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Assessment of Urinary Fetuin - Aasa Marker for Diabetic Nephropathy in Type2 Diabetic patients

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Abstract

Background: Type 2 diabetes mellitus (DM) is the most common type of diabetes, accounting foraround 90% of all cases of diabetes. Diabetic nephropathy (DN) is the largest single cause of end-stage kidney disease, therefore, there is an urgent need to identify more sensitive and specificbiomarkersthan microalbuminuriaforearly detection ofDN.

Aim of study: This study is aimed for early detection of diabetic nephropathy in type 2 DM.**SubjectsandMethods**:This case controlstudywascarriedout onsixty-nine individualsadmittedtodepartmentandoutpatientclinicofinternalmedicineatZagazigUniversityHospitals.Theparticipantsweredividedintothreegroups:GroupA:(23subjects)Controlgroup.healthyindividuals,ageandsex-matched,withnegativefamilyhistoryofhypertensionandischemicheartdiseases.GroupB:(23patients)Type2Diabeticpatients.GroupC:(23patients):Type2Diabeticpatientswithdiabeticnephropathy.Allpatientsweresubjectedto(I)Fullhistory:priormedicalrecordsincludingthefollowing:Familyhistoryofdiabetesmellitusandobesity.Type2diabetes(evidenceofraisedbloodglucosemeasurements,HA1C,FBS,2HPP,orRBSrecordedondifferentdaysbeforestudyduringadmission,(III)RoutineLaboratorytesting(IV) Specificlaboratory;HbA1C,FastingInsulin,andMeasurementofUrinaryLeveloffetuin-Aintype2diabeticpatientsand control group by ELISA.

Results: A highly significant difference in duration of DM and diabetic retinopathy among studiedgroups; with ($p < 0.001$). A significant difference in family history of DM among studied groups;with($p<0.05$UrinaryAngiostatin;withhighlysignificantstatisticaldifference($p<0.01$).Activity and chronicity indices had a highly significant positive correlation with Urinary Angiostatin; withhighly significant statistical difference ($p < 0.01$ respectively). A highly significant difference inhemoglobin,platelets,albumin,TGs,urea,creatinine,andUrinaryAlbumin/Creatinineratio,amongstudied groups; with ($p < 0.001$). A significant difference in ALT among studied groups; with ($p < 0.05$). High Urea level in Type 2 DM and Type 2 DM with nephropathy patients in comparison toControl group; with ($p < 0.01$), and high Urea level in Type 2 DM with nephropathy patients incomparison to Type 2 DM patients; with ($p < 0.01$). High creatinine level in Type 2 DM withnephropathypatients in comparison to Control group and Type2DM.
High Albumin/Creatinine ratio in Type 2 DM with nephropathy patients in comparison to

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Controlgroup. A highly significant difference in FBS and HbA1c, fasting insulin, HOMA-IR, and UrinaryFetuin-A among studied groups; with ($p < 0.001$). High Urinary Fetuin-A level in Type 2 DM andType 2 DM with nephropathy patients in comparison to Control group; with ($p < 0.01$), and highUrinary Fetuin-A level in Type 2 DM with nephropathy patients in comparison to Type 2 DMpatients;with ($p <0.01$).

Conclusion: FromthisstudywecouldconcludethatUrinaryexcretionofFetuin-Acanbeusedforearlydetection ofDNinType2 DM patients withexcellent accuracy.

Keywords: DiabetesMellitus (DM),DiabeticNephropathy(DN),UrinaryFetuin-A.

