FETUIN-A AS A MARKER OF INSULIN RESISTANCE

Ganavi P Yamasandhi and Mala Dharmalingam

Department of Endocrinology, M.S. Ramaiah Medical College and Hospital, Karnataka, India

FETUIN-A KAO MARKER INSULINSKE REZISTENCIJE

Ganavi P Iamasandhi i Mala Dharmalingam

Odeljenje za endokrinologiju, M.S. Medicinski fakultet i bolnica Ramaiah, Karnataka, Indija

Received/Primljen: 09.12.2020. Accepted/Prihvaćen: 03.04.2021.

ABSTRACT

Fetuin–A is a glycoprotein which helps in the regulation of metabolism. It is an early marker of insulin resistance (IR). The aim of this study was to evaluate the role of Fetuin–A as a predictive biomarker in cases of newly detected type 2 diabetes (NDD). The study involved 60 NDD and 60 Normal Healthy Controls (NHC). All the demographics and anthropological characteristics were noted. Fasting blood samples were drawn and various biochemical parameters were analyzed. The homeostatic model assessment of insulin resistance (HOMA-IR) and the quantitative insulin sensitivity check index (QUICKI) score was calculated. Chi-square, student T-test and Mann Whitney U tests were employed to associate and compare the mean and median between the NDD and NHC groups. Pearson's and Spearman's correlation analysis were employed to examine the relationship of Fetuin–A levels with parametric and nonparametric variables. The independent predictors of Fetuin–A was determined by employing multiple forward linear regression. Fetuin–A was significantly high in NDD compared to NHC (1323 vs. 306.98 mcg/mL; p<0.001). Majority of NDD patients demonstrated IR based on the HOMA-IR (88.33% vs. 66.67%) and QUICKI score (96.67% vs. 85%). The multiple linear regression analysis showed that systolic blood pressure, age and QUICKI score were independently associated with Fetuin–A (p value <0.01). Fetuin–A may be used as a biomarker to detect NDD. Therefore, early detection of Fetuin–A levels in NDD gives an opportunity for suitable patient management.

Keywords: *alpha-2-HS-Glycoprotein, biomarkers, endocrinology, insulin resistance, type 2 diabetes mellitus.*

SAŽETAK

Fetuin - A je glikoprotein koji pomaže u regulaciji metabolizma. To je rani marker insulinske rezistencije (IR). Cilj ove studije bio je da se proceni uloga Fetuina - A kao prediktivnog biomarkera u slučajevima novootkrivenog dijabetesa tipa 2 (NDD). Studija je obuhvatila 60 NDD i 60 normalnih zdravih kontrola (NHC). Zabeležene su sve demografske i antropološke karakteristike. Uzeti su uzorci krvi natašte i analizirani su različiti biohemijski parametri. Izračunati su homeostatski model procene insulinske rezistencije (HOMA-IR) i kvantitativni indeks provere osetljivosti na insulin (KUICKI). Hi-kvadrat, studentski T-test i Mann Vhitnei U testovi su korišćeni za povezivanje i upoređivanje srednjih vrednosti i medijane između NDD i NHC grupa. Pearsonova i Spearmanova analiza korelacije korišćene su za ispitivanje odnosa nivoa Fetuin - A sa parametarskim i neparametarskim promenljivim. Nezavisni prediktori Fetuin -A određeni su primenom višestruke linearne regresije unapred. Fetuin -A je bio značajno visok u NDD u poređenju sa NHC (1323 naspram 306, 98 mcg/mL; p <0,001). Većina pacijenata sa NDD pokazala je IR na osnovu HOMA-IR (88, 33% naspram 66,67%) i KUICKI skora (96,67% naspram 85%). Analiza višestruke linearne regresije pokazala je da su sistolni krvni pritisak, starost i KUICKI rezultat nezavisno povezani sa Fetuin – A (vrednost p <0,01). Fetuin - A se može koristiti kao biomarker za otkrivanje NDD. Stoga rano otkrivanje nivoa Fetuin - A u NDD -u daje priliku za odgovarajuće lečenje pacijenata.

