ROLE OF FETUIN-A IN ANGIOPATHY IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

Sara Mahmoud Samy Mohamed
EbtehagHelmy Hassan,HodaAbdeen,AmiraShokry
Department of clinical Pathology, Faculty of Medicine, Zagazig University, Egypt

ABSTRACT

Background: Fetuin-A is a negative acute phase protein known to inhibit insulin signaling and pathologic calcification, has emerged as a promising candidate biomarker for diabetes risk.
Aim of work: Evaluate the role of fetuin-A in type 2 diabetes patients &its relation to angiopathy.
Subjects & Method: Serum Fetuin-A levels, were assessed in 52 patients with T2DM and 30 normal glucose tolerance (NGT) using enzyme linked immunosorbont assay (Elisa).
Results: T2DM subjects had significantly higher Fetuin-A levels than NGT subjects (420.7± 115.8 mg/l vs 215.6± 19.7 mg/l, *P*< 0.001).In the Pearson's correlation coefficients, Fetuin-A levels and clinical parameters: Fetuin-A was positively correlated with HOMA-insulin resistance index (HOMA-IR),), HbA1c, triglyceride (TG), Low density lipoprotein cholesterol (LDL-C) , fasting plasma glucose (FBG) and 2 h post-prandial blood glucose (2 h pp) (*P* < 0.001), but negatively with High-density lipoprotein cholesterol (HDL-c).
Conclusions: Our study suggests that the serum Fetuin-A levels may be associated with macro angiopathies in T2DM patients. Therefore, detecting early serum Fetuin-A levels inT2DM provides an opportunity to intervene of Atherosclerosis in diabetic patients and giving timely treatment for the prevent of diabetic vascular complications.
Keywords: type 2 diabetes mellitus, Fetuin-A, Atherosclerosis.

INTRODUCTION

Atherosclerosis (AS) may be the most common macrovascular complication of type 2 diabetes(T2DM) and therefore, will emerge as the leading causes of death in these patients. The risk of AS patients with T2DM is higher than those with normal glucose tolerance [1]. Patients with type 2 diabetes mellitus (T2DM) may not have typical of clinical manifestations. Thehigh blood sugar is only for the main performance of T2DM. So, early detection in high risk groups can take effective preventive measures to reduce cardiovascular mortality inpatients with T2DM. [2].Fetuin-A, which is a glycoprotein secreted by the liver, has emerged as a promising candidate biomarker for diabetes risk [3-7]. High levels of Fetuin-A appear to be related with insulinresistance [8]. A previous study showed that Fetuin-A induces insulin resistance by inhibiting insulin receptor autophosphorylation[9].

Additionally, Fetuin-A is shown to inhibit ectopic calcium deposition and protect from vascular calcification [10].

Thus, the aim of this study was to investigate serum Fetuin-A levels in patients with T2DM and to analyze the association of Fetuin-A levels with Atherosclerosis in patients with T2DM.

ROLE offetuin-A in type 2 diabetes:

Several studies have demonstrated that fetuin-A was found to affect glucose metabolism and play a significant role in regulating insulin sensitivity, weight gain, and fat accumulation..[8].

High levels of circulating fetuin-A are directly associated with insulin resistance and dyslipidaemia because it inhibits the insulin-stimulated autophosphorylation of the insulin receptor by its reversible binding to the extracellular domain of insulin receptor-tyrosine kinase in peripheral tissues and decrease the rate of auto-phpsphorylation and subsequent downstream intracellular signaling cascades[11].

MATERIALS AND METHODS

Collection of samples:

5 ml of venous blood were collected under complete a septic conditions, 2 ml collected on EDTA for glycated HB, 3 ml in serum separator tube(SST) and allow to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Serum sample was separated and used in routine laboratory investigation and store samples at ≤ -20 C.

All Patients and controls were subjected to the following assessment:

1-Complete history taking
2-clinical evaluation
3-Complete blood count
4-Liver function test

3-Routine laboratory investigations:

1- Fasting serum glucose
2-hour post prandial serum glucose
3-Glycatedheamoglobin
4-Fasting insulin
5-Lipids profile (Cholesterol, Triglyceride, HDl ,LDl).
6-Specific laboratory investigations: determination of serum level of fetuin-A by ELISA.

Measurement of serum Fetuin-A levels :

Serum Fetuin-A levels were measured with a commercially available ELISA kit.

