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ORIGNAL ARTICLE

Serum N-acetyl-β-D-glucosaminidase level assessment in type 2 diabetes mellitus patients with ischemic heart disease.

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ABSTRACT

Background: Ischemic heart disease has a great impact on morbidity and mortality in type2 diabetes mellitus patients. Type2Diabetes Mellitus (Type 2D.M) is risk factor for coronary atherosclerosis. N-acetyl-β-D-glucosaminidase (NAG) is an enzyme present in humans (IHD) in patients suffering from type 2 DM.

methods: cross-sectional study included 84 subjects; 21 healthy individuals, 21 patients with IHD; 21 with type 2 DM and 21 with type2DM with IHD, subjected to history taking, clinical examination, liver function tests, postprandial blood sugar, HbA1c, lipid profile, ECG, echocardiography, cardiac catheterization and carotid Doppler ultrasound. and N-acetyl –B-D-glucosaminidase level measurement

Results: There are a statistically significant differences between our groups as regard basic and laboratory data. There are differences between control group and diabetic group regarding WBCS (P=0.028), urea (p<0.001), creatinine (p=0.003). AST,fasting, 2hour postprandial blood sugar and HBA1C (p<0.001). There is a difference regarding NAG (p<0.001). There are direct correlations between NAG and BMI, SBP, DBP, WBCS,, triglycerides, fasting, 2 hours postprandial blood sugar and HbA1c. The best cutoff of serum NAG in diagnosis of ischemia in diabetic patients is > 0.692 U/L, AUC of 0.949, sensitivity of 95% and specifcity of 90.91%. The best cutoff of serum NAG in diagnosis of ischemia in diabetic patients is > 6.77 U/L, AUC of 0.884, sensitivity of 90.48% and specifcity of 95.24%.

Conclusion: Level of serum N-acetyl-β-D-glucosaminidase increases in type diabeticsmsuffering from ischemic heart disease.

Keywords: type 2 diabetes mellitus, ischemic heart disease, acetyl beta D glucosaminidase.

INTRODUCTION

International Diabetes Federation has reported that, in 2011 365 million people suffered from diabetes and the number is expected to increase up to 552 million come the year 2030 **(1)**.

Along many of complications of diabetes on the long run, cardiovascular diseases are responsible for greatly increasing morbidity and mortality in people suffering from D.M. About 80% of deaths in diabetics are due to cardiac diseases **(2).**

So, ischemic heart disease is an enormous cause of morbidity and mortality in type 2 diabetics, as diabetes enhances the possibility of ischemic heart diseases by two or three folds in comparison with patients without diabetes mellitus.Therefore, developing methods for detection of the risk of presence of ischemic heart disease in diabetic patients will help to prevent further complications of diabetes and ischemic heart disease **(3).** As it was found that Type 2 diabetics are in a great danger for coronary atherosclerosis but the mechanism is still not known for sure. Chronic hyperglycemia is highly associated with the pathophysiology of macrovascular and microvascular diseases **(4).** Even small changes in glucose metabolism may affect the onset of cardiovascular disease so it's important to find new approach for early detection of cardiac conditions as ischemic heart disease as in type 2 diabetic patients suffering from coronary atherosclerosis **(5).** Alot of markers for damage in renal tubules have raised great awareness because of their sensitivity and specificity for predicting development and deterioration of renal disease in patients with type 2 diabetes mellitus including N-acetyl-β-D-glucosaminidase **(6)**.

N-acetyl-β-D-glucosaminidase is a lysosomal enzyme which is distributed in human body. It is

released into serum as a result of cellular breakdown and because of its high molecular weight it is released from cells by exocytosis **(7).**

If there is a glomerular lesion or damage of kidney tubules, urinary levels of N-acetyl-β-D-glucosaminidase activity increases and so it posses great importance in diagnosis of renal diseases **(8)**.However, it was reported that serumN-acetyl-β-D-glucosaminidase activity is elevated in different diverse diseases as in hypertension, diabetes mellitus, and renal disease, which may indicate the presence of ischemic heart disease in these patients, so it is though

t to be related to changes in the cardiovascular system either functional or structural. **(9).**

Undiagnosed and untreated ischemic heart disease especially in patients with type 2 diabetes is also acompanied with a decreased quality of life and therefore, early diagnosis and treatment of ischemic heart disease among the older population is an important topic **(10).**

Despite its role as a marker in IHD, the role of N acetyl β D-glucosaminidase in Egyptian type 2 diabetic ischemic heart disease patients has not been fully investigated. The aim of this study is to use N-acetyl-β-D-glucosaminidase as an early marker of ischemic heart disease in patients with type 2 diabetes mellitus.

