



## Original Article

## Proinsulin/insulin ratio as a predictor of insulin resistance in patients with diabetic nephropathy



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## ABSTRACT

Patients with Diabetic nephropathy (DN) have an increase in cardiovascular mortality, and since IR may be a contributing factor. Therefore, **the aim of this study** was to assess the role of pro insulin/insulin ratio as a predictor of insulin resistance in patients with diabetic nephropathy

**Patients and methods:** A Case-control study was conducted in a total of 50 patients who diagnosed with type 2 diabetes mellitus from July 2017 to March 2018. The patients were divided into 2 groups according to presence of diabetic nephropathy. Demographic and clinical data were collected.

**Results:** There is a significant increase in serum pro insulin/insulin ratio in patients with diabetic nephropathy patients compared to patients without diabetic nephropathy. An association was found between increase serum pro insulin/insulin ratio and increase predicting of insulin resistance. Cut-off value of serum pro insulin/insulin ratio  $\geq 0.1145$  with sensitivity and specificity of 92.3 and 60.3 respectively as a predictor for insulin resistance

**Conclusion:** This study demonstrate a strong relationship between insulin resistance and CKD and this relationship was stronger in the presence of obesity. Pro insulin/insulin ratio was found to be a significant predictor for insulin resistance.

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## 1. Introduction

Diabetic nephropathy is the leading cause of end-stage kidney disease in the United States and most developed countries. Diabetes accounts for 30%–50% of the incident cases of end-stage kidney disease [1]. Insulin resistance (IR) is a condition in which normal response to a given amount of insulin is reduced. So hyperinsulinemia occur due to both increased production and in patient with diabetic kidney disease also decreased clearance by the diseased kidney [2].

Insulin resistance is a syndrome rather than single entity, it is a major risk factor for both cardiovascular morbidity and mortality. Insulin resistance in patients with Diabetic nephropathy, somewhat unclear is it a cause especially in the light of insulin responsiveness of renal epithelial cells or a consequence [3].

## 2. Patients and Methods

This is a case-control study included 50 patients with type 2 diabetes. They were selected from patients attending the Internal Medicine of Zagazig University Hospitals in the period between July 2017 and March 2018.

All included patients diagnosed with type 2 diabetes mellitus according to American Diabetes Association (ADA) criteria [4].

After excluded patients with type I diabetes mellitus, patients with end stage renal disease on regular hemodialysis and Proteinuria for another cause. The patients divided to two groups:

- **Group A:** Control group, includes 25 Patients with type II diabetes Mellitus without diabetic nephropathy.
- **Group B:** Include 25 Patients with type II diabetes Mellitus with diabetic nephropathy.

Patients were subjected to thorough history taking regarding age, sex, and body mass index, and arterial blood pressure, family history of diabetes mellitus, hypertension and obesity. Full clinical examination was done. Routine investigations were carried to

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verify the inclusion and exclusion criteria of studied patients. Specific investigations included HbA1c, measurement of insulin resistance by homeostasis model assessment (HOMA IR) and albumin/creatinine ratio

### 2.1. Ethical

Written Informed consent was taken from the patient to participate in the study. Approval for performing the study was obtained from Internal Medicine and Medical Biochemistry Departments, Zagazig University Hospitals after taking Institutional Review Board (IRB) approval.

### 2.2. Statistical analysis

Data analysis was performed using the software SPSS (Statistical Package for the Social Sciences) version 20. Quantitative variables were described using their means and standard deviations. Categorical variables were described using their absolute frequencies. Kolmogorov-Smirnov (distribution-type) and Levene (homogeneity of variances) tests were used to verify assumptions for use in parametric tests. To compare means, 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 assess the best cutoff for study variables, ROC curve analysis was used. The level of statistical significance was set at 5% ( $P < 0.05$ ). A highly significant difference was present if  $p \leq 0.001$ .

## 3. Discussion

This study was conducted aiming to evaluate insulin resistance in patients with diabetic nephropathy through estimation of proinsulin/insulin ratio and to study its relation to diabetic nephropathy and predicting of mortality of these patients.