1.All reagents, samples and standards were prepared

2. Prepared samples, standards and ELISA solutions were added. Let them react for 60 minutes at 37 ℃.

3. The plate was washed five times .Chromogen solution A and B was added. Incubate for 10 minutes at 37 ℃; for color development.

4. Stop solution was added

5. Reading the OD value within 10minutes. 6. Calculated.

Statistical Analysis

Data collected throughout history, basic clinical examination, laboratory investigations and outcome measures coded, entered and analyzed using Microsoft Excel software. Data were then imported into Statistical Package for the Social Sciences(SPSS version20.0) (Statistical Package for the Social Sciences)software for analysis. According to the type of data qualitative represent as number and percentage, quantitative continues group represent by mean ± SD , the following tests were used to test differences for significance;. difference and association of qualitative variable by Chi square test (X^2). Differences between parametric quantitative independent groups by t test, correlation by Pearson's correlation and agreement by Kappa agreement. P value was set at <0.05 for significant results &<0.001 for high significant result

RESULTS

Table 1:

Characteristic	T2DM	Control
Age (years)	54.1± 8.8	51.7 ± 6.1
Gender (female/male)	27 /25	17/13
FPG(mg/dl)	151.01± 19.7	83.36±7.47
2Hpp (mg/dl)	249.67 ±60	113.23 ± 6.9
FINS (IU/ml)	8.26±.449	5.18±.448
TC(mg/dl)	249.4± 47.5	171.6 ± 17.8
TG (mg/dl)	188.1 ±33.5	120.9 ± 18.8
LDL-C(mg/dl)	182±48	95.7± 20.7
HDL-C(mg/dl)	27.4 ±7.9	51.30 ± 6.9
HOMA-IR	3.02± .44	1.02 ± .14
HbA1C(%)	8.09±1.13	4.47±.78
Fetuin-A(mg/l)	420.7±115.8	215.6± 19.7

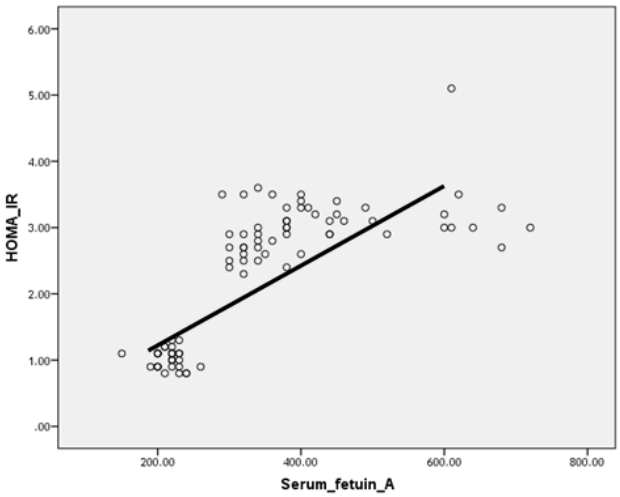
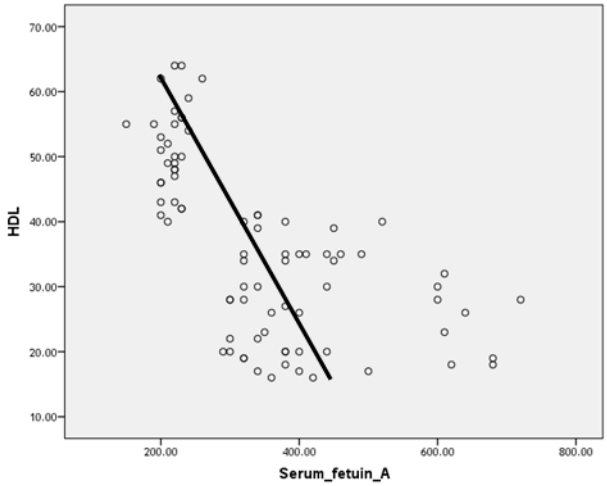
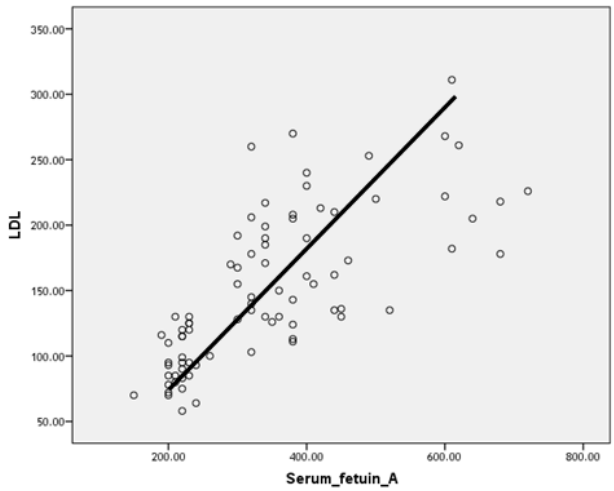