1. Introduction:

Diabetic nephropathy (DN), a microvascular complication occurring in approximately 20-40% ofpatients with type 2 diabetes mellitus (T2DM), is characterized by the progressive impairment ofglomerularfiltrationandthedevelopmentofKimmelstiel-Wilsonlesionsleadingtoend-stagerenaldisease(ESRD)**(1).**

Diabetic nephropathy is primarily classified according to the extent of albuminuria in addition totheglomerularfiltration rate**(2).**

There are many markers that may be more sensitive than urinary albumin, (the current goldStandard,inthedetectionofincipientnephropathyandriskAssessmentofcardiovasculardisease);however,thesensitivityofthesemarkerscomparedwithalbuminrequiresfurtherinvestigation**(3).**These markers include biomarkers of renal dysfunction such as transferrin, type IV collagen andN-acetyl-b-D-glucosaminidase,inflammatorymarkersincludingorosomucoid,tumornecrosisfactor-α,transforminggrowthfactor-β,vascularendothelialgrowthfactorandmonocytechemoattractantprotein-1,aswellasoxidativestressmarkerssuchas8-hydroxy-29deoxyguanosine**(3).**

Fetuin-A (α2-Heremans Schmid glycoprotein: AHSG) is an abundant circulating glycoprotein thatisprimarilysynthesizedintheliverandplaysseveralfunctionsinhumanphysiologyandpathology.Amongthese, insulin resistanceinductionis well recognized**(4).**

Fetuin-A may be a useful urinary marker to predict the development of microalbuminuria andreductionofGFR in diabeticnephropathy**(5).**

There are several mechanisms that can explain the association between higher fetuin-A level andincreased risk of developing T2DM, First, fetuin-A level is positively associated with insulinresistanceSecond, fetuin-A level wasfoundto berelatedto obesity**(6).**

Higher fetuin-A may contribute the development of insulin resistance, diabetes and subsequentobesity-relatedCKDanddiabeticnephropathy**(7).**

Fetuin-A induced low-grade inflammation and repressed adiponectin production in animals andhumans. Moreover, adiponectin is a key regulator of albuminuria andis inversely related toalbuminuria**(7).**

We aimed in this study to find out a biomarker that would help for early detection of diabeticnephropathyin type 2 DM.

2. PatientsandMethods:

This was a case control study and was carried out on sixty-nine individuals admitted to

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departmentandoutpatientclinicofinternalmedicineatZagazigUniversityHospitalsfromJanuary2019to June2019.
Written Informed consent was taken from the subjects to participate in the study. Approval forperforming the study was obtained from internal medicine and medical biochemistry departments,ZagazigUniversityHospitals aftertakingInstitutional ReviewBoard(IRB) approval.

Theparticipantsweredividedintothreegroups.**GroupA:**(23subjects)Controlgroup.Apparentlyhealthy individuals,ageandsex-matched,withnegativefamilyhistoryofhypertensionandischemicheart diseases. **Group B:** (23 patients) Type 2 Diabetic patients diagnosed according to Americandiabetesassociation(ADA2010),Ageandsexmatchedwithnegativefamilyhistoryofhyperten sion and ischemic heart disease. **Group C:** (23 patients): Type 2 Diabetic patients withdiabetic nephropathy diagnosed according to American diabetes association (ADA 2010) Age andsexmatched with negativefamily history ofhypertensionand ischemic.
Patients included in the study were patients with age group 35 years old or more, of both sexes,diagnosedwithtype2DMaccordingtoAmericandiabeticassociation(ADA2010):Fastingplasma glucose value of ≥126 mg/dL (≥7.0 mmol/L) or 2-h PG ≥200 mg/dl (≥11.1 mmol/L) during oralglucose tolerance test (oGTT) or glycated hemoglobin (HbA1c) ≥6.5% (≥48 mmol/molHb) orpatient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucosevalue of ≥ 200 mg/dL (≥11.1 mmol/L) and patients were categorized according to their basalmetabolic rate into underweight <18.5, normal 18.5 to 24.9, overweight 25 to 29.9 and obese whenBMIisover 30.
All the following patients were excluded: type 1 DM, Hypertensive, gestational diabetes, smoking,centralnervoussystemcomplications,chestdisease,endstagerenaldiseases,chronicliverdisea se,ischemic heart disease, acute inflammatory conditions, hypo and hyperthyroidism dyslipidemia,hypoand hypercalcemiapatients.