SUBJECTS AND METHODS

This cross-sectional study was carried out on patients suffering from type 2 diabetes mellitus over a period of 6 months during the year 2018 in the Departments of Internal Medicine and Medical Biochemistry, Zagazig University Hospitals to estimate the level of N-acetyl-β-D-glucosaminidase as an early marker of ischemic heart disease in patients suffering from diabetes mellitus type 2. 84 patients included in the present study were classified into 4 groups. **Group i:** 21 Healthy subjects as a control group. They are healthy volunteers invited by public invitation; they are age and sex and matched, **Group ii:** 21 Non-diabetic patients with ischemic heart disease, **Group iii:** 21 Patients with type 2 diabetes mellitus without ischemic heart disease, **Group iv:** 21 Patients with type 2 diabetes mellitus and ischemic heart disease.

Inclusion criteria includes Patients age from 50 to 70 years old, Both male, and females were included. **Exclusion criteria** includes age below 50 or above 70 years and associated malignancy.

METHODS

Careful history taking and Clinical examination with special emphasis on blood pressure, heart rate, body temperature, respiratory rate, Manifestations of terminal liver disease (jaundice, liver cirrhosis, splenomegaly and ascites), uremic manifestations, etc….

Full routine investigations for all groups including: CBC, Liver function tests including (SGPT and SGOPT) and kidney function tests (serum urea and serum creatinine), Investigations to diagnose diabetes in form of fasting and 2 hours postprandial blood sugar and glycosylated Hemoglobin A1C, Lipid profile in the form of HDL-Cholesterol, LDL-Cholesterol, serum triglycerides and serum Cholesterol

Special investigations in the form of: Electrocardiography (ECG), Echocardiography, Cardiac catheterization for all patients with ischemic heart disease. And Carotid Doppler ultrasound in diabetic patients without ischemic heart disease.

Serum –N-acetyl –B-D-glucosaminidase Detection range: 4-50 U/L. Serum –N-acetyl –B-D-glucosaminidase level measurement in all patients. This kit was based on standard sandwich enzyme-linked immune-sorbent assay technology NAG was measured in a serum sample that was obtained from each participant . The serum NAG level was measured by a colorimetric method.

Principle of the assay: This kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. The purified anti-NAG antibody was precoated onto 84-well plates, and the HRP-conjugated anti-NAG antibody was used as detection antibodies. The standards, test samples and HRP-conjugated detection antibody were added to the wells subsequently, mixed and incubated, then, unbound conjugates were washed away with wash buffer. TMB substrates (A and B) were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the NAG amount of sample captured in plate. Read the OD absorbance at 450 nm in a microplate reader, and then the concentration of NAG can be calculated.

STATISTICAL ANALYSIS

:Data analysis was performed using the software SPSS(statistical package for the social science)version 20.qusantitative variables were described using their means and standard deviations.categorical variation were described using their absolute frequencies and to compare the proportion of categorical data, chi square test and fisher exact test were used when appropriate . Pearson correlation method will be used to analys results statistically. Kolmogrov-smirnov (distribution type) and Levene (homogeneity of variances) tests were used to verify assumptions for use in parametric test. To compare means of two groups , independent sample t test was used when

appropriate .nonparametric test(mann whitney) was used to compare means when data was not normally distributed and to compare medians in categorical data. To compare means of more than two groups , one way ANOVA was used for normally distributed data and kruskal wallis test was used for data which was not normally distributed . ROC curve analysis was used to asses the best cut off of studied parameters . the level statistical significance was set at 5% (*p<0.05)*. highly significant difference was present if *p*≤0.001 .