In our study, there is a highly statistically significant difference between patients with diabetic nephropathy (case group) and patients without diabetic nephropathy (control group) regarding BMI. The mean of BMI is  $30.62 \text{ kg/m}^2$  in cases but the mean of BMI is  $27.45 \text{ kg/m}^2$  in control and ( $p$  value is  $< 0.001$ ). The present results support previous findings which show that patients with high BMI are more at risk for insulin resistance and diabetic nephropathy than those patients with low BMI. This was consistent with the results of Dennis et al. [5]. Obesity ( $\text{BMI} \geq 30 \text{ kg/m}^2$ ) is a significant risk for insulin resistance thus obese patients are more at risk for insulin resistance than those patients with low BMI (see Table 1).

Also, Kim and Park [6], study shows that body mass index (BMI) has been widely used as a significant predictor for diabetes mellitus, hypertension, and dyslipidemia.

Lotta et al. [7] shows that ectopic fat accumulation and 'lipotoxicity' in the liver and muscle and the disruption of adipocyte secretion may cause both hyperinsulinemia and hypertriglyceridemia that support obesity as a potential cause of the primary insulin resistance.

Goffredo et al. [8] found that in obesity-induced insulin resistance (IR). The gut microbiota has recently been reported to disturb insulin signaling, which interferes with glucose regulation in obesity. This finding suggests that alterations in the gut microbiota in obesity lead to chronic inflammation, causing IR and chronic inflammation mainly originates from adipose tissue. Adipose tissue macrophages (ATMs) correlate with increased production of inflammatory molecules and are therefore a crucial factor in inflammation. This result is in agreement with our study that obesity is a risk factor for insulin resistance.

In our study there is a non-significant increase of diabetic

**Table 1**

Shows the clinical and biochemical characteristics of patients with diabetic nephropathy and patients without diabetic nephropathy. The patients with diabetic nephropathy had higher fasting blood glucose, BMI, Serum Creatinine, Blood urea nitrogen, Albumin/creatinine ratio, ALT, Proinsulin/insulin ratio and HOMA-IR and lower eGFR (estimated glomerular filtration rate).

	DN Group (N = 25)	T2DM Group (N = 25)	P
Age (years)	60.88 ± 7.31	56.36 ± 7.78	.039
Mean ± SD			
Female n (%)	20 (80%)	17 (68%)	.333
BMI ( $\text{kg/m}^2$ )	30.62 ± 1.47	27.45 ± 2.81	<b>&lt; 0.001</b>
Mean ± SD			
Duration of DM (yrs)	10 (3–15)	11 (5–16)	.062
Mean ± SD			
S. Creatinine (mg/dL)	3.6 ± .95	.71 ± .1	<b>&lt; 0.001</b>
Mean ± SD			
BUN (mg/dL)	63.76 ± 22.17	22.51 ± 8.35	<b>&lt; 0.001</b>
Mean ± SD			
eGFR ( $\text{ml/min/1.73 m}^2$ )	32.61 ± 7.07	119.13 ± 22.71	<b>&lt; 0.001</b>
Mean ± SD			
Albumin/creatinine ratio	635 ± 200.86	11.68 ± 4.69	<b>&lt; 0.001</b>
Mean ± SD			
S. Albumin (mg/dL)	3.27 ± .32	3.52 ± .9	.195
Mean ± SD			
Total protein (mg/dL)	6.06 ± .61	5.96 ± 1.08	.666
Mean ± SD			
Total bilirubin	.53 ± .297	0.6 ± 0.2	.572
Mean ± SD			
Direct bilirubin	0.11 ± 0.11	0.11 ± 0.13	.514
Mean ± SD			
SGOT (U/L)	26.6 ± 5.05	25.12 ± 6.12	.356
Mean ± SD			
SGPT (U/L)	29.4 ± 3.64	25.88 ± 7.19	<b>.036</b>
Mean ± SD			
Hb (g/dL)	11.79 ± 1.24	12.78 ± 1.23	<b>.007</b>
Mean ± SD			
HbA1c (%) (mmol/mol)	8.09 ± 2.5(65 ± 4)	8.4 ± 2.15(68 ± 2)	.638
Mean ± SD			
FBS (mg/dL)	182.32 ± 43.22	103.52 ± 13.46	<b>&lt; 0.001</b>
Mean ± SD			
Proinsulin/insulin	0.51 ± 0.58	0.18 ± 0.18	<b>&lt; 0.001</b>
Mean ± SD			
HOMA-IR (%)	17.58 ± 11.37	3.35 ± 2.13	<b>&lt; 0.001</b>
Mean ± SD			