Allpatients of thestudy weresubjected to the following:
2.1. Full history: all participants prior medical records including the following: Duration of DM,Familyhistoryofdiabetesmellitusandobesity,Treatmentofdiabeteswithinsulinororalhypoglycem ic agents, Calculation of the body mass index (BMI), also called Quetelet's index wasderived by dividing weight by the square of height. BMI = Weight (Kg) / Height $(m)^2$, Type 2diabetes(evidence of raised blood glucose measurements ,HA1C ,FBS ,2HPP,or RBS recorded ondifferentdaysbeforestudy(aphysiciandiagnosisoruseofmedication)duringadmission,Hyperlipidem ia(useofmedication,serumcholesterolconcentration>220mg/dLorserumtriglyceride concentration >150 mg/dl), Cardiac diseases [ischemic heart disease (documentedhistory of angina pectoris or myocardial infarction)], Stroke ,Transient ischemic attacks (TIA) oracuteneurological deficit , Complications of diabetes.

2.2. Fullclinicalexamination:
Anthropometricmeasurementincludingwaistandhipcircumferenceincm,weightinkg,andheightin cm.

2.3. <u>Collectionofsamples</u>:

- Blood sampling: 5 ml of peripheral venous blood were taken from each subject under

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completeaseptic conditions and were divided into 3 portions. 1. 1 ml collected on fluoride oxalate (2:1)2mg/ml for estimation of plasma glucose (fasting & 2 hours postprandial). 2. 1 ml collected withpotassiumEDTA1mg/mlformeasurementforglycohemoglobin(HbA1c).3.3mlwereleftfor30-60 minutes for spontaneous clotting then centrifuged at 3000rpm for 10 minutes; serum sampleswereseparated into another setof tubesand keptfrozenat-80º Ctill use.

- Fresh, mid-stream urine was collected from all patients and refrigerated at -20˚C. Using BayerCLINITEKMicroalbuminReagentStrips,asemiquantitativemethodforMicroalbuminuria.Analysiswasdoneusing theCLINITEKAnalyzer.Accordingtothemanufacturer,theBayerMicroalbustixtesthasasensitivityof90%andaspecificityof88%fortheurinaryalbumin/creatinineratio.

2.3.1. RoutineLaboratorytesting:

- **Completeblood picture:** Byautomatedbloodcounter.
- **Liverfunctiontests:**
  Serumalbumin,serumALTandASTby colorimetricmethodbyusingaspectrophotometer.
- **Renalfunctiontests:**
  serumcreatinineandserum ureabycolorimetric method.
- **Randombloodsugar,fastingbloodsugarand2hourpostprandialusingcolorimetricmethod.**
- **Lipidprofile:**
  Bloodsampleswеredrawnfromallparticipantsafter12hoursovernightfast.Allseraobtainedwereanalyzedforhdl, ldl,totalcholesteroland triglycerides.

2.3.2. Specificlaboratory:

- **HbA1C:**
  The HbA1c determination is based on the turbid metric inhibition immunoassay (TINIA) forhemolyzedwholeblood.
- **FastingInsulin:**
  HOMA-IR: Insulin resistance was determined using HOMA-IR was calculated according to theformula:fasting insulin (microU/L)x fasting glucose(mgl/dL)/ 405.
- **MeasurementofUrinaryLeveloffetuin-AbyELISAkitsdevelopdbySunRedbiotechnologyco.:**
  - Urinaryfetuin-Aintendeduse:
    ThisELISA(enzyme-linkedimmunosorbentassay)kitisintendedforthequantitativedeterminationof human Fetuin-A, also known as alpha-2-HS glycoprotein (AHSG), in serum, plasma (EDTA orHeparin), cell culture supernatant, tissue extraction and urine. This Fetuin-A ELISA kit is forlaboratoryprofessional use.
  - Testprinciple:
  - Reagents:PreparationandStorage:
    - Thistestkitwasstoredat2–8°Cuponreceipt.Allcomponentsarestableat2–8°Cuntilthisexpirationdate.
    - Priortouse,allreagentswereallowedtocometoroomtemperature.
  - SpecimenCollection:
    Urine collected using a sterile container, centrifugated 20 minutes at the speed of 2000-3000 r.p.m.removesupernatant.
  - Assayprocedure:

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1. Asufficientnumberofantibodycoatedmicrowellstripswereplaced(Cat.300010)inaholdertorunhuman Fetuin-A standards, controls and unknown samplesin duplicate.
2. TestConfiguration

- Performancecharacteristics

- Sensitivity:

  Theanalyticalsensitivityofthehuman Fetuin-AELISAasdeterminedbythe95%confidencelimiton20 duplicate determination ofzero standard is5.0 ng/mL.

- Precision:

  The intra-assay precision is validated by measuring two samples in a single assay with 20-replicatedeterminations.

2.4. StatisticalAnalysis:

Dataentry,processingandstatisticalanalysiswascarriedoutusingMedCalcver.18.2.1(MedCalc,Ostend , Belgium). Tests of significance (ANOVA, and Chi square tests, logistic and multipleregressionanalysis,Spearman'scorrelationandROCCurveanalysis)wereused.Datawerepresented and suitable analysis was done according to the type of data (parametric and non-parametric)obtainedforeachvariable.P-valueslessthan0.05(5%)wasconsideredtobestatisticallysignificant.

P-value:levelofsignificanceP > 0.05: Non-significant (NS).P<0.05: Significant (S).
P<0.01:Highly significant(HS).

Descriptivestatistics: Mean,Standarddeviation(±SD)andrangeforparametricnumericaldata,whileMedian andInter-quartilerange(IQR)fornon-parametricnumericaldata.

3. Results:

Wefoundregardingsociodemographicdataoftheparticipantsanon-significantdifferenceasregardtoageand sexamong studied groups (p > 0.05)(Table1).

A highly significant difference in duration of DM and diabetic retinopathy among studied groups;with (p <0.001). A significant difference in family history of DM among studied groups; with (p <0.05).Anon-significantdifference as regardstheremainingclinical data (p >0.05)(Table2).

Ahighlysignificantdifferenceinhemoglobin,platelets,albumin,TGs,urea,creatinine,andUrinaryAlbumin/Creatinineratio,amongstudiedgroups; with(p<0.001).AsignificantdifferenceinALTamong studied groups; with (p < 0.05). A non-significant difference as regards TLC, AST, HDL,LDL,and total cholesterol among studied groups; with (p >0.05)(Table 3).

HighUrealevelinType2DMandType2DMwithnephropathypatientsincomparisontoControlgroup;with(p<0.01),andhighUrealevelinType2DMwithnephropathypatientsincomparisontoType2 DM patients; with (p <0.01)(Table 4).

High creatinine level in Type 2 DM with nephropathy patients in comparison to Control group andType 2 DM; with (p < 0.01), but creatinine level in Control group and Type 2 DM patients

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werecomparable (p >0.05)(Table 5).

High Albumin/Creatinine ratio in Type 2 DM with nephropathy patients in comparison to Controlgroup and Type 2 DM; with (p < 0.01), but Albumin/Creatinine ratio in Control group and Type 2DMpatients werecomparable; with (p >0.05).

AhighlysignificantdifferenceinFBSandHbA1c,fastinginsulin,HOMA-IR,andUrinaryFetuin-Aamong studied groups; with (p <0.001)(Table7).
HighUrinaryFetuin-AlevelinType2DMandType2DMwithnephropathypatientsincomparisontoControlgroup;with(p<0.01),andhighUrinaryFetuin-AlevelinType2DMwithnephropathypatientsin comparison to Type2 DM patients;with (p <0.01)(Table 8).