Administrative design:

Written informed consent was obtained from all participants, the study was approved by the research ethical committee of Faculty of Medicine, Zagazig University. The study was done according to The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

RESULTS

There is statistically significant difference regarding (WBCS, Urea, Creatinine, AST, Triglycerides, Total serum Cholesterol , LDL - Cholesterol, Fasting and 2 Hour post prandial blood suger, HBAIC). BMI, Kg/m2 is highly significant P<0.001, in Ischemic, Diabetic and DM & Ischemic group in comparison to control group.

There is statistically significant difference regarding (WBCS, Urea, Creatinine, AST, Triglycerides,Total Cholesterol , LDL, Fasting and 2 Hour post prandial blood sugar, HBAIC). (table 1).In IHD patients, Electrocardiography (ECG), Echocardiography, Cardiac catheterization showed ischemic change and they were managed by cardiology staff doctors either medically or by interventional treatment.

In diabetic patients without ischemic heart disease, there was an overall increase in IMT measured by carotid doppler. A significant number of patients (18 patients) with increased lesion intima media thickness (≥ 1.1 mm).

There is statistically significant difference regarding NAG values among the studied groups (table 3).

There is direct correlation in table (4) between NAG and certain parameters which are: (BMI, SBP, DBP, WBCS, Urea, Triglycerides, Fasting and 2 Hour post prandial blood sugar , HBA1C).

There is direct correlation in table (5) between NAG and certain parameters in the control group which are (SBP, HBA1C).

In our study, the best cutoff of serum NAG in diagnosis of ischemia in diabetic patients is > 0.692 U/L with AUC of 0.949, sensitivity of 95%, specifcity of 90.91%, PPV of 90.5% and NPV of 95.2%. But, the best cutoff of serum NAG in diagnosis of ischemia in non-ischemic diabetic patients is > 6.77 U/L with AUC of 0.884, sensitivity of 90.48%, specifcity of 95.24%, PPV of 95% and NPV of 90.9% (figures 1, 2).

Table (1): Comparison of the Laboratory values among the studied groups

	Group				***Total N=84***	***F***	***Sig.***
	Control N=21	**Ischemic N=21**	**Diabetic N=21**	**DM & Ischemic N=21**			
WBC, x109L	6.1 ± 1.9	7.7 ± 2.1	7.6 ± 2.1	7.6 ± 1.9	7.2 ± 2.1	3.2	0.028
Hb, g/dl	12.6 ± 1	12 ± 1.2	12.3 ± 1.1	12.6 ± 1.2	12.4 ± 1.1	1.1	0.362
PLT, x109L	249.8 ± 87.4	254.2 ± 48.7	333.8 ± 361.1	248.3 ± 75.8	271.5 ± 191.1	1.0	0.4
Urea, mg/dL	28.3 ± 3.4	28.6 ± 5.6	48.9 ± 12.1	34 ± 7.3	35 ± 11.3	32.5	**<0.001**
Cr, mg/dL	1 ± 0.2	0.9 ± 0.2	1.2 ± 0.4	0.9 ± 0.2	1 ± 0.2	4.9	0.003
ALT, IU/L	25.5 ± 4.8	23.1 ± 8	27.8 ± 5.7	26 ± 7.7	25.6 ± 6.8	1.7	0.165
AST, IU/L	22.7 ± 4.8	31.1 ± 8.4	32.5 ± 7.3	24.8 ± 6.2	27.8 ± 7.9	10.3	**<0.001**
TG, mg/dL	73.6 ± 11.9	193.2 ± 73.4	104.9 ± 52.3	150 ± 65.6	130.4 ± 71.5	18.3	**<0.001**
TC, mg/dL	140 ± 41.3	279.4 ± 84.4	167.9 ± 79	192 ± 92.6	194.8 ± 91.9	12.9	**<0.001**
HDL, mg/dL	41.8 ± 7.6	42 ± 12.7	40.4 ± 9.7	40.8 ± 12.1	41.2 ± 10.5	0.1	0.958