nephropathy in female more than male between them regarding gender that disagrees with Qiu et al. [9] who found that women have a lower incidence of insulin resistance than men of a similar age. However, this protective effect disappears after menopause, which suggests that estrogen could have a protective effect. That may indicate that  $17\beta$  oestradiol protects pro-opiomelanocortin (POMC) neurons from developing insulin resistance.

In our study, there is a positive correlation between age and diabetic nephropathy. The mean of age is 60.88 (SD ± 7.31) in patients with diabetic nephropathy but the mean of age is 56.36 (st±7.78) patients without diabetic nephropathy ( $p$  value is  $< 0.039$ ) and that agrees with Frago et al. [10] who showed a significantly higher age in group with diabetic nephropathy.

Klessens et al. [11] who found that age and insulin resistance are important risk factors for diabetic nephropathy in elderly. Although in general, the incidence of diabetic kidney disease is higher among elderly persons who have had long standing type 2 diabetes, the role of age in the development of diabetic kidney disease is unclear.

In our study, there is a highly statistically significant increase in albumin creatinine ratio in group with diabetic nephropathy in comparison to that of control group ( $p < 0.001$ ). The mean of albumin creatinine ratio is 635 with (SD ± 200.86) in group with diabetic nephropathy but the mean of albumin creatinine ratio is 11.68 with (SD ± 4.69) in patients without diabetic nephropathy and that agrees with Purohit and Tiwari [12], who found that 11

patients had albumin creatinine ratio  $>30$  mg/dl, of which 5 patients had HOMA IR  $>3.31$  and show the higher albumin creatinine ratio, the higher HOMA IR (see Table 1).

In our study, there is highly statistically significant decrease in eGFR in group with diabetic nephropathy in comparison to that of control group ( $p < 0.001$ ). The mean of eGFR is  $22.61$  ( $SD \pm 7.07$ ) in group with diabetic nephropathy but the mean in group without diabetic nephropathy is  $119.13$  ( $SD \pm 22.71$ ). This is consistent with Silva et al. [13] who found a slightly decrease in eGFR and increase in ACR respectively in group with diabetic nephropathy

The decreased eGFR in group with diabetic nephropathy can be explained by increased glomerular hydrostatic pressure, increase renal vascular permeability, aggravate glomerular hyperfiltration and enhance renal sodium reabsorption in insulin resistance. All together, the steady state of renal endothelial functions and hemodynamic harmonization may be caused by insulin resistance [14].

In our study, there is a negative correlation between eGFR and Homa IR and that was explained by Umanath and Lewis, (2018) who found that diabetes mellitus and insulin resistance leading to the generation and circulation of advanced glycation end products, elaboration of growth factors, and hemodynamic and hormonal changes. These lead to the release of oxygen free radicals and inflammatory mediators. These changes result in glomerular hyperfiltration, glomerular hypertrophy, renal hypertrophy, and altered glomerular composition, which is manifested clinically as albuminuria and hypertension

Our study revealed that there is highly statistically significant increase in serum insulin levels in patients with diabetic nephropathy with mean is  $(40.37 \pm 23.81)$  in relation to patients without diabetic nephropathy with mean  $(13.42 \pm 8.79)$   $p$  value is  $< 0.001$ , this was in agreement with Purohit and Tiwari [12], which found slightly difference between the studied group in median value of fasting plasma insulin in group with diabetic nephropathy was  $7.45 \mu\text{U/ml}$  with mean  $10.53 \pm 9.42 \mu\text{U/ml}$  while in control was  $7.19$  with mean  $7.43 \pm 2.26 \mu\text{U/ml}$  ( $p$  value  $0.026$ ) that disagree with Saisho et al. [15] who show significant negative correlations with insulin ( $r = -0.330$ ).