Ključne reči: *alfa-2-HS-glikoprotein, biomarkeri, endokrinologija, insulinska rezistencija, dijabetes melitus tip 2.*

sciendo
DOI: 10.2478/sjecr-2021-0042

Corresponding author:
Dr. Ganavi P Yamasandhi
Assistant Professor, Department of Endocrinology,
M.S. Ramaiah Medical College and Hospital,
Bangalore – 560054, Karnataka, India,
Contact: +91–8040503022,
Email: ganavipattabhi@gmail.com

INTRODUCTION

The global incidence of diabetes mellitus was 463 million (9.3%) in 2019 and is expected reach to 700 million (10.9%) by 2045 (1). Type 2 diabetes mellitus (T2DM) is a major public health issue, caused by either insulin resistance (IR) or insulin deficiency (2). Insulin is the primary regulator of glucose homeostasis and its deficiency occurs due to damaged β cells of the islets of Langerhans, thereby stopping insulin production (2). However, IR is due to the decreased response or sensitivity towards insulin and plays a crucial role in T2DM pathophysiology (3). IR is associated with prediabetes and can be preferably treated using biguanides and thiazolidinediones (4, 5).

Various inflammatory and metabolic biomarkers, such as Fetuin-A, glycated hemoglobin A1c (HbA1c), adiponectin, retinol binding protein–4, myostatin, IL-6, fibroblast growth factor–21, irisin chemerin, adipocyte fatty acid-binding protein have been studied to identify T2DM and IR (6, 7). Previous studies have documented a relatively higher level of serum Fetuin–A in newly detected type 2 diabetes (NDD), cardiovascular disease, metabolic syndrome and obesity patients and is an emerging novel biomarker of IR (8-10).

Fetuin-A is a multifunctional glycoprotein secreted by the liver, also known as α-2 Heremans Schmid glycoprotein (AHSG). The gene encoding locus of Fetuin-A is located on chromosome 3q27 region and is identified as the T2DM susceptibility locus (11). Fetuin-A can induce IR by inhibiting insulin receptor autophosphorylation in the liver and skeletal muscle (12). It is a major carrier of free fatty acids (FFA) in the circulation and is required for FFA interaction with toll-like protein receptor 4 (TLR4) in adipocytes, thereby triggering pro-inflammatory adipokine expression and IR (13). Thus, Fetuin-A promotes lipid-induced IR via inflammatory signaling pathway, causing inflammatory cytokines production. Chronic inflammation caused by free radicals is thought to be the reason for IR (14). Therefore, this study was aimed to elucidate the clinical relevance of Fetuin-A, to identify individuals at increased risk of developing T2DM and for therapeutic purposes.

PARTICIPANTS AND METHODS

This two-year cross-sectional study was conducted at the endocrinology department of a multispecialty hospital, from January 2017 to December 2018. Ethical clearance was procured from the institutional review board and participants were enrolled after obtaining written informed consent.

A total of 120 participants, aged between 18-80 years, were included in this study. They were divided into two groups - normal healthy controls (NHC, n=60) and NDD (case, n=60). Participants with normal fasting blood sugar (FBS) and fasting serum insulin levels were considered as NHC. Participants who were euthyroid, met the 2017 American Diabetic Association (ADA) criteria, with HbA1c <12%, were considered as NDD.

Exclusion criteria considered for this study were pregnant women, patients with secondary diabetes, Type 1 DM, known T2DM patients receiving insulin or oral antidiabetic drugs/not on treatment, patients with BMI >30 kg/m^2, smokers, drinkers, alcohol consumers of ≥20 g/d for a year prior to study, patients with hepatitis B, hepatitis C and other causes of liver diseases (hemochromatosis, α1antitrypsin deficiency, Wilson's disease, primary sclerosing cholangitis or primary biliary cirrhosis), serum creatinine >1.5 mg/dl and any chronic or acute inflammatory disease (leukocyte count >10,000/mm^3) with any clinical signs of infection or any other major diseases, including advanced malignant diseases.

Sample size

A previous study reported serum Fetuin–A levels of 337±96 and 291±63 mcg/ml in cases and controls, respectively (15). Based on the above-mentioned findings and considering the power of the study at 80%, α error of 5% and effect size of 0.53, a minimum of 59 subjects were required per group. Hence, sample size of 120 patients in total was justified for this study.