	Group				Total N=84	F	Sig.
	Control N=21	Ischemic N=21	Diabetic N=21	DM & Ischemic N=21			
LDL, mg/dL	83.6 ± 40.6	199 ± 74.9	106.5 ± 65	121.3 ± 79.2	127.6 ± 78.6	11.9	**<0.001**
FBS, mg/dL	80.1 ± 11.8	83.2 ± 13.8	213.5 ± 49.1	193.5 ± 68.8	142.6 ± 74.9	56.5	**<0.001**
2HPP, mg/dL	96.8 ± 10.2	108.5 ± 5.4	326.1 ± 63	285.6 ± 96.3	204.3 ± 117.9	88.2	**<0.001**
HbA1C, g/dL	4.2 ± 0.4	3.9 ± 0.5	7.5 ± 1.1	6.9 ± 1.3	5.6 ± 1.9	88.3	**<0.001**

Wbc : white blood cells
Hb : hemoglobin
Plt : platelets
Cr : creatinine
Tg: triglycerides
Tc: total cholesterol
Fbs : fasting blood sugar
2hpp : 2 hour post prandial
Alt : alanine transaminase
Ast : aspartate transaminase
Hdl : high density lipoprotein
Ldl : low density lipoprotein

Table (2): Comparison of clinico-demographic data and lab values among the studied groups

		Group				Total N=84	Test	P
		Control N=21	Ischemic N=21	Diabetic N=21	DM & Ischemic N=21			
Sex	F	11 (52.4%)	14 (66.7%)	10 (47.6%)	17 (81.0%)	52 (61.9%)	6.1*	0.109
	M	10 (47.6%)	7 (33.3%)	11 (52.4%)	4 (19.0%)	32 (38.1%)		
Age, years		61.4 ± 5.7	59.9 ± 5.9	60.5 ± 14.2	60.8 ± 5.7	60.6 ± 8.5	0.1	0.953
BMI, Kg/m2		23.3 ± 2.7	29.1 ± 4.4	31 ± 4.5	29.8 ± 4.1	28.3 ± 4.9	15.2	**<0.001**
WC, Cm		79.6 ± 12.4	87.2 ± 11.8	84.1 ± 20.5	87.3 ± 9.3	84.5 ± 14.2	1.4	0.252
SBP, mmHg		111 ± 14.1	121.9 ± 23.4	119.1 ± 15.1	121.4 ± 16.5	118.3 ± 17.9	1.7	0.166
DBP, mmHg		74.8 ± 9.8	78.1 ± 15	76.7 ± 10.2	85.2 ± 12.1	78.7 ± 12.4	3.1	0.032
WBC, x109L		6.1 ± 1.9	7.7 ± 2.1	7.6 ± 2.1	7.6 ± 1.9	7.2 ± 2.1	3.2	0.028
Hb, g/dl		12.6 ± 1	12 ± 1.2	12.3 ± 1.1	12.6 ± 1.2	12.4 ± 1.1	1.1	0.362
PLT, x109L		249.8 ± 87.4	254.2 ± 48.7	333.8 ± 361.1	248.3 ± 75.8	271.5 ± 191.1	1.0	0.4
Urea, mg/dL		28.3 ± 3.4	28.6 ± 5.6	48.9 ± 12.1	34 ± 7.3	35 ± 11.3	32.5	**<0.001**
Cr, mg/dL		1 ± 0.2	0.9 ± 0.2	1.2 ± 0.4	0.9 ± 0.2	1 ± 0.2	4.9	0.003
ALT, IU/L		25.5 ± 4.8	23.1 ± 8	27.8 ± 5.7	26 ± 7.7	25.6 ± 6.8	1.7	0.165
AST, IU/L		22.7 ± 4.8	31.1 ± 8.4	32.5 ± 7.3	24.8 ± 6.2	27.8 ± 7.9	10.3	**<0.001**
TG, mg/dL		73.6 ± 11.9	193.2 ± 73.4	104.9 ± 52.3	150 ± 65.6	130.4 ± 71.5	18.3	**<0.001**
TC, mg/dL		140 ± 41.3	279.4 ± 84.4	167.9 ± 79	192 ± 92.6	194.8 ± 91.9	12.9	**<0.001**

