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ORIGINAL ARTICLE

Tryptophan Level as a Potential Prognostic Marker of Diabetic Nephropathy

Ahmed Reda Faheem*[1], Ezzat Moustafa Mohammed[1], Hoda Fathi Mohamed[2], Said Mohammed El Barshomy[1]

[1] *Internal Medicine Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt*
[2] *Clinical Pathology Department, Faculty of Medicine, Zagazig University, Zagazig,Egypt*

***Corresponding author:**
Ahmed Reda Mohamed Faheem
Internal Medicine Department,
Faculty of Medicine,
Zagazig University,
Zagazig, Egypt

Email:
a.reda202020@yahoo.com

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ABSTRACT

Background: Diabetic nephropathy (DN) is one of the most common diseases in the world and developing countries such as Egypt. DN is leading to end stage renal disease. Tryptophan is a recent good marker for diabetic nephropathy. So, the aim of our study is to evaluate the value of tryptophan in diabetic patients either with or without nephropathy and to investigate the association between tryptophan and eGFR.

Patients and Methods: A Cohort study that includes 60 patients who are diagnosed with diabetes mellitus from January 2018 to August 2018. The patients were divided into 2 groups according to presence of diabetic nephropathy or not and follow up the same patients in internal medicine department in Zagazig university after six monthes. Demographic and clinical data were collected.

Results: Tryptophan is associated with positive correlation with eGFR and negative correlation with serum creatinine level and albumin/creatinine ratio in diabetic nephropathy patients.

Conclusion: A low level of tryptophan, especially <39.5 ng/L, was associated with a rapid decline in eