Evaluation of Some Parameters to Detection of Type 2 Diabetes Induced Nephropathy in the Population Sample of Al Dewaniyah Province

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Abstract

Background: Diabetic nephropathy (DN is a dreaded consequence of Type2 diabetes mellitus (T2D), accounting for about 40% of end-stage renal disease (ESRD). **Aim:** finding a predictive and tracking function of kidney failure and generalizing it as a clinical diagnostic method effective, and finding related to the development of complications of this disease with age, gender, familiar History, smoking and area of residence. **Subjects:** Study was conducted at a single center in Iraq from the beginning of February to the end of July 2021,The 90 individual were divided into 2 groups, of which 50 were chronic renal failure patients with T2D, and 40 were age and sex matched controls. **Methods:** The Insulin and HbA1C% levels was measured by using sandwich ELISA technique and a sandwich immune detection method respectively, A spectrophotometric method was applied to measure concentrations of urea, creatinine and glucose, in serum samples, then the urea-creatinine ratio and HOMA-IR were calculated mathematically. **Results:** There were significant elevated differences ($p<0.05$) for all parameters in the patient group compared to healthy controls. Moreover, ANOVA test showed significant ($p<0.05$) variation when Urea, Creatinine, Glucose, HOMA-IR and HbA1C % levels were compared between the same sex in the two subgroups, while the test showed significant ($p<0.05$) variation for only males when Urea: Creatinine ratio and Insulin levels were compared between the same sex. **Conclusion:** It can be dependable on investigations of Urea, Creatinine, Urea: Creatinine ratio Glucose, Insulin, HOMA-IR and HbA1C % as good prognostic indicators for diagnosis renal failure resulting from complications of DM.

Keywords: Urea, Creatinine, Diabetes Mellitus, Glucose, Insulin, HOMA-IR, HbA1C %

Introduction

Accumulating studies have demonstrated that type 2 diabetes (T2D) is primarily associated with insulin secretory defects related to inflammation, and metabolic stress among other contributors including genetic factors. It is a public endocrine disease categorized by hyperglycemia and insulin resistance[1].If the beta cell cannot recompense, there is a comparative lack of insulin in the blood permitting lipolysis of visceral fat garages using increased construction of free fatty acids(FFA)[2]. The increased FFA flux to peripheral tissues, including the liver and skeletal muscle, inhibits insulin signaling. With hepatic insulin resistance and an abundance of FFA substrate, gluconeogenesis is increased, further contributing to hyperglycemia. Over time, The pancreatic beta cell is no longer able to recompense for the amplified insulin requirement, and T2D is the unsuccessful outcome[3].

Complications are the important cause of disease and mortality in T2D and are confidential as microvascular (kidney disease, poly neuropathy, retinopathy) and large blood vessel (cardiovascular disease or CVD)[4]. Diabetic nephropathy is the leading cause of kidney failure. early in the Kidney disease, pathological changes happen in the glomeruli and agonize from enlargement of the mesangium, a thickening of the basement membrane, and hyper filtration. Then a loss of podocytes allows the protein to leak into the urine[5].Clinically, nephropathy is diagnosed by the presence of microalbuminuria which can progress to overt proteinuria with nephritic syndrome[6]. Chronic kidney disease (CKD) is characterized by progressive destruction of renal mass with irreversible sclerosis and loss of nephron over a period of time[7]. CKD is slowly progressing, It is usually asymptomatic until histopathological features such as tubulointerstitial fibrosis and tubular atrophy are observed in renal biopsies[8].Accompanied renal failure a rise in serum urea and creatinine. always, accompanied by a fall in urine output. Present as anuria or oliguria and is usually an indicative of the failure of both the glomerular and tubular function[9].The blood glucose concentration is regulated by two counteracting controllers, insulin and

glucagon. So these two hormones are parts of an integral rein control system then they must be linked. It is now well established that the b-cells are glucose sensitive. The rate of glucose metabolism is responsible for generating the signal for insulin secretion[10].Urea and creatinine are chemical compounds that indicate normal kidney function, while creatinine is an endogenous metabolism that is useful for assessing glomerular function. Creatinine is produced in equal amounts and excreted in the urine every day. Urea is a nitrogen product that is excreted by the kidneys from dietary protein. In patients with renal failure, serum urea levels provide the best sign for the emergence of toxic urea and are a detectable symptom compared to creatinine[11].