Patient demographic data (age and gender) was collected by direct interview method. All patients underwent further clinical examination, and all findings were recorded on a predesigned and pretested proforma. Anthropometric details such as height, weight, waist and hip circumferences were measured according to the standard procedures and body mass index (BMI), waist to hip ratio were calculated. Whole blood samples were withdrawn under aseptic precautions after an 8 hour overnight fasting from participants, and were evaluated for various hematological and biochemical parameters, such as complete blood count, glycated HbA1c, FBS, fasting insulin and Fetuin–A levels. After blood collection, serum was separated within 1 hour and stored at –80^0 C.

Serum Fetuin-A and serum insulin levels were measured using Quantikine ELISA Human Fetuin-A Immunoassay kit (R&D Systems, Inc.) and DRG Insulin Elisa kit (DRG Instruments, GmbH, Germany), respectively. All participants were divided on the basis of four quartile range of Fetuin-A (quartile 1: 0–<305 µg/ml; quartile 2: 305 – 757 µg/ml; quartile 3: 758 – 1328 µg/ml; quartile 4: >1328 µg/ml). The IR was calculated using homeostatic model assessment of insulin resistance (HOMA-IR) and patients were considered as insulin resistant if HOMA-IR (fasting insulin x fasting glucose) was ≥2 (16). Quantitative insulin sensitivity check index (QUICKI) was calculated using the formula QUICKI=1/ [log (fasting insulin (µU/L) + log (fasting glucose (mg/dL)] and patients were considered as insulin resistant if QUICKI was ≤0.36 (16).

Statistical analysis

Statistical analysis was performed using SPSS statistical software version 18. Shapiro test was employed to check

normality of the data. Normally distributed data are represented as mean ± standard deviation (SD) and the means between the two groups were compared by performing student T-test. Non-normalized data are represented as median (interquartile range, [IOR]). Mann Whitney U test was employed to evaluate the difference in the serum Fetuin-A levels among the groups in case of non-normalized data. Chi-square test was employed to assess the association between the Fetuin-A level and various variables for categorical data. Pearson's and Spearman's correlation analysis was employed to examine the relationship of Fetuin-A levels with parametric and non-parametric variables, respectively. Multiple forward linear regression was employed to determine the independent predictors of Fetuin-A. Multivariate logistic regression was employed to calculate the odds ratio (OR) and 95% confidence interval (CI). Level of significance was set at $p \leq 0.05$.

RESULTS

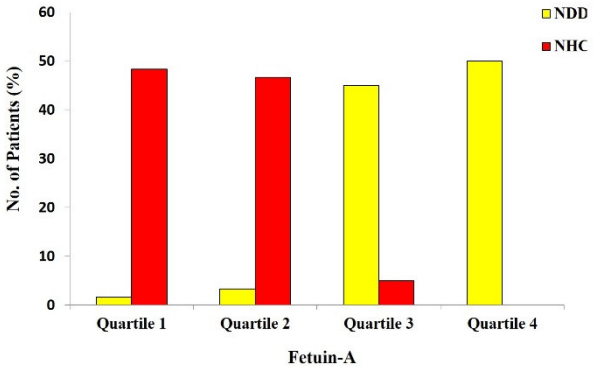
The mean age of patients in the NDD and NHC groups were 51.63±12.01 and 48.72±11.87 years respectively, with male predominance, with male to female ratio of 1.72:1 in NDD and 1.60:1 in NHC groups. On comparing the anthropometry variables, such as waist circumference (WC) and waist to hip ratio, a significantly higher WC (98 cm vs. 89.6 cm; p=0.01) and waist to hip ratio was noted (0.99 vs. 0.87; p=0.009) in NDD compared to the NHC group. However, BMI was found to change insignificantly between both the groups. The difference in the biochemical variables between NDD and NHC groups were significant ($p<0.05$) along with platelet count except for the serum albumin levels. NDD group had a significantly higher systolic (125 mmHg vs. 116 mmHg) and diastolic blood pressure (80 mmHg vs. 77 mmHg), HbA1c (8.4 vs. 5.4), FBS (157.5 mg/dL vs. 92.5 md/dL), fasting serum insulin (16.08 µIU/mL vs. 10.98 µIU/mL), fetuin–A (1322.98 µg/mL vs. 306.97 µg/mL) and platelet count (256.38 ± 74.26 mm^3 vs. 246.73 ± 83.25 mm^3) as compared to NHC group. The NDD group also had significantly higher IR based on HOMA-IR (8.07 vs. 2.39) and QUICKI score (0.29 ± 0.03 vs. 0.33 ± 0.02) compared to NHC group ($p<0.05$) (Table 1).