Table(1):Comparisonofsocio-demographicdataamongstudiedgroups

		Control group(Group1) Mean±SD	Type2 DM (Group2) Mean±SD	Type 2 DM withnephropathy(Group3) Mean±SD	Test ofsignificance(F-test)	Pvalue
Age(years)		47 ± 7	48 ± 8	51 ± 7	1.343	0.268
Sex	Female	9(39.1%)	11(47.8%)	12(52.2%)	X^{20} .816	0.665
	Male	14(60.9%)	12(52.2%)	11(47.8%)		

Table(2):Comparisonofbasicclinicaldata amongstudied groups

Variable	Controlgroup (Group1) Mean±SD	Type2 DM (Group2) Mean±SD	Type2 DMwith nephropathy(Group3) Mean±SD	Test ofSignificance (F-test)	P value
Waist circumference(cm)	**79 ± 7**	**85 ± 10**	**82 ± 7**	**2.65**	**0.078**
Hipcircumference (cm)	**99 ± 3**	**101 ± 3**	**101 ± 2**	**4.9**	**0.010**
WaisttoHipratio	**0.81 ± .06**	**0.84 ± 0.08**	**0.81 ± 0.06**	**1.6**	**0.2**
Height(m)	**1.74 ± 0.1**	**1.79 ± 0.12**	**1.77 ± 0.8**	**1.53**	**0.224**
Weight(kg)	**71.5 ± 7.98**	**74.3 ± 10.56**	**70.96 ± 7.7**	**0.945**	**0.394**
BMI(kg/m^2)	**23.69 ± 1.51**	**23.16 ± 1.95**	**22.66 ± 1.49**	**2.183**	**0.121**
SBP(mmHg)	**117.39 ± 4.23**	**120 ± 6.03**	**119.57 ± 6.2**	**1.454**	**0.241**
DBP (mmHg)	**76.96 ± 3.91**	**78.91 ± 4.51**	**78.7 ± 3.76**	**1.593**	**0.211**
DurationofDM (years)	**-**	**5.6 ± 1.49**	**11 ± 2.41**	**82.869**	**< 0.001***

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	Controlgroup (Group1)	**Type2 DM (Group2)**	**Type2 DMwith nephropathy(Group3)**	**X^2**	**P value**
Family history ofDM					
+ve	**5(21.7%)**	**16(69.6%)**	**15(65.2%)**	**12.894**	**0.0016***
-ve	**18(78.3%)**	**7(30.4%)**	**8(34.8%)**		
DiabeticRetinopathy					
+ve	**0(0%)**	**5(21.7%)**	**14(60.9%)**	**21.935**	**0.0001***
-ve	**23(100%)**	**18(78.3%)**	**9(39.1%)**		

Table(3):Comparison ofBiochemicalparametersamongstudiedgroups

Variable	Control group(Group1) Mean±SD	Type2 DM (Group2) Mean±SD	Type2 DM with nephropathy(Group3) Mean±SD	Test ofsignificance(F-test)	Pvalue
Hb (g/dL)	13.62 ± 1.2	13.45 ± 0.8	12.17 ± 1.17	12.478	<0.001**
PLT(10^3/µL)	264 ± 53.51	293.91 ± 58	205 ± 30.9	19.659	<0.001**
TLC (10^3/µL)	5.95 ± 1.77	6.19 ± 1.76	6.67 ± 1.71	1.014	0.368
AST (U/L)	26.22 ± 5.77	26.04 ± 5.59	26.83 ± 7.23	0.0997	0.905
ALT(U/L)	29.96 ± 6.98	37.96 ± 11.75	38.57 ± 11.52	4.985	0.010*
S.Albumin(g/dL)	4.34 ± 0.43	4.37 ± 0.44	3.84 ± 0.49	9.787	<0.001**
T.Cholesterol (mg/dL)	148.22 ± 23.34	153.17 ± 25.11	154.65 ± 25.35	0.431	0.652
TGs(mg/dL)	113.65 ± 25.21	142.48 ± 13.07	144.61 ± 21.37	16.334	<0.001**
HDL(mg/dl)	50 ± 7	47 ± 6.6	50 ± 6.6	1.52	0.226
LDL(mg/dl)	81.69 ± 12.3	88.26 ± 7.78	86.52 ± 6.58	3.116	0.051
B.Urea(mg/dL)	32.6 ± 7.89	41.91 ± 6.2	60.91 ± 9.1	78.216	<0.001**
S.Creatinine (mg/dL)	0.97 ± 0.1	1.04 ± 0.13	2.06 ± 0.3	216.159	<0.001**
UrinaryAlbumin/Creatinineratio (mg/mmol)	13.43 ± 3.24	17.6 ± 3.8	45.17 ± 9	193.746	<0.001**