Prolonged hemodialysis results in infection of the stomach which results in an increase in amino acids in the stomach so that patients with chronic kidney failure result in reduced appetite and even significant bodyweight loss[12].

Subjects and Methods

During the period from the beginning of February to the end of July 2021, sera samples were collected from 90 individuals (50 diagnosed type 2 diabetic patients presented to hemodialysis (28 males their ages ranged between 42 and 65 years and 22 females their ages ranged between 40 and 63 years.), in addition to 40 healthy controls (23 males their ages ranged between 40 and 64 years and 17 females their ages ranged between 40 and 62 years)). From the dialysis center/teaching hospital in Al-Diwaniyah Governorate-Iraq.

patients samples were collected before performing the dialysis process. Early diagnosis of this group was made clinically by specialists. Healthy individuals were selected as a control group based on several criteria. the two study groups were divided according to their gender and age. The Insulin and HbA1C% levels was measured by using sandwich ELISA technique (Human Insulin ELISA Kit, SunLong Biotech Co., LTD) and a sandwich immune(ichroma™, Boditech Med Incorporated 43, Geodudanji

1-gil, Dongnae-myeon Chuncheon-si, Gang-won-do, 24398 Republic of Korea) detection method respectively, A spectrophotometric method(Enzymatic Colorimetric Test, Human Gesellschaft für Biochemica und Diagnostica mbH Max-Planck-Ring 21-D-65205 Wiesbaden-Germany)was applied to measure concentrations of urea, creatinine and glucose, in serum samples, then the urea-creatinine ratio and HOMA-IR were calculated mathematically. Statistical analysis of the results in the current study was done using version 24 of SPSS. The results were expressed in terms of mean±SD. Using independent student's t-test, where the statistical comparison was made between the two main study groups, while ANOVA test was used to compare the results of the study subgroups. The results were statistically significant at a probability value less than 5%. Criteria of Patients Inclusion the current study required the exclusion All patients with type 1diabetis, People with cancer or who has been cured, All cases of renal failure caused by diseases other than diabetes, and Cases with diabetic complications other than nephropathy complicated.

Results and Discussion

The current study included 90 individuals distributed in two main groups, The first is the group of type Π diabetic patients with renal failure undergoing to dialysis. The second group included the healthy individuals. The socio-demographic characteristics of the present study groups are shown as observational data in **Table 1**.

No significant differences were recorded when the patients and controls groups were compared together where no statistically significant differences in weight (p=0.091), height(p=0.082), BMI(p=0.095), and age(p=0.130) leveles, as shown in **Table 1.**

Table 1: Detailed Information about the Participants in the Study groups

Parameters	Type 2 Diabetic Patients 50	Healthy Controls 40	p-value
Weigh (kg)	65.536±12.327	66.536±10.327	0.091
Height (m)	1.622±0.302	1.635±0.341	0.082
BMI (kg/m^2)	25.524±4.794	26.675±3.328	0.095
Age of Onset (years)	52.96±6.718	48.3±5.734	0.130
Familiar History (Yes/No)	37/13	6/34	-
Sex (Female/Male)	22/28	17/23	-
Treated/Untreated	41/9	-	-
Smoking (Yes/No)	31/19	3/21	-
Rural/Urban	38/12	25/15	-

The Mean Difference is Significant at 0.05 Level

A significant variation (p=0.000) in urea, creatinine, and glucose concentrations and also in HOMA-IR and HbA1C % levels and (p=0.001) in insulin concentration was recorded when the patients and controls groups were compared together using independent student's t-test, as shown in **Table 2** .

Table 2: levels of some biochemical parameters in the Study Individuals

The Studied Parameter	Subjects(n)		*p-value*
	Patients (50)	Controls (40)	
	Mean ± S.D. Min-Max Range	Mean ± S.D. Min-Max Range	
Urea (mg/dL)	127.960±71.709 1-291 290	21.675±6.061 10-32 22	0.000
Creatinine (mg/dL)	8.280±2.505 3.6-14.5 10.9	0.785±0.174 0 .6-1.2 0 .6	0.000
Urea: Creatinine Ratio	20.752±10.731 7.7-67.3 59.6	27.965±7.441 12.800-43.500 30.700	0.118
Glucose (mg/dL)	199.360±61.640 80-430 350	103.025±12.476 78-141 63	0.000
Insulin (mU/L)	17.442±18.968 3.4-82.3 78.9	6.627±2.684 0.9-13.2 12.3	0.001
HOMA-IR (ulU/mL)	8.784±10.503 1.4-51.9 50.5	1.690±0.711 0.2-3.1 2.9	0.000
HbA1C %	8.490±1.294 6.8-11.5 4.7	5.02±0.322 4.2-5.6 1.4	0.000