Our study revealed that there is highly statistically significant increase serum fasting glucose in group with diabetic nephropathy as mean of serum fasting glucose is  $182.32$  ( $\pm 43.22$ ) with median is  $174$  comparison to group without diabetic nephropathy as the mean of serum fasting glucose is  $103.052$  ( $\pm 13.46$ ) with median is  $98$  and that agree with study by Purohit and Tiwari [12], that show that the mean of fasting blood glucose is  $164.12$  ( $\pm 54.43$ ) with median is  $146$  mg/dl and controls had median  $112$  with mean  $(110.6 \pm 7.07)$  mg/dl ( $p$  value  $p < 0.000$ ).

In the current study, a significant increase in HOMA-IR in the group with diabetic nephropathy as compared to in group without diabetic nephropathy, these findings cope with Purohit and Tiwari [12], and Silva et al. [13], who reported that HOMA-IR is increased in patients with diabetic nephropathy and also it was reported that increased HOMA-IR index predicted insulin resistance. That was against a study by Srikanthan and Karlamangla, [16]. Who showed that the participants in the highest quartile of skeletal muscle had a reduction of HOMA-IR compared to the lowest quartile of skeletal muscle with hemodynamic alteration in the kidney

In the present study, there is a highly statistically significant difference between study groups regarding in HOMA-IR as group with diabetic nephropathy had a higher level than group without diabetic nephropathy. The mean value of group with diabetic nephropathy is  $(17.58 \pm 11.37)$  and mean value of group without diabetic nephropathy is  $(3.35 \pm 2.13)$  ( $p < 0.001$ ). In agree with Purohit and Tiwari [12], who revealed that statistical significant increase of the median value of HOMA IR score is  $2.91$  in group with diabetic

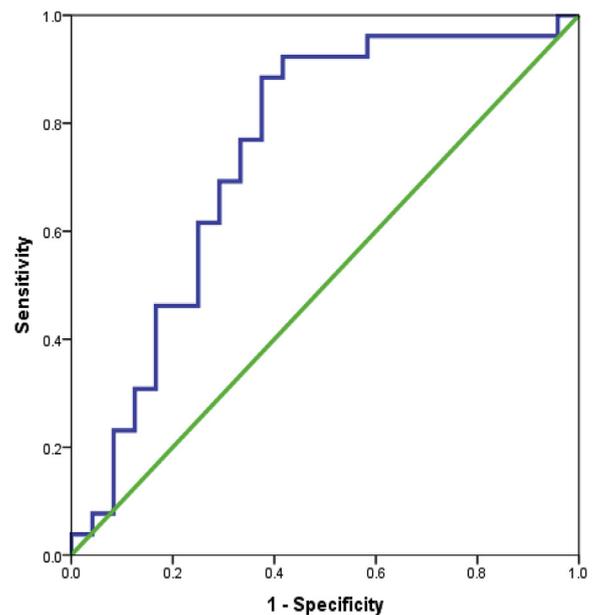
nephropathy with mean value of  $(4.15 \pm 3.56)$  and the median value of group without diabetic nephropathy is  $1.97$  with mean  $(2.03 \pm 0.64)$  ( $p < 0.0001$ ).

Our study revealed that a fasting pro insulin  $>26.65$  mIU/L would be of predictable value in determining the development of insulin resistance of type 2 DM, with sensitivity and specificity of  $61.5$  and  $45.8$  respectively. This was near to study of Heaton et al. [17] who found that at an elevated pro insulin concentrations have been demonstrated in patients with type2 DM and were interpreted as an indication of early subclinical B-Cell dysfunction.