FIG.(I) :Correlation between the level of Fetuin- A and HOMA_IR among diabetic patients.



FIG(II) :Correlation between the level of Fetuin - A and HDL (mg/dl)among diabetic patients



FIG(III) :Correlation between the level of Fetuin-A andLDL(mg/dl) among diabetic patients

DISCUSSION

Fetuin-A is a multi functional molecule secreted by the liver [15].In the context of previous studies have demonstrated that Fetuin-A hasemerged as a biomarker for risk of type 2 diabetes[3].

Type 2 diabetes mellitus is a major risk factor for cardiovascular disease[12].In the present study, we found that serum Fetuin-A levels were elevated in patients withT2DM compared with NGT group.Dyslipidemia is elevation of total cholesterol, triglyceride and

small dense LDL-C concentrations with a low HDL-C levels. HDL-C has a protective role in atherosclerosis, because it can remove cholesterol from cells in the artery wall [13].

The present study showed that the mean values of the level of fetuin-A were significantly increase in all diabetic patients when compared to the control group ($p<0.001$).

This is in agreement with ***(Keskin .et al.,2017)*** who found a significant increase in Fetuin-A level in type 2 diabetes patients when compared to non diabetics.

This is also in agreement with***(Ishibashi ,et al.,2010)***who found that the higher serum fetuin-A levels are associated with insulin resistance and type 2 diabetes mellitus.

The relationship between fetuin-A and T2DM may be genetic origin because of The gene encoding fetuin-A located on chromosome 3q27.This chromosomal region was previously mapped as a type 2 diabetes and metabolic syndrome susceptibility locus***(Cheyssac,et al.,2006).***

Dutta et al. 2014investigated the effect of fetuin-A in pre diabetic population in India and found that fetuin-A is important predictor for progression of pre diabetes to diabetes .

Similarly to our study,***Yin et al. 2015***evaluated fetuin-A levels in T2DM for preventing diabetic vascular complication and found that fetuin-A was positively correlated with carotid intima media thickness, TG, LDL, HOMA-IR, fasting plasma glucose .

A previous study have demonstrated that plasma Fetuin-A levels is absolutely related with visceral obesity and dyslipidemia[19]. In agreement with previous studies, we demonstrated that higher fetuin-A concentrations were independently associated with clinical parameters. In the current study, we found that serum Fetuin-A levels was positively and significantly correlated with LDL-C and triglyceride, but negatively with HDL-C. In addition, serum Fetuin-A levels were associated with HbA1c, FBG and 2 hpp. However, in contrast to our study, a previous study reported that Fetuin-A showed no significant correlations with clinical parameters [20].Another study reported that Fetuin-A levels do not correlate with some clinical parameters as total cholesterol, HDL and triglyceride in T2DM patients with early diabetic nephropathy [21]

CONCLUSION

From this study, it could be concluded that:

1-Fetuin-A serum level was significantly increased in diabetic patients when compared to control group,thus Fetuin-A should be consider a diagnostic marker in type 2 diabetes.

2-High human fetuin-A level is strongly associated with insulin resistance (which play a major role of atherosclerosis) and dyslipidemia and its level could beneficial in early detection and prevention of this disease.

REFERENCES

[1]**Pyörälä K, Laakso M, Uusitupa M.*(1987):*** Diabetes and atherosclerosis :an epidemiologic view.Diabetes Metab Rev 1987; 3: 463-524.

[2]Chatterjee, KKhunti, MJ Davies - The Lancet,(2017):Type 2 diabbetes.

[3]**Sun Q, Cornelis MC, Manson JE, et al.,(2013):.** Plasmalevels of fetuin-A and hepatic enzymes and riskof type 2 diabetes in women in the U.S. Diabetes 2013; 62: 49-55.