The Mean Difference is Significant at 0.05 Level

Also, the comparison among the sub-study groups were carried out using analysis of variants (ANOVA) test. The elevation in the urea, creatinine, glucose and HbA1C % levels in the female patients comparing to their peer in the control subgroup was observed(p=0.000) as for in the HOMA-IR levels (p=0.036), same results were recorded in the urea, creatinine, urea: creatinine ratio glucose, HOMA-IR and HbA1C %levels when the two male subgroups were compared (p=0.00o)as for in the insulin levels (p=0.002), as shown in **Table 3**.

Table 3: levels of some biochemical parameters in in the Study Subgroups

The Studied Parameter	Subjects (n)			
	Patients (50)		Controls (40)	
	1) Female (22)	2) Male (28)	3) Female (17)	4) Male (23)
	Mean ± S.D. Min-Max Range	Mean ± S.D. Min-Max Range	Mean ± S.D. Min-Max Range	Mean ± S.D. Min-Max Range
	p-value			
Urea (mg/dL)	100.095±87.064 1-291 290	148.137±50.797 70-266 196	19.647±5.337 10-29 19	23.173±6.235 10-32 22
	1 *vs*2 0.002	2 *vs*4 0.000	1 *vs*3 0.000	3 *vs*4 0.830
Creatinine (mg/dL)	7.795±2.429 3.9-14.5 10.6	8.631±2.542 3.6-14.5 10.9	0.748±0.150 0 .600-1.200 0.600	0.813±0.188 0.6-1.2 0.6
	1 *vs*2 0.122	2 *vs*4 0.000	1 *vs*3 0.000	3 *vs*4 0.914
Urea/Creatinine Ratio	23.738±13.033 11.3-67.3 56	18.589±8.286 7.7-43.6 35.9	26.688±7.534 12.8-43.5 30.7	28.908±7.395 12.8-43.5 30.7
	1*vs*2 0.057	2 *vs*4 0.000	1 *vs*3 0.333	3 *vs*4 0.457
Glucose (mg/dL)	198.190±48.579 112-330 218	200.206±70.439 80-430 350	100.058±8.422 78-112 34	105.217±14.578 78-141 63
	1*vs*2 0.882	2 *vs*4 0.000	1 *vs*3 0.000	3 *vs*4 0.734
Insulin (mU/L)	14.404±16.685 3.4-82.3 78.9	19.641±20.466 5.7-80.7 75	5.929±2.744 0.9-10.9 10	7.143±2.577 3-13.2 10.2
	1*vs*2 0.204	2*vs*4 0.002	1*vs*3 0.073	3*vs*4 0.791

HOMA-IR (ulU/mL)	6.938±8.348 1.4-40.8 39.4	10.120±11.784 2.4-51.9 49.5	1.488±0.714 0 .2-2.8 2 .6	1.839±0.686 1-3.1 2.1
	1*vs*2 0.161	2*vs*4 0.000	1*vs*3 0.036	3*vs*4 0.889
HbA1C%	8.119±1.246 6.8±11.2 4.4	8.758±1.283 7.1-11.5 4.4	4.97±0.303 4.2-5.4 1.2	5.056±0.338 4.2-5.6 1.4
	1*vs*2 0.025	2*vs*4 0.000	1*vs*3 0.000	3*vs*4 0.783

1: Diabetic Female Patients with Renal Failure 2:Diabetic Male Patients with Renal Failure,3:Healthy Female Control, and 4: Healthy Male Control. The Mean Difference is Significant at0.05 Level