In our study, there are highly statistically significant differences between study groups regarding Pro insulin/insulin. Pro insulin/insulin had higher level in group with diabetic nephropathy in comparison to group without diabetic nephropathy. The mean of cases is  $0.51 \pm 0.58$  while the mean of control is  $0.18 \pm 0.18$  ( $p < 0.001$ ). Saisho et al. [15] who revealed P/I ratio showed significant positive correlations with plasma glucose ( $r = 0.465$ ), HbA1c ( $r = 0.434$ ) and significant negative correlations with insulin ( $r = -0.330$ ) and HOMA- $\beta$  ( $r = -0.520$ ) even after adjustment for age, sex, duration of diabetes, family history of diabetes, use of sulfonylureas, smoking and body mass index.

The best cutoff value of Pro insulin/insulin ratio in prediction of insulin resistance was  $\geq 0.1145$  with sensitivity  $92.3$ , specificity  $60.3$ , PPV  $63.2$ , PNV  $83.3$ , +LR  $1.58$ , -LR  $0.18$ , accuracy  $68\%$  ( $p < 0.05$ ). Estimation of pro insulin level and pro insulin\insulin ratio provide us with a clear vision about beta cell function in patients with diabetic nephropathy. Provide the most utility as a clinical tool for predicting insulin resistance and diabetic nephropathy (see Fig. 1). That result agree with (Ohkura et al. [18] who found that an elevated P/I ratio reflects impairment of insulin secretory capacity of  $\beta$ -cells and cardiovascular risk in patients with IGT or T2DM.

Klessens et al. [11] found that the peak incidence (3%/year) is usually found in persons who have had diabetes for 10–20 years In Pima Indians with type 2 diabetes, the onset of diabetes at a younger age was associated with a higher risk of progression to end-stage kidney disease that against the result in our study where there are non-significant differences between the studied group in



**Fig. 1.** ROC curve of Pro insulin/Insulin ratio in prediction of insulin resistance The best cutoff value of Pro insulin in prediction of insulin resistance was  $\geq 0.1145$  with sensitivity  $92.3$ , specificity  $60.3$ , PPV  $63.2$ , PNV  $83.3$ , +LR  $1.58$ , -LR  $0.18$ , accuracy  $68\%$  ( $p < 0.05$ ).

duration of diabetes.

In our study, there are non-significant differences between groups in ultrasonographic renal grading regarding age, BMI or gender, Insulin, pro insulin, pro insulin/insulin ratio, HbA1c, type of treatment and HOMA-IR. This result is against with Silve et al. [13] who revealed that the higher nephropathy grade, the higher HOMA IR will be and that patients with type 2 diabetes predisposed to higher insulin resistance are more likely to develop microalbuminuria. Future research should aim at stopping renal function deterioration by improving insulin sensitivity for type 2 diabetes with high insulin resistance and that confirmed by HSU et al. [14].

In our study, there are highly statistically significant difference increase between both ultrasonographic renal impairment grading group regarding albumin creatinine ratio as p value of ACR is 0.765 and that in agree with Silva et al. [13] that show with progression of diabetic nephropathy from stage1 to stage 2, there is increase in albumin creatinine ratio level. We reconfirm that patients with type 2 diabetes predisposed to higher insulin resistance are more likely to develop microalbuminuria.

In our study, there is statistically significant difference between both ultrasonographic renal impairment grading group regarding direct bilirubin as grade2 nephropathy group had higher level in bilirubin than grade1 nephropathy group while there are statistically non-significant differences between both groups regarding serum albumin, total protein, total bilirubin, SGPT, SGOT and CBC findings.

We can conclude that the high level of pro insulin/insulin ratio in type2 diabetes with diabetic nephropathy due to hyperglycemic state may lead to many vascular complications in type2 diabetes. Therefore, we recommend screening patients with any kidney function abnormality in type 2 diabetes for early management to guard against vascular complications that may occur in type 2 diabetes.

### Conflicts of interest

The authors declare that they have no competing interests.

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