	Group				*Total N=84*	*Test*	*P*
	Control N=21	**Ischemic N=21**	**Diabetic N=21**	**DM & Ischemic N=21**			
HDL, mg/dL	41.8 ± 7.6	42 ± 12.7	40.4 ± 9.7	40.8 ± 12.1	41.2 ± 10.5	0.1	0.958
LDL, mg/dL	83.6 ± 40.6	199 ± 74.9	106.5 ± 65	121.3 ± 79.2	127.6 ± 78.6	11.9	**<0.001**
FBS, mg/dL	80.1 ± 11.8	83.2 ± 13.8	213.5 ± 49.1	193.5 ± 68.8	142.6 ± 74.9	56.5	**<0.001**
2HPP, mg/dL	96.8 ± 10.2	108.5 ± 5.4	326.1 ± 63	285.6 ± 96.3	204.3 ± 117.9	88.2	**<0.001**
HbA1C, g/dL	4.2 ± 0.4	3.9 ± 0.5	7.5 ± 1.1	6.9 ± 1.3	5.6 ± 1.9	88.3	**<0.001**

Wc : waist circumference
Sbp : systolic blood pressure
Dbp : diastolic blood pressure
U/L : unit / litre
Nag u/l values: –N-acetyl –B-D-glucosaminidase value

Table (3): Comparison of the NAG values among the studied groups

		Group				*Total N=84*	*F*	*Sig.*
		Control N=21	**Ischemic N=21**	**Diabetic N=21**	**DM & Ischemic N=21**			
NAG (U/L)	*Mean±SD*	0.4 ± 0.2	4.5 ± 1.2	5.2 ± 0.9	8.8 ± 0.8	4.7 ± 3.1	330	**<0.001**
	Median (range)	0.4 (0.1-0.7)	4.2 (2.7-6.7)	4.8 (4.2-6.8)	9 (7.4-9.9)	4.7 (0.1-9.9)		

Nag u/l values: –N-acetyl –B-D-glucosaminidase value
U/L : unit / litre

Table (4): LSD Post-hoc test, to indicate which group is significantly different from each other

	Control Vs. Ischemic	**Control Vs. Diabetic**	**Control Vs. DM & Ischemic**	**Ischemic Vs. Diabetic**	**Ischemic Vs. DM & Ischemic**	**Diabetic Vs. DM & Ischemic**
WBC, x109L	**0.011**	**0.017**	**0.013**	**0.884**	**0.963**	**0.921**
Hb, g/dl	**0.132**	**0.409**	**1**	**0.491**	**0.132**	**0.409**
PLT, x109L	**0.94**	**0.159**	**0.979**	**0.181**	**0.92**	**0.151**
Urea, mg/dL	**0.905**	**<0.001**	**0.021**	**<0.001**	**0.029**	**<0.001**
Cr, mg/dL	**0.791**	**0.004**	**0.894**	**0.002**	**0.894**	**0.002**
ALT, IU/L	**0.261**	**0.261**	**0.782**	**0.026**	**0.163**	**0.396**
AST, IU/L	**<0.001**	**<0.001**	**0.332**	**0.527**	**0.003**	**<0.001**
TG, mg/dL	**<0.001**	**0.075**	**<0.001**	**<0.001**	**0.014**	**0.011**
TC, mg/dL	**<0.001**	**0.243**	**0.031**	**<0.001**	**<0.001**	**0.314**
HDL, mg/dL	**0.954**	**0.677**	**0.774**	**0.636**	**0.731**	**0.897**
LDL, mg/dL	**<0.001**	**0.269**	**0.071**	**<0.001**	**<0.001**	**0.473**
FBS, mg/dL	**0.817**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.138**
2HPP, mg/dL	**0.512**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.026**
HbA1C, g/dL	**0.329**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.037**

Table (5): Correlations between NAG and certain studied parameters in the whole group