Kidneys regulate the volume and composition of the extracellular fluid to maintain homeostasis. Renal damage reduces glomerular filtration capacity of kidneys and leads to increased serum levels of metabolic byproducts. Among the byproducts, urea and creatinine are important indicators of renal function alterations. Dialysis is used to remove excess metabolic byproducts in cases of renal failure.During renal failure, continuous monitoring of serum levels of metabolic byproducts decides the need for dialysis[13]. Pancreatic beta cells release insulin when blood sugar levels rise. which moves glucose from the blood into muscles and other tissues, for use as energy. Helps the liver absorb glucose, storing it as glycogen in case the body needs energy during stress or exercise. When blood sugar falls, pancreatic alpha cells release the hormone glucagon. Glucagon causes glycogen to be broken down into glucose in the liver. The glucose then enters the bloodstream, restoring blood sugar levels to normal[14].Renal failure is common in patients with diabetes, and HbA1c is widely used as an index of mean blood glucose in these patients. Many factors can affect interpretation of HbA1c measurements in patients with chronic renal failure (CRF). Several reports havesuggested that erythrocyte survival is substantially lowered in most patients with CRF; this would be expected to lower HbA1c results. Although a shortened erythrocyte lifespan would presumably not interfere with the measurement of HbA1c, it could adversely affect the interpretation of HbA1c results. In chronic renal failure

CarbamylatedHb (carbHb) is increased due to abnormal urea concentration, which is dissociated in vivo to yield cyanate ions[15]. Humanly, HOMA estimated insulin resistance values were significantly associated with the prevalence of diverse diseases when it known as enhancement factor for them. hyperinsulinaemia operate on the liver to increase production of insulin-like growth factor-I (IGF-I) which stimulate tumors growth and block apoptosis. Rising confirmation supports the concept that insulin resistance is represented one of an important affect in the pathogenesis of cognitive impairment and neuro degeneration[16].

Table 4: Relationships among the variables in patients and controls groups

Patients 50			r p				Controls 40			r p		
Criteria	*Urea*	*Creatinine*	*Glucose*	*HOMA-IR*	*HbA1C %*	*U:C Ratio*	*Urea*	*Creatinine*	*Glucose*	*HOMA-IR*	*HbA1C %*	*U:C Ratio*
Urea	1 ---	0.099 0.492	0.066 0.647	0.071 0.623	0.119 0.409	0.130 0.367	1 ---	0.536** 0.000	0.151 0.351	0.124 0.445	0.003 0.983	0.689** 0.000
Creatinine	0.099 0.492	1 ---	0.009 0.953	0.063 0.665	0.074 0.609	0.524** 0.000	0.536** 0.000	1 ---	0.308 0.054	0.147 0.364	0.294 0.065	0.232 0.149
Glucose	0.066 0.647	0.009 0.953	1 ---	0.276 0.052	0.418** 0.003	0.100 0.488	0.151 0.351	0.308 0.054	1 ---	0.299 0.061	0.596** 0.000	0.102 0.533
HOMA-IR	0.071 0.623	0.063 0.665	0.276 0.052	1 ---	0.086 0.551	0.166 0.250	0.124 0.445	0.147 0.364	0.299 0.061	1 ---	0.120 0.459	0.001 0.994
HbA1C %	0.119 0.409	0.074 0.609	0.418** 0.003	0.086 0.551	1 ---	0.105 0.468	0.003 0.983	0.294 0.065	0.596** 0.000	0.120 0.459	1 ---	0.253 0.115
U:C Ratio	0.130 0.367	0.524** 0.000	0.100 0.488	0.166 0.250	0.105 0.468	1 ---	0.689** 0.000	0.232 0.149	0.102 0.533	0.001 0.994	0.253 0.115	1 ---

Correlation is significant at the 0.05 level, **Correlation is significant at the 0.01 level

In addition to increasing the correlation between (Urea, Creatinine, Urea: Creatinine ratio Glucose, Insulin, *HOMA*-IR, and HbA1C %) expressions and interstitial inflammation in diabetic nephropathy patients, the results of the present work showed a significant positive correlation among variables following: between creatinin and urea:creatinin ratio with (r=0.536), glucose and HbA1c with (r=0.418), insulin and HOMA-I.R with (r=0.961) for patients group, Also in the healthy controls group the results showed a significant positive correlation among variables following: urea and creatinin with (r=0.536), urea and urea/creatinin ratio with(r=0.689), glucose and HbA1c with (r=0.596), insulin and HOMA-I.R with (r=0.957), as shown in **Table 4**.