Table(4):LSDforUreacomparison amongstudiedgroups:

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Urea	Type2 DM	Type2 DMwith nephropathy
Control group	<0.01**	<0.01**
Type2 DM	---	<0.01**

Table(5):LSDforUreacomparison amongstudiedgroups:

Creatinine	Type2 DM	Type2DMwithn ephropathy
Control group	>0.05	<0.01**
Type2 DM	---	<0.01**

Table(6):LSDforUrinaryAlbumin/Creatinineratiocomparison amongstudiedgroups

Albumin/Creatinineratio	**Type2 DM**	**Type 2 DM withnephropathy**
Controlgroup	>0.05	<0.01**
Type2 DM	---	<0.01**

Table(7):ComparisonofglycemicparametersandUrinaryFetuin-Aamongstudiedgroups

Variable	**Controlgroup(Group1)**	**Type2 DM (Group2)**	**Type 2 DMwithnephropathy (Group3)**	**Test ofsignificance(F-test)**	**P value**
	Mean±SD	**Mean±SD**	**Mean±SD**		
FBS (mg/dL)	**91.48 ± 8.08**	**185.35 ± 31.79**	**181.17 ± 31.61**	**93.52**	**< 0.001****
HbA1C (mg/dL)	**5.36 ± 0.34**	**7.83 ± 0.66**	**7.93 ± 0.58**	**165.64**	**< 0.001****
FastingInsulin (mU/L)	**6.83 ± 0.87**	**8.26 ± 0.87**	**11.37 ± 2.7**	**42.205**	**< 0.001****
HOMA-IR	**1.53 ± 0.17**	**3.74 ± 0.5**	**4.96 ± 0.92**	**185.828**	**< 0.001****
UrinaryFetuin-A(ng/gcr)	**67.79 ± 12**	**86.78 ± 10.8**	**111.51 ± 20.58**	**48.457**	**< 0.001****

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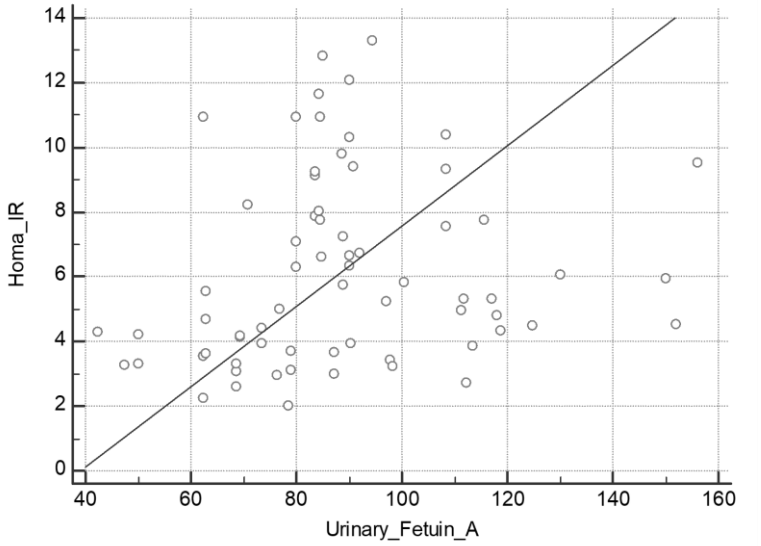
Table(8):LSDforUrinaryFetuin-Acomparisonamongstudiedgroups:

UrinaryFetuin-A	Type2 DM	Type 2 DM withnephropathy
Controlgroup	<0.01**	<0.01**
Type2 DM	---	<0.01**



Figure(1): CorrelationbetweenUrinaryFetuin-A andHOMA-IR.