All patients	***NAG, U/L***	
	r	***P***
Age, years	-0.031	0.78
BMI, Kg/m2	0.417	**<0.001**
WC, Cm	0.167	0.129
SBP, mmHg	0.256	0.019
DBP, mmHg	0.31	0.004
WBC, x109L	0.287	0.008
Hb, g/dl	-0.05	0.652
PLT, x109L	-0.02	0.859
Urea, mg/dL	0.259	0.018
Cr, mg/dL	0.044	0.689
ALT, IU/L	-0.007	0.946
AST, IU/L	0.093	0.4
TG, mg/dL	0.34	0.002
TC, mg/dL	0.163	0.139
HDL, mg/dL	-0.014	0.901
LDL, mg/dL	0.13	0.237
FBS, mg/dL	0.549	**<0.001**
2HPP, mg/dL	0.604	**<0.001**
HbA1C, g/dL	0.576	**<0.001**

r = Correlation Coefficient

P ≤ 0.05= significantP<0.001highly significant and P >0.05 Non-significant

Table (6): Correlations between NAG and certain studied parameters within each group

	Control		***Ischemic***		***Diabetic***		***DM & Ischemic***	
	r	P	r	P	r	P	r	P
Age, years	-0.398	0.074	-0.355	0.114	0.092	0.692	0.291	0.2
BMI, Kg/m2	-0.315	0.164	-0.205	0.372	-0.351	0.119	-0.285	0.211
WC, Cm	0.125	0.589	-0.033	0.886	-0.103	0.655	-0.054	0.817
SBP, mmHg	0.455	0.038	0.231	0.314	0.326	0.149	0.063	0.786
DBP, mmHg	0.169	0.465	0.148	0.523	0.149	0.52	0.047	0.84
WBC, x109L	0.326	0.15	0.228	0.32	-0.047	0.838	0.01	0.966
Hb, g/dl	-0.157	0.498	-0.055	0.814	-0.263	0.25	-0.356	0.113
PLT, x109L	0.303	0.182	-0.014	0.951	-0.242	0.291	-0.116	0.616
Urea, mg/dL	0.023	0.92	-0.019	0.935	0.364	0.104	-0.111	0.632
Cr, mg/dL	0.31	0.172	-0.003	0.99	0.105	0.651	0.175	0.447
ALT, IU/L	0.409	0.066	-0.217	0.344	-0.298	0.19	-0.255	0.265
AST, IU/L	0.151	0.515	0.003	0.99	-0.258	0.258	-0.135	0.561
TG, mg/dL	0.036	0.875	0.128	0.579	0.145	0.531	-0.33	0.144
TC, mg/dL	0.203	0.377	0.053	0.82	0.212	0.355	-0.426	0.054
HDL, mg/dL	0.205	0.373	0.094	0.685	0.389	0.081	-0.245	0.284
LDL, mg/dL	0.168	0.467	0.019	0.936	0.176	0.445	-0.406	0.068
FBS, mg/dL	-0.375	0.094	-0.167	0.471	-0.195	0.397	-0.154	0.505
2HPP, mg/dL	-0.292	0.199	-0.105	0.649	0.244	0.287	-0.187	0.418
HbA1C, g/dL	0.609	0.003	0.105	0.649	0.258	0.258	-0.068	0.768

r = Correlation Coefficient

P ≤ 0.05= significantP<0.001highly significant and P >0.05 Non-significant

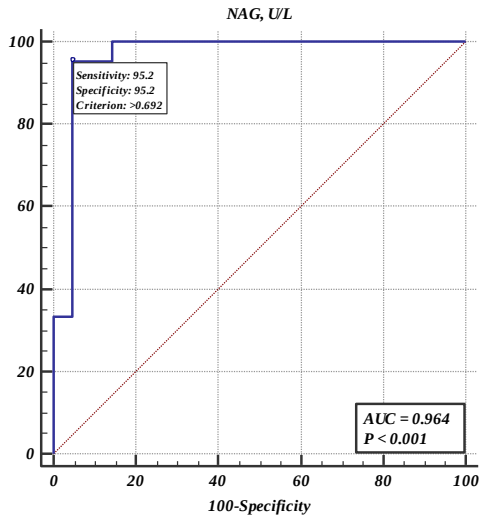

Figure (1): The ROC curve of NAG as a diagnostic marker for Ischemia in patients with DM compared to healthy controls.