Conclusions

Urea, Creatinine, Urea/Creatinine ratio Glucose, Insulin, HOMA-IR and *HbA1C* % are Good prognostic indicators for predicting complications of diabetes mellitus that cause renal failure.

References

[1] Y. H. Chang *et al.*, "Interleukin-4 Promotes Myogenesis and Boosts Myocyte Insulin Efficacy," *Mediators Inflamm.*, vol. 2019, 2019.

[2] S. L. Samson and A. J. Garber, *Type 2 diabetes*, 2nd ed. Elsevier Inc., 2018.

[3] P. Re-, "Classification and diagnosis of diabetes," *Diabetes Care*, vol. 40, no. January, pp. S11–S24, 2017.

[4] L. R. Mack and P. G. Tomich, "Gestational Diabetes: Diagnosis, Classification, and Clinical Care," *Obstet. Gynecol. Clin. North Am.*, vol. 44, no. 2, pp. 207–217, 2017.

[5] D. Care and B. P. Control, "Cardiovascular disease and risk management," *Diabetes Care*, vol. 40, no. January, pp. S75–S87, 2017.

[6] J. E. W. Boell, D. M. G. V. da Silva, M. E. E. Guanilo, K. Hegadoren, B. H. S. Meirelles, and S. R. Suplici, "Resilience and self-care in people with diabetes

mellitus," *Texto e Context. Enferm.*, vol. 29, pp. 1–12, 2020.

[7] G. B. Piccoli, M. Al Rukhaimi, Z. H. Liu, E. Zakharova, and A. Levin, "What we know and do not know about women and kidney diseases; questions unanswered and answers unquestioned: Reflection on world kidney day and international woman's day," *Brazilian J. Med. Biol. Res.*, vol. 51, no. 7, pp. 1–12, 2018.

[8] M. Ruiz-Ortega, R. R. Rodrigues-Diez, C. Lavoz, and S. Rayego-Mateos, "Special issue 'diabetic nephropathy: Diagnosis, prevention and treatment,'" *J. Clin. Med.*, vol. 9, no. 3, pp. 2–5, 2020.

[9] A. Connell *et al.*, "Evaluation of a digitally-enabled care pathway for acute kidney injury management in hospital emergency admissions," *npj Digit. Med.*, vol. 2, no. 1, pp. 1–9, 2019.

[10]J. H. Koeslag, P. T. Saunders, and E. Terblanche, "A reappraisal of the blood glucose homeostat which comprehensively explains the type 2 diabetes mellitus-syndrome X complex," *Journal of Physiology*, vol. 549, no. 2. pp. 333–346, 2018.

[11]I. Arjani, "Gambaran Kadar Ureum Dan Kreatinin Serum Pada Pasien Gagal Ginjal Kronis (Ggk) Yang Menjalani Terapi Hemodialisis Di Rsud Sanjiwani Gianyar," *Meditory J. Med. Lab.*, vol. 4, no. 2, pp. 145–153, 2017.

[12]A. M. Syara, S. Suhaimi, A. S. G. Purba, J. M. Simarmata, and C. Y. Saragih, "Hubungan Lama Hemodialisis Dengan Nafsu Makan Pada Pasien Gagal Ginjal Kronik Di Unit Hemodialisis Rumah Sakit Grandmed Lubuk Pakam Tahun 2019," *J. Keperawatan Dan Fisioter.*, vol. 3, no. 1, pp. 79–86, 2020.

[13]B. S. Bagalad, K. P. Mohankumar, G. S. Madhushankari, M. Donoghue, and P. H. Kuberappa, "Diagnostic accuracy of salivary creatinine, urea, and potassium levels to assess dialysis need in renal failure patients," *Dent. Res. J. (Isfahan).*, vol. 14, no. 1, pp. 13–18, 2017.

[14]S. Falck, "Pancreas: Functions and possible problems," no. May 2017. pp. 1–8, 2017, [Online]. Available: https://www.medicalnewstoday.com/articles/10011.php.

[15]R. R. Little *et al.*, "Measurement of Hba1C in patients with chronic renal failure," *Clin. Chim. Acta*, vol. 418, pp. 73–76, 2013.

[16]K. Inoue *et al.*, "Possible discrepancy of HbA1c values and its assessment among patients with chronic renal failure, hemodialysis and other diseases," *Clin. Exp. Nephrol.*, vol. 19, no. 6, pp. 1179–1183, 2015.