[4]**Ix JH, Wassel CL, Kanaya AM, et al.,(2008);** Health ABC Study.Fetuin-A and incident diabetes mellitus in older persons. JAMA 2008; 300: 182-188

[5]**Ix JH, Biggs ML, Mukamal KJ,et al(2012):.** Associationof fetuin-a with incident diabetes mellitus incommunity-living older adults: thecardiovascular health study. Circulation 2012; 125: 2316-2322.

[6]**Stefan N, Fritsche A, Weikert C,et al.,(2008):.**Plasma fetuin-Alevels and the risk of type 2 diabetes. Diabetes2008; 57: 2762-2767.

[7]**Rasul S, Wagner L, Kautzky-Willer A.(2012);**Fetuin-Aand angiopoietins in obesity and type 2 diabetes mellitus. Endocrine 2012; 42: 496-505.

[8]**Song A, Xu M, Bi Y,et al.(2011):** Serum fetuin-A associates with type 2 diabetes and insulin resistance in Chinese adults. PLoS One 2011; 6: e19228.

[9]**Mathews ST, Chellam N, Srinivas PR, et al(2000):**Alpha2-HSG, a specific inhibitor of insulin receptor autophosphorylation, interacts with theinsulin receptor. Mol Cell Endocrinol 2000;164: 87-98.

[10]**Schafer C, Heiss A, Schwarz A, et al(2003):,** The serum protein alpha 2-Heremans-Schmid glyco-protein/fetuin-A is a systematically acting inhibitor of ectopic calcification. J Clin Invest 2003; 112: 357-366.

[11]**Emoto M *et al.(2010):*** Fetuin-A and atherosclerotic calcified plaque in patients with type 2 diabetes mellitus. Metabolism 2010*;* 59*:* 873–878.

[12]Denecke B, Graber S, Schafer Cet al (2003). Tissue distribution and activity testing suggest a similar butnot identical function of fetuin-B and fetuin-A.Biochem J 2003; 376: 135-145.

[13]**Alberti KG, Zimmet PZ.**Definition, diagnosisand classificationofdiabetes mellitus and itscomplications.

Part 1: diagnosis and classification of diabetes mellitus, provisional report ofa WHO consultation. Diabetic Med 1998; 15:539-55317: 2295-2301.

[14]Keskin M,Cavit Çulha1, NeşeErsözGülçelik, EsranurAdemoğlu, AhmetKeskin and Yalçın Aral(2017): Fetuin-A levels determine cardiovascular risk in young diabetic patients**.**

[15]**Ishibashi A, Ikeda Y, Ohguro T, Kumon Y, Yamanaka S, Takata H, Inoue M, Suehiro T, Terada Y.(2010)*:*** Serum fetuin-A is an independent marker of insulin resistance in Japanese men. J AtherosclerThromb 2010; 17: 925-933

[16]**Cheyssac C, Dina C, Lepretre F, (2006):**EIF4A2 Is a positional candidate gene at the 3q27 locus linked to type 2 diabetes in French families. Diabetes 2006; 55: 1171-1176.

[17]Dutta D, Mondal SA, Kumar M, et al(2014):. Serum fetuin-A concentration predicts glycaemic outcomes in people with prediabetes: a prospective study from eastern India. Diabet Med 2014; 31: 1594-1599.

[18]**Yin L, Cai WJ, Zhu LY, et al.,(2003) (2015):** Association of plasma Fetuin-A and clinical characteristics in patients with new-onset type 2 diabetes mellitus. Int J Clin Exp Med.; 8(1): 991-9.

[19] **Chen HY, Chiu YL, Hsu SP, et al.,(2009):.**Association of serum fetuin A with truncal obesity and dyslipidemia in non-diabetic hemodialysis patients.Eur J Endocrinol 2009; 160:777-783.

[20] **Roos M, Oikonomou D, von Eynatten M, (2010):**Associations offetuin-A levels with vascular disease in type 2 diabetes patients with early diabetic nephropathy. CardiovascDiabetol 2010; 9: 48.

[21]**Ramadan A, Shoukry A, Ismail MI, (2011):.**Serum fetuin-A levels in type 2 diabetes patients with early diabetic nephropathy: it's relation to diabetes control. J Am Sci 2011; 7: 759-765.