4. Discussion:

Diabetes mellitus is one of the most common chronic diseases, and its incidence continues toincreasenot just in adults but also in thepediatricpopulation.**(8)**

Theearlystagein thenaturalhistory ofincreasingalbuminuriain DKDwassubclinicalproteinuria"microalbuminuria",and sometimes termedas“incipientdiabeticnephropathy.”**(9)**

Development of diabetic nephropathy has a genetic component that is likely polygenetic. Theprevalence of diabetic nephropathy varies regarding the risk factors associated which includesirreversibleriskfactorsas(age,sex,ethnicity,familyhistory, anddurationof diabetes) andmodifiableoneas(hyperglycemia,hypertension,albuminuria,dyslipidemia,andsmoking).Despitet heroleofgeneticsusceptibilityandfamilialaggregation,associationsbetweengene(s)andindexesofDK Dhavenot yet identified.**(9)**

MicroalbuminuriaisthemostwidelyusedasearlyclinicalindicatorofDNandhasbeenrecognizedasapredictorofprogressiontoend-stagekidneydiseaseinbothtype1andtype2diabetes,however,microalbuminuria is not specific for the presence of diabetic kidney disease because it can occur inpatientswithdiabeteswithoutconcurrentorfutureDN,andinnondiabeticpatientswithprogressivechronickidney disease(CKD). **(10).**

Hence,DNisthelargestsinglecauseofend-

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stagekidneydisease,therefore,thereisanurgentneedtoidentifymoresensitiveandspecificbiomarkersthanmicroalbuminuriaforearlydetectionofDN.The current study was made to evaluate urinary Fetuin-A as a biomarker for DN in type 2 diabeticpatients.

In agreement with us **Motawi et al., (11)** who discussed the potential serum biomarkers for earlydetection of diabetic nephropathy found no significant difference between patient and controlsubjectsasregardingtheBMI,whileWCwasnotdiscussedinhisstudy,andalsoinagreementwith**Assal et al., (12),** and **Nakhjavani et al., (13),** they added that BMI was not significantly differentinpatients'groups, P=(> 0.05).

DKD requires hyperglycemia to develop, and glycemic control is the primary determinant of theonset of nephropathy. Hyperglycemia generates advanced glycation end products (AGEs) withintissue and plasma. These are generated via non-enzymatic oxidative reaction of amino acids fromproteinspresent in renal tissueand plasma**(14).**

The glycemic parameters of the current study showed highly significant difference among studiedgroupsincluding (FBS, HBA1C,Fasting insulin, HOMA-IR),P =(<0.001)for all.

In agreement with us **Motawi et al., (11)**, **Sun et al., (15),** and **Assal et al., (12);** their studiessuggestingthathyperglycemiawasthedrivingforceforthedevelopmentofDNandelevatedHbA1chasbeenassociatedwiththedevelopmentofmicroangiopathyindiabetesduetothespecialaffinityforoxygen, and therebycausing tissue anoxia.

NotinconcordancewithourstudyNakhjavanietal.,(13),foundHbA1Cwasnotsignificantlydifferent between type 2 diabetic patients and type 2 diabetic patients with Diabetic nephropathy.Theroleofdyslipidemiaintheinitiationand progressionofDKDstillispoorlydefined.Resultsofseveralcross-sectionalandprospectivestudiesrevealassociationsbetweendys-lipoproteinemia,elevatedapoB-100–containinglipoproteinlevels,lowhigh-densitylipoprotein(HDL)cholesterollevels,and albuminuriain individuals with diabetes**(14).**

Asregardingourlipidprofile,including(HDL,LDL,S.CholesterolandTriglycerides),asignificantelevation in Type 2 DM and Type 2 DM with nephropathy patients than in control, Triglyceridesonlyshowed significant statistical difference, P=(<0.001)

InagreementwithusAssaletal.,(12);foundasignificantdifferenceasregardingTGonlybetweenpatients'groupsandasignificant differencebetweenpatientsand healthygroups,P=(<0.05).

Also,inagreementwithusMotawietal.,(11),BonnetandCooper,(16)andTazawaetal.,(17),found only the serum level of TGs was significantly higher in patients than in control, whileCholesterol was not significantly differed. **Mahfouz et al., (18),** found that the serum levels ofCholesterolandTGsweresignificantlyhigherindiabeticgroupsthaninthecontrolandinbetweenthediabeticgroups.Thesediscriminateresultsmaybeduetodisruptioninlipidmetabolisminthatpromoteglomerularand tubuleinterstitial injury**(19)**

Not in concordance with our results **Motawi et al., (11)** reported that, urea only was significantlyhigherinpatients'groupsthancontrolgroupandalsoinbetweenpatients'groups,whileCreatinineandUACR had no significant difference.

Nakhjavani et al., (13), found Creatinine was not significantly different between patients' groupsandhealthysubjects,andinnewlydiagnosedpatientsandthosewithdiabetesdurationofmorethan5 years.

In the current study, UACR was significantly elevated in the patient Type 2 DM with

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nephropathythancontrol group and Type2 DM group, P=(0.001)**.**
inagreementwith**Motawietal.,(11),**hefoundsignificantdifferencebetweenpatientsandcontrolsubjects,and between patients' groups.
In the present study; we used HOMA-IR index to evaluate the difference between T2DM patientsandhealthysubjects,whichwassignificantlyelevatedinpatientsgroupsthancontrolone,andwhencomparing the patients group together, a highly significant statistical difference among studiedgroupswererecorded, P=(<0.001).
Also, we found a highly significant positive correlation between HOMA-IR and urinary Fetuin-A,P=(<0.001).
Inagreementwithus**Waheed,(20)**whostudiedtheroleofsomebiochemicalmarkersinpredictionof DN, and his results showed significant difference (P<0.001) in HOMA-IR mean values betweenpatientsgroups andalsobetweendiabeticpatientsgroups andcontrol group;P=(<0.001).
The current study evaluated the role of urinary excretion of fetuin-A, and estimated its role as anearlydetector ofDN.
In diabetic nephropathy, tubular involvement may precede glomerular involvement, as severaltubular proteins and enzymes are detectable even before the appearance of microalbuminuria orrisingin serum creatinine**(21)**
Inoueetal.,(22)demonstratedthattheurinaryexcretionoffetuin-Aisacandidateforthebiomarkertopredicttheprogressionofdiabeticnephropathy.Althoughtwoprevious publishedstudiesidentified fetuin-A in urines samples of the patients with diabetic nephropathy, the quantificationswere limited to inaccurate estimations by fluorescence 2-D differential in-gel electrophoresis **(21)**andcapillaryelectrophoresis coupled to massspectrometry**(22).**

Higher excretion of fetuin-A into urine has been reported to reflect the insulin resistance andinflammatory responses in obesity and type 2 diabetes and it may reflect the increase in the serumlevelsoffetuin-Aandalterationsinthechangesinthepermeabilityofglomerularcapillaries.Fetuin-A is reported to pass through the slit diaphragm and re-introduced to proximal tubular cells bymegalin-mediatedendocytosis**(23).**
Urinary Fetuin-A in the current study showed significant elevation in patients' groups than controlgroupandincomparingType2DMandType2DMwithnephropathytogether,ahighlysignificantstatisticaldifferenceamong studiedgroups was reported, P=(<0.001),**(Table 7)**.
In agreement with **(22)** as they found that urinary excretion of fetuin-A positively correlated withCreatinine,Ureaand ACR and negatively correlatedwith serum albumin.
Higherurinaryfetuin-Aexcretiondemonstratedahigherriskforthedevelopmentofmicroalbuminuriaand reduction ofrenal function**(22)**

5. Conclusion

WecouldconcludethatUrinaryexcretionofFetuin-Acanbeusedforearlydetectionof DNinType2DM patients with excellent accuracy.

6. **ConflictofInterest**: Noconflictofinterest.

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