Cholesterols determination was by the method of Liberman and Burchard (1970).

Statistical analysis:
Results of this study were given in mean $\pm$ SD. All statistical analyses were performed with SSPS 13 computer software (SSPS INC. Chicago, 11, USA). $P< 0.05$ was considered as statistically significant.

RESULTS

The summary of the clinical profile of the subjects were shown in Table 1. Body Mass Index (BMI) which serves as useful marker of obesity in this study was highly elevated in the patients where obesity and diabetes mellitus co-existed. These patients also had extremely high levels of adenosine deaminase (ADA) activity, protein glycosylation (GHbAic), blood pressure and fasting blood glucose (FBG) respectively when compared with the control group.
Table 2 shows the correlation and comparison of the Body Mass Index with the Fasting blood Glucose, Glycated Haemoglobin, and Adenosine Deaminase in the test groups.

In table 2, the absence of a significant correlation between BMI versus FBG, GHbA1c and ADA respectively in all the groups rules out the exclusive usefulness of BMI as an independent predictor of metabolic disease.

Table 3 shows the correlation and comparison of fasting blood glucose (FBG) versus Glycated Haemoglobin,and Adenosine Deaminase in the test groups. The positive correlation between FBG and GHbAic as well as ADA in this study reaffirmed the facts that protein glycosylation (shown by GHbA1c levels) is one of the complications of diabetes mellitus, and that ADA plays an important role in the in the modulation of carbohydrate metabolism and glucose regulation (Onyeanusi et al, 2003).

Table 4 shows the correlation and comparison of the Glycated Hemoglobin (GHbAic) with the Adenosine Deaminase (ADA) in the test groups. The highly significant correlation between GHbA1c and ADA among the obese diabetics than in the other two (obese non-diabetics and non-obese diabetics) may imply that serum ADA activity may be used to monitor long term diabetic control especially when it is accompanied with obesity.

Table 5 shows that the three groups of dyslipidemic patients had significantly higher levels of total cholesterol, HDL- cholesterol and LDL- cholesterol when compared to control ($p<0.001$ respectively).

Figure 1 shows that ADA activity in the three groups of hyerlipidaemic patients was significantly

higher than control (p< 0.001). ADA activity appeared higher in the LDL-cholesterolaemia than

Table 1. Clinical profile of subjects, Mean±SD

Characteristic	Control subjects (N=25)	Obese NIDDM (N=25)	Obese-Non NIDDM (N=25)	Non-Obese NIDDM (N=25)
Age(years)	54±2	53±8	55±4	55±5
Height(cm)	170±8	150±6	156±5	167±6
Weight(kg)	66.1±6	98.8±13	76±11	68±6
BMI(Kg/m2)	22.67±1	41.1±0.5*	30.6±4*	24.1±2*
FBG(mmol/l)	4.5±0.7	12.5±2*	4.8±0.4*	11.3±1.3*
BP (mmHg)	121±2/80±1	135±4/80/±4*	134±5/79±3*	133±9/78±3*
GHbAic (%)	7.88±1.25	17.35±1.53*	12.87±0.6*	14.15±0.75*
ADA (iu/L)	11.5±1.3	22.88±2.65*	18.23±0.87*	18.076±1.46*

BMI=Body Mass Index, FBG=Fasting Blood Glucose, BP=Blood Pressure Systole/Diastole, GHBAic=Glycated Hemoglobic, ADA= Adenosine Deaminase Activity.*P<0.05

Table 2. Correlation of BMI in the metabolic disease subjects

Variable	*Obese Diabetic (r ; P)*	*Obese non-diabetic. (r ; P)*	*Non-obese diabetic. (r ; P)*
BMI vs FBG	0.265; 0.200 NS	0.044; 0.834 NS	-0.112 ; 0.593 NS
BMI vs GHb	0.349; 0.088 NS	0.185; 0.376 NS	-0.182 ; 0.385 NS
BMI vs ADA	0.020; 0.335 NS	0.028; 0.896 NS	-0.149 ; 0.476 NS

BMI=Body Mass Index, FBG=Fasting Blood Glucose, GHBAic=Glycated Hemoglobic, ADA= Adenosine Deaminase Activity. NS, Not significant

Table 3. Correlation of FBG in the metabolic disease subjects

Variables	*Obese Diabetic (r ; P)*	*0bese non-diabetic (r ; P).*	*Non-0bese diabetic (r ; P)*
FBG vs GHb	0.886; 0.001	0.671; 0.001	0.909; 0.001
FBG vs ADA	0.934; 0.001	0.770; 0.001	0.593; 0.002

FBG=Fasting Blood Glucose, GHBAic=Glycated Hemoglobic, ADA= Adenosine Deaminase Activity

Table 4. Correlation between GHbA1c and ADA in the metabolic disease subjects

Variables	*Obese Diabetic(r ; P)*	*0bese non-diabetic (r ; P)*	*Non-0bese diabetic (r ; P)*
GHbA1c vs ADA	0.763; 0.001	0.582; 0.002	0.523; 0.007

GHBAic=Glycated Hemoglobic, ADA= Adenosine Deaminase Activity

Table 5. Cholesterol concentration (mg/dl) in control and test subjects of dyslipeademic patients

Variables	***Control* (mg/dl)**	***Test* (mg/dl)**
Hypercholesterol,n=25	158.0±31	386.0±60*
HDL-cholesterol,n=25	73.0±20	23.0±80*
LDL-cholesterol,n=25	87.9±26	231.5±40*

*P<0.05

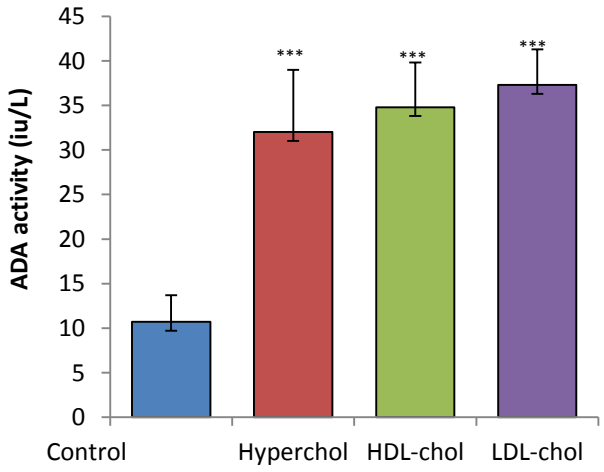

Figure 1. Comparison of ADA activity in apparently healthy subjects (control) and hyperlipidaemic patients. ***P<0.001

Table 6. Correlation of ADA activity in hypercholester-olaemic, HDL-cholestrolaemic and LDL- cholesterolaemic patients

Variables	*R*	*P-Value*
ADA vs Hypercholesterolaemia	0.640	0.001
ADA vs HDL-cholesterolaemia	0.646	0.001
ADA vs LDL-cholesterolaemia	0.932	0.001

HDL-cholesterolaemia and hypercholesterolaemia patients but the difference was not statistically significant (p< 0.05).

Table 6 shows the correlation of ADA activity in Hypercholesterolemia, HDL-cholesterolemia and LDL –cholesterolemia, showing highest correlation (r=0.932) between ADA activity and LDL-cholesterolaemic patients. The significant correlation between ADA and dyslipidemia shows that ADA is also an important factor in these three types of dyslipidemia. So, the higher the cholesterolaemia the higher the ADA activity.

DISCUSSION

The results of this study affirmed that metabolic syndrome is associated with deregulations in adenosine deaminase activity. This was shown in significantly

higher fasting blood glucose (FBG), glycated haemoglobin(GHbAic) and adenosine deaminase (ADA) activity in the obese diabetic (Obese NIDDM) and Obese non-diabetics (Obese non-NIDDM) groups of subjects as well as the dyslipidaemic (hypercholesterolaemic, LDL-cholesterolaemic and HDL-cholesterolaemic) subjects. The high positive correlation between fasting blood glucose (FBG), glycated haemoglobin (GHbAic) and adenosine deaminase in this study corroborates the fact that protein glycosylation is one of the complications of diabetes and obesity (Onyeanusi et al, 2003; Lelieurve et al, 2000). Our study also showed that all clinical profiles (especially FBG and ADA activity) of obese diabetic (Obese NIDDM) and non-Obese diabetics (non- Obese NIDDM) were significantly higher compared to control. The Obese non-diabetics (Obese non-NIDDM) subjects, however, showed higher metabolic compensatory hyperinsulinaemia which was reflected by the lower level of FBG in this group compared to obese diabetic and non-obese diabetic subjects. In the obese diabetics (obese-NIDDM), the compensatory mechanism was very low, reflected by increased FBG and the Adenosine Deaminase activity.

The elevated ADA activities found in the patients of dyslipidemias (hypercholesterolaemia, LDL-Cholestrolaemias, and HDL-Cholestrolaemia) had only been implied in an earlier study by Mensa et al (2002). Our study here showed increased ADA activity in the dyslipidaemic subjects and other cases of metabolic diseases, which in the presence of hyperglycemia forms the basis of the pathophysiology associated with these metabolic diseases.

Our results showed there was a significantly positive correlation between ADA activity and total cholesterol in the hypercholestrolaemic, and HDL-cholesterol (good cholesterol) in the HDL-cholestrolaemic subjects. However, there was a stronger positive correlation between ADA and LDL-cholesterol in the LDL-cholesterolaemic subjects. This research findings therefore indicate that ADA activities might be a predicting factor in the dyslipidaemias in particular, and metabolic diseases in general, and may be used as a diagnostic kit in the daily assessment of the metabolic syndrome. In conclusion, ADA can be used in daily routine laboratory assessment of most metabolic diseases especially in obese and diabetic patients. Thus, targeting ADA in the treatment of metabolic diseases would be very appropriate.

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