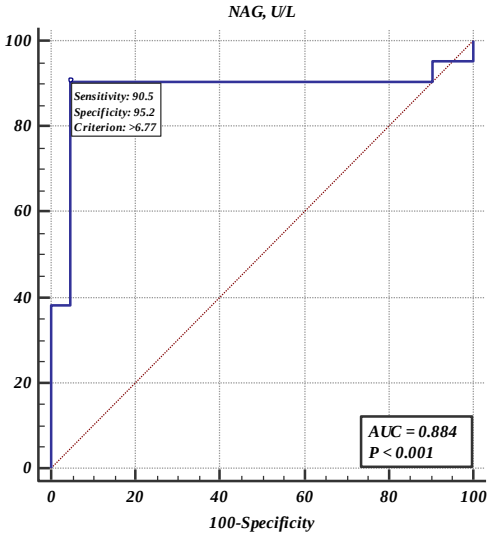

Figure (2): The ROC curve of NAG as a diagnostic marker for Ischemia in patients with DM compared to non-ischemic diabetics

DISCUSSION

Cardiovascular disease (CVD) has a great impact on morbidity and mortality in type 2 diabetes mellitus (T2D) patients. The risk of CVD incidence increases two to fourfold in comparing to non-diabetes. IHD is the most common single cause of morbidity and mortality in the Western world. According to the American Heart Association Statistics Committee one third of individuals has a forms of CVD **(11).**Estimation the risk of CVD in diabetic patients with more accuracy is highly advocated and will aid in preventing CVD events. Several surrogate measures for CVD that estimate subclinical atherosclerosis have been reported. Approved surrogates include pulse wave velocity (PWV), carotid artery intima-media thickness (IMT), the presence of carotid plaques, and albuminuria. Type 2 diabetes mellitus (T2D)-related complications has also become a public health issue **(5).**Several markers of renal tubular damage have gained considerable attention because of their clinical significance as sensitive and specific biomarkers for predicting ischemic heart disease **(12)**.NAG excretion in urine is increased by injury of proximal renal tubular cells. Furthermore, increases in NAG already occur in normal to mildly increased albuminuric patients with T2D **(13)**.NAG is associated with complications rather than nephropathy like vascular complications of T2D, including retinopathy, neuropathy, and macrovascular disease **(14)**. Regarding clinico-demographic data, there is high statistically significant difference between the four groups regarding BMI (P<0.001). Diabetic and DM and Ischemic group compared to control group. obesity has been found to contribute to approximately 55% of type 2 diabetes **(15)** So obesity and diabetes all appear to aggravate many of the vascular alterations elicited by ischemia and reperfusion. In the study of **Kim et al (12) They** found that urinary NAG was associated with age, duration of diabetes, and BMI with positive

correlation between urinary NAG and both age and diabetes duration and negative correlation between urinary NAG and BMI. **Han et al (15)** Showed that a distinctive pattern was observed between urinary NAG with both age and BMI: an elevated urinary NAG was more closely associated with older age and was not significantly linked with BMI.

In our study, There was a statistically significant difference between the four groups regarding WBCS, urea, creatinine, AST, triglycerides, total cholesterol, LDL, fasting and 2 hour postprandial blood suger and HbA1c. On LSD comparison, there are significant differences between control group and ischemic group regarding WBCS, AST, Triglycerides, Total Cholesterol and LDL. There are significant differences between control group and diabetic group regarding WBCS, urea, creatinine, AST, fasting, 2hour postprandial blood sugar and HBA1C. There are significant differences in control group versus diabetic and ischemic groups regarding WBCS, urea, triglycerides, total cholesterol, fasting, 2hour postprandial blood sugar and HbA1c. we observed significant differences between ischemic group and diabetic group regarding urea, creatinine, ALT, triglycerides, total cholesterol, LDL, fasting, 2 hour postprandial blood sugar and HBA1C. There are significant differences in ischemic group versus diabetic group versus diabetic and ischemic groups regarding urea, AST, triglycerides, total cholesterol, LDL, fasting, 2 hour postprandial blood sugar and HBA1C. There are significant differences in diabetic group vs diabetic and ischemic groups regarding urea, creatinine, AST, triglycerides, 2 hour postprandial blood sugar and HBA1C. Regarding NAG values measurement, there was a statistically significant difference regarding NAG values among the studied groups.

Kim et al (16) found that, Group II patients had significantly higher blood glucose levels [HbA1C, GA, and basal and stimulated (postprandial) glucose] than those in Group I. Lipid profiles and serum creatinine did not significantly differ between the groups. **Han et al (15)** study showed that urinary NAG was positively correlated with HbA1c (r = 265, P<0.00) .Also in our study there is direct correlation between HBA1C levels and NAG levels in all of the studied groups.

In their study**, Inoue et al (17)** found that in all 168 patients, there is no significant difference in serum NAG activity between males and females (8.7± 2.3 vs. 8.1±2.8 U/l). The serum NAG activity was 9.2±2.3 U/l in the multi-vessel disease group, which was higher than 7.8±1.8 U/l (*P*<0.01) in the no stenotic lesion group and 8.2 ± 2.2 U/l (*P*<0.05) in the single-vessel disease group .In 126 patients without apparent diabetes mellitus, the value was 8.2±1.7, 8.5±2.8 and 9.3±2.3 U/l, respectively in the groups of no stenotic lesion (*n*=30), single-vessel disease(*n*=47) and the multi-vessel disease (*n*=49), and also higher (*P*<0.05) in the multi-vessel disease group than in the no stenotic lesion group. **Kim et al (16)** found that the median value of urinary NAG was 7.21 U/g creatinine.

In our whole group, there are direct correlations between NAG and certain parameters which are BMI, SBP, DBP, WBCS, urea, triglycerides, fasting, 2 hour postprandial blood sugar and HbA1c. But, there are direct correlations between NAG and certain parameters in the control group which are and SBP and HbA1c and carotid IMT . **Kim et al. (2017)** correlated urinary NAG positively with maximum carotid IMT and mean of maximum carotid IMT.

In our study, the best cutoff of serum NAG in diagnosis of ischemia in diabetic patients is > 0.692 U/L with AUC of 0.949, sensitivity of 95%, specifcity of 90.91%, PPV of 90.5% and NPV of 95.2%. But, the best cutoff of serum NAG in diagnosis of ischemia in non-ischemic diabetic patients is > 6.77 U/L with AUC of 0.884, sensitivity of 90.48%, specifcity of 95.24%, PPV of 95% and NPV of 90.9%.

In the study done by **Kim et al (16)**, when increased carotid IMT was defined as an IMT of >1 mm, the sensitivity of urinary NAG (>7.21 U/g creatinine; median value) and urinary ACR (≥30 mg/g creatinine) to identify an increased mean of maximum carotid IMT was 64.9 and 31.6%, respectively. **Weitgasser et al (18)** reported the relation between urinary NAG and macrovascular disease in elderly T2D patients. They investigated patients with T2D during a median follow-up of 7 years and stated that urinary NAG is of value as albuminuria as an indicator of the preexistence and development of severe macrovascular disease, including myocardial infarction and peripheral vascular disease. **Jungbauer et al (19)** assessed whether NAG is related to deterioration of chronic kidney disease in patients with chronic heart failure. And concluded that NAG is a potential cardiorenal biomarker due to its prognostic capability regarding cardiac and renal events in high-risk patients. **Kim et al (16)** found that elevated urinary NAG, a marker of renal tubular damage, was related to increased carotid IMT and the presence of carotid plaques in patients with T2D. Urinary NAG may be a more sensitive biomarker than urinary albumin for early detection of atherosclerosis. Regarding subjects without diabetes, **Ouchi et al (20)** showed that elevated urinary NAG is associated with arterial stiffness assessed by brachial-ankle PWV. In a study including a low-risk general population, urinary NAG and ACR were independently associated with first-ever myocardial infarction, first-ever

ischemic stroke, and all-cause mortality. However, NAG did not add to the risk predicted by traditional cardiovascular risk factors, such as eGFR and ACR **(21).**This study has limitations such as small number of subjects, wide further improvements are needed in future study.

Conclusion: Serum level of serum N-acetyl-β-D-glucosaminidase is elevated in patients with ischemic heart diseae and type 2 diabetes mellitus.

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