Table 2 represents the distribution of patients based on the different quartile of Fetuin-A and IR as per HOMA–IR and QUICKI. Majority of patients in the NDD group demonstrated IR based on the HOMA-IR (88.33% vs. 66.67%) and QUICKI score (96.67% vs. 85%) and belonged to the Fetuin-A 3rd and 4th quartile compared to NHC group.

Patients in the NDD group belonging to Fetuin-A 3rd quartile had a significantly higher value of HOMA-IR (median: 7.04; IQR: 3.37–11.01) compared to NHC group (median: 4.7; IQR: 4.18–5.23) with p=0.038 (Table 3).

Percentage of patients with NDD was highest in the 4th quartile than the 1st quartile (50% vs 1.66%, $p<0.001$) (Figure 1).

Figure 1. Comparing different quartiles of Fetuin-A amongst NDD and NHC groups.

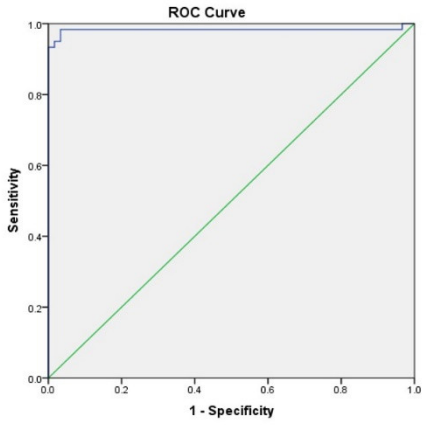


NDD - newly detected type 2 diabetes;
NHC - normal healthy control.

The correlation of Fetuin-A levels with various clinical and biochemical parameters for entire population are represented in Table 4. A significant weak positive correlation of Fetuin-A was noted with age, systolic blood pressure (SBP), diastolic blood pressure (DBP), FBS, HbA1c, fasting insulin and HOMA-IR score ($p<0.01$).

Fetuin-A cut-off value of 733.6296 mcg/ml yielded maximum sensitivity of 98.3% and specificity of 96.7% to differentiate NDD patients from NHC with an AUC of 0.983 (Figure 2).

Figure 2. ROC curve for Fetuin-A.



Multiple regression models were employed to choose the best fit model for selecting independent marker of Fetuin-A. The multiple linear regression analysis showed that QUICKI, SBP and age were independently associated with Fetuin-A ($p<0.01$; adjusted $R^2=0.368$), indicating these may serve as independent predictor of Fetuin-A. Age and SBP were positively associated where as QUICKI was negatively associated with Fetuin-A (Table 5).

Table 1. Baseline characteristics of NDD and NHC patients.

Parameters		NDD group (n=60)	NHC group (n=60)	p value
Demographic				
Gender [a]	Male	38 (63.3)	37 (61.6)	0.85
	Female	22 (36.6)	23 (38.3)	
Age (Years) [b]		51.63 ± 12.01	48.72 ± 11.87	0.119
Anthropometry				
BMI (Kg/m^2) [c]		26.35 (24.4-28.3)	26.25 (22.64-27.89)	0.277
WC (cm) [c]		98 (85.5-106)	89.6 (82.5-100.7)	0.010*
Waist to hip ratio [c]		0.99 (0.88-1.04)	0.87 (0.82-1.01)	0.009*
SBP (mmHg) [c]		125 (118-134)	116 (110-124)	0.000***
DBP (mmHg) [c]		80 (75-87)	77 (71-80)	0.001**
Biochemical				
Platelet count (mm^3)		256.38 ± 74.26	246.73 ± 83.25	0.038*
Albumin (g/dL)		4.2 (4-4.4)	4.5 (4.35-4.8)	0.246
HbA1c (%) [c]		8.4 (7.1-9.85)	5.4 (5.3-5.5)	0.000***
FBS (mg/dL) [c]		157.5 (131-226.5)	92.5 (85-97)	0.000***
Fetuin–A (µg/mL) [c]		1322.98 (1133.11-1575.15)	306.97 (260.84-530.05)	0.000***
Insulin (µIU/mL) [c]		16.08 (8.55-27.58)	10.98 (8.02-17.6)	0.000***
HOMA-IR [c]		8.07 (3.62-12.19)	2.39 (1.84-4.12)	0.0286*
QUICKI score [b]		0.29 ± 0.03	0.33 ± 0.02	0.000***

BMI - body mass index; DBP - diastolic blood pressure; FBS – fasting blood sugar; HOMA-IR - homeostatic model assessment of insulin resistance; NDD - newly detected type 2 diabetes; NHC - normal healthy control; QUICKI - quantitative insulin sensitivity check index; SBP - systolic blood pressure; WC - waist circumference.
*, **, and *** indicates statistically significant P value of ≤0.05, ≤0.001 and ≤0.0001, respectively.
[a] data represented as frequency (%); [b] data represented as mean ± SD; [c] data represented as median (IQR).
Chi-square test was used for categorical data analysis.
Student T-test and Mann Whitney U test were used for normalized and non-normalized data analysis.

Table 2. Distribution of patients based on Fetuin–A quartile and insulin resistance as per HOMA-IR and QUICKI.

Fetuin–A (IQR)	Groups - No. of Patients (%)							
	NDD (n=60)				NHC (n=60)			
	IR based on HOMA-IR		IR based on QUICKI Score		IR based on HOMA-IR		IR based on QUICKI Score	
	No	Yes	No	Yes	No	Yes	No	Yes
1 (0-304.94)	0	1 (1.67)	0	1 (1.67)	12 (20)	17 (28.33)	4 (6.67)	25 (41.67)
2 (305 - 757.4576)	1 (1.67)	0 (0)	0	1 (1.67)	8 (13.33)	21 (35)	5 (8.33)	24 (40)
3 (757.5 - 1327.5)	2 (3.33)	26 (43.33)	1 (1.67)	27 (45)	0 (0)	2 (3.33)	0	2 (3.33)
4 (>1328 – 00)	4 (6.67)	26 (43.33)	1 (1.67)	29 (48.33)	0 (0)	0 (0)	0	0
Total	7 (11.67)	53 (88.33)	2 (3.33)	58 (96.67)	20 (33.33)	40 (66.67)	9 (15)	51 (85)
p value [¥]	0.039 *		0.995		0.32		0.779	

IR - insulin resistance; HOMA-IR - homeostatic model assessment of insulin resistance; IQR – inter quartile range; NDD - newly detected type 2 diabetes; NHC - normal healthy control; QUICKI - quantitative insulin sensitivity check index.
[¥] denotes Chi-square p values; * indicates statistically significant p value of ≤0.05.

Table 3. Comparison of insulin resistance as per HOMA-IR and QUICKI score among Fetuin–A quartiles in NDD and NHC groups.

Fetuin–A (IQR)	IR in Patients					
	Based on HOMA-IR [Median (IQR)]			Based on QUICKI Score [Mean ± SD]		
	NDD	NHC	p value	NDD	NHC	p value
1 (0-304.94)	12.31 (12.31–12.31)	2.24 (1.74–2.63)	0.149	0.27±0	0.34±0.167	–
2 (305 - 757.4576)	1.53 (1.53–1.53)	3.28 (1.99–5.17)	0.094	0.36±0	0.32±0.032	–
3 (757.5 - 1327.5)	7.04 (3.37–11.01)	4.7 (4.18–5.23)	0.038*	0.29±0.03	0.31±0.01	0.283
4 (>1328 – 00)	8.29 (4.07–13.31)	0	–	0.29±0.033	0	–

IR - insulin resistance; HOMA-IR - homeostatic model assessment of insulin resistance; IQR – inter quartile range; NDD - newly detected type 2 diabetes; NHC - normal healthy control; QUICKI - quantitative insulin sensitivity check index; SD – standard deviation.
* indicates statistically significant p value of ≤0.05.
'–' represents analysis could not be performed.

Table 4. Correlation of Fetuin–A with various clinical parameters (n=120).

Parameters	Correlation Coefficient	p value
Age	0.1684	0.066
BMI (kg/m2)	0.0077	0.934
Waist circumference (cm)	0.0904	0.326
Waist hip ratio	0.1255	0.172
SBP (mmHg)	0.3754	0.000***
DBP (mmHg)	0.2504	0.002**
Platelet count (mm3)	0.1582	0.282
Albumin (g/dL)	-0.4025	0.000***
HbA1c (%)	0.7679	0.000***
Fasting Blood Sugar (mg/dL)	0.7247	0.000***
Insulin (µIU/mL)	0.2426	0.008**
HOMA-IR	0.5193	0.000***
QUICKI score	-0.5257	0.000***

BMI - body mass index; DBP - diastolic blood pressure; HOMA-IR - homeostatic model assessment of insulin resistance; QUICKI - quantitative insulin sensitivity check index; SBP - systolic blood pressure.
** and *** refer to <0.01 and <0.001 level of significance respectively.

Table 5. Multiple linear regression analysis showing independent predictors of Fetuin–A.

Parameters	OR (95% CI)	p value	Adj. R^2 value
***QUICKI	-7073.65 (-9341.22, -4806.07)	0.000***	0.368
*SBP	13.25 (6.53, 19.98)	0.004**	
*Age	6.56 (0.11, 13.01)	0.005**	

Adj - adjusted; CI – confidence interval; OR - odds ratio; QUICKI - quantitative insulin sensitivity check index; SBP - systolic blood pressure.
and * refer to <0.01 and <0.001 level of significance respectively.

DISCUSSION

Fetuin-A is a natural inhibitor of insulin receptor tyrosine kinase along with terminating the downstream signal cascades, thereby resulting in IR and the onset of T2DM (17, 18). Fetuin-A binds to tandem fibronectin type 3 (Fn3) domains of the insulin receptor-subunit. Insulin and Fetuin-A exhibit affinity towards the same insulin receptor ectodomain, with insulin activating the receptor's intrinsic tyrosine kinase activity through its binding to the α-subunit, causing a conformational change that promotes fetuin binding to the β-subunit, thereby resulting in the receptor inactivation (19). Fetuin-A also promotes lipid-induced IR by binding to TLR4 through an endogenous ligand function that is mediated by its terminal galactoside moiety, being able to directly bind the Leu100–Gly123 and Thr493–Thr516 residues in TLR4, causing adipose tissue inflammation and subsequent onset of IR (13, 20).

The emergence of Fetuin-A as a biomarker was demonstrated by many studies (10, 11, 21, 22). However, only few evaluated the correlation between serum Fetuin-A levels with T2DM patients' characteristics (23). Therefore, this study was attempted to pitch some light on the potential role of Fetuin-A as a biomarker and a therapeutic target in IR.

This study demonstrated that NDD group significantly differed from NHC group for various baseline characteristics, such as WC, waist to hip ratio, SBP, DBP, platelet count, HbA1c, FBS, Fetuin-A, fasting insulin levels, HOMA-IR and QUICKI score ($p<0.05$) except age, gender BMI and albumin ($p>0.05$). Similar findings were reported in a previously conducted study (24). Sharma and colleagues (24) suggested that WC can be better predictor of diabetes than BMI and reported that a strong correlation between WC and FBS (R=0.813) compared to BMI and FBS (R=0.539). Demographic factors, such age and gender were not significantly associated in this study ($p>0.05$) and supported by the findings of previous researchers (25-27). Dabrowska and colleagues (25) and Vörös and colleagues (26) stated that Fetuin-A concentrations were independent of gender and Sun and colleagues (27) showed no association between age and Fetuin-A levels.

The NDD group had higher levels of Fetuin-A levels compared to NHC group ($p<0.001$) and was concurred with Ou and colleagues (15) and Song and colleagues (28). The current study also showed that NDD patients majorly distributed in the Fetuin-A 3rd and 4th quartile in comparison to the NHC group, who were majorly distributed in the 1st and 2nd quartile. On comparing the HOMA-IR and QUICKI score among the four quartiles of Fetuin-A levels, HOMA-IR was significantly higher in the NDD compared to the NHC group ($p<0.05$). This finding was in accordance with the study conducted by Song and colleagues (28). Whereas QUICKI score varied insignificantly among both the groups ($p>0.05$).

The independent predictors of Fetuin-A by multiple linear regression analysis were QUICKI, SBP and age ($p<0.01$) and were in contrast with findings of Yin and colleagues (23) and Song and colleagues (28). Yin and colleagues (23) reported fasting plasma glucose, 2 h oral glucose tolerance test, HOMA-beta-cell insulin secretion index, triglycerides and carotid intima media thickness as independent associated predictors of Fetuin-A. Song and colleagues (28) reported the association of Fetuin-A levels with fasting serum insulin and HOMA-IR. Our findings suggested that Fetuin-A was positively associated with SBP and age, but negatively with QUICKI and were independent predictors of Fetuin-A. This finding suggested that Fetuin-A and QUICKI represents a counteracting mechanism. Higher Fetuin-A were related to IR indicated as QICIKI score and might lead to development of T2DM and act as predictive marker for NDD cases that may be used as a therapeutic target in IR cases.

Limitations of this study was the small sample size considered. Since this study was not prospective in design, it did not allow for causal inference between serum Fetuin-A concentrations and the progression of T2DM. Moreover, as the study cases were mostly men, our results may not be applicable to women.

CONCLUSION

High levels of Fetuin-A were associated with NDD compared to NHC group, which in turn associated independently with SBP, albumin and HbA1c. Thus, Fetuin-A may be used as a biomarker for detection of NDD. This can help in for accurate diagnosis, thereby facilitating appropriate treatment for NDD and helps in managing patients with the risk of T2DM.

ACKNOWLEDGEMENTS

None

CONFLICTS OF INTEREST

The authors declare that there are no competing interests associated with the manuscript.

FUNDING

Nil

LITERATURE

1. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas. Diabetes Res Clin Pract 2019; 157: 107843.
2. Standards of Medical Care in Diabetes. Summary of revisions. Diabetes Care 2016; 39 (Supplement 1): S4–5.

3. Kahn SE, Cooper ME, Del Prato S. Pathophysiology and treatment of type 2 diabetes: perspectives on the past, present, and future. Lancet 2014; 383(9922): 1068-83.
4. Cai X, Xia L, Pan Y, He D, Zhu H, Wei T, et al. Differential role of insulin resistance and β-cell function in the development of prediabetes and diabetes in middle-aged and elderly Chinese population. Diabetol Metab Syndr 2019; 11: 24.
5. Pala L, Barbaro V, Dicembrini I, Rotella CM. The therapy of insulin resistance in other diseases besides type 2 diabetes. Eat Weight Disord 2014; 19(3): 275-83.
6. Scirica BM. Use of biomarkers in predicting the onset, monitoring the progression, and risk stratification for patients with type 2 diabetes mellitus. Clin Chem 2017; 63(1): 186–195.
7. Park SE, Park CY, Sweeney G. Biomarkers of insulin sensitivity and insulin resistance: Past, present and future. Crit Rev Clin Lab Sci 2015; 52(4): 180-90.
8. Maruo S, Mori K, Motoyama K, Nakamura M, Kawarabayashi R, Kakutani Y, et al. Correlation analysis of monocyte subsets and insulin resistance considering fetuin-A involvement in patients with type 2 diabetes. Clin Transl Med 2018; 7: 9.
9. Komsa-Penkova RS, Golemanov GM, Radionova ZV, Tonchev PT, Iliev SD, Penkov VV. Fetuin-A – alpha2-heremans-schmid glycoprotein: from structure to a novel marker of chronic diseases part 1. Fetuin-A as a calcium chaperone and inflammatory marker. J Biomed Clin Res 2017; 10(2): 90-7.
10. Ismail NA, Ragab S, El Dayem SM, Elbaky AA, Salah N, Hamed M, et al. Fetuin-A levels in obesity: differences in relation to metabolic syndrome and correlation with clinical and laboratory variables. Arch Med Sci 2012; 8(5): 826-33.
11. Alireza E, Mohsen A, Sahar F, SinaN, Manouchehr N. Comparative effects of metformin and pioglitazone on fetuin-A and osteoprotegerin concentrations in patients with newly diagnosed diabetes: A randomized clinical trial. Diabetes & Metabolic Syndrome. Clin Res Rev 2015; 9(4): 258-65.
12. Mathews ST, Chellam N, Srinivas PR, Cintron VJ, Leon MA, Goustin AS, et al. Alpha2-HSG, a specific inhibitor of insulin receptor autophosphorylation, interacts with the insulin receptor. Mol Cell Endocrinol 2000; 164(1-2): 87-98.
13. Pal D, Dasgupta S, Kundu R, Maitra S, Das G, Mukhopadhyay S, et al. Fetuin-A acts as an endogenous ligand of TLR4 to promote lipid-induced insulin resistance. Nat Med 2012; 18(8): 1279-85.
14. Oguntibeju OO. Type 2 diabetes mellitus, oxidative stress and inflammation: examining the links. Int J Physiol Pathophysiol Pharmacol 2019; 11(3): 45-63.
15. Ou HY, Yang YC, Wu HT, Wu JS, Lu FH, Chang CJ. Serum fetuin-A concentrations are elevated in subjects with impaired glucose tolerance and newly diagnosed type 2 diabetes. Clin Endocrinol 2011; 75(4): 450-455.
16. Shivakumar. N, Kumar M, Ashwathanarayana M, Venkatesh M, Sheshadri M, Deshmukh S, et al. Role of retinol-binding protein 4 in obese Asian Indians with metabolic syndrome. J Med Biochem 2012; 31(1): 40-6.
17. Khadir A, Kavalakatt S, Madhu D, Hammad M, Devarajan S, Tuomilehto J, et al. Fetuin-A levels are increased in the adipose tissue of diabetic obese humans but not in circulation. Lipids Health Dis 2018; 17(1): 291.
18. Jin C, Lin L, Han N, Zhao Z, Liu Z, Luo S, et al. Effects of dynamic change in fetuin-A levels from the first to the second trimester on insulin resistance and gestational diabetes mellitus: a nested case–control study. BMJ Open Diab Res Care 2020; 8(1): e000802.
19. Goustin AS, Abou-Samra AB. The "thrifty" gene encoding Ahsg/Fetuin-A meets the insulin receptor: Insights into the mechanism of insulin resistance. Cell Signal 2011; 23(6): 980-90.
20. Bourebaba L, Marycz K. Pathophysiological implication of fetuin-a glycoprotein in the development of metabolic disorders: A concise review. J Clin Med 2019; 8(12): 2033.
21. Stefan N, Kantartzis K, Häring HU. Causes and metabolic consequences of Fatty liver. Endocr Rev 2008; 29(7): 939-60.
22. Yin L, Cai WJ, Chang XY, Li J, Su XH, Zhu LY, et al. Association between fetuin-A levels with insulin resistance and carotid intima-media thickness in patients with new-onset type 2 diabetes mellitus. Biomed Rep 2014; 2(6): 839-42.
23. Yin L, Cai WJ, Zhu LY, Li J, Su XH, Wang XL, et al. Association of plasma Fetuin-A and clinical characteristics in patients with new-onset type 2 diabetes mellitus. Int J Clin Exp Med 2015; 8(1): 991-9.
24. Sharma B, Sarmah D. WC is better than BMI as a predictor of diabetes and prediabetes in Hindu priests of India. Asian J Med Sci 2015; 6(1): 91-4.
25. Dabrowska AM, Tarach JS, Wojtysiak-Duma B, Duma D. Fetuin-A (AHSG) and its usefulness in clinical practice. Review of the literature. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2015; 159(3): 352-9.
26. Vörös K, Gráf L, Prohászka Z, Gráf L, Szenthe P, Kaszás E, et al. Serum fetuin-A in metabolic and inflammatory pathways in patients with myocardial infarction. Eur J Clin Invest 2011; 41: 703-9.
27. Sun Q, Cornelis MC, Manson JE, Hu FB. Plasma levels of fetuin-A and hepatic enzymes and risk of type 2 diabetes in women in the US. Diabetes 2013; 62(1): 49-55.
28. Song A, Xu M, Bi Y, Xu Y, Huang Y, Li M, et al. Serum fetuin-A associates with type 2 diabetes and insulin resistance in Chinese adults. Plos One 2011; 6(4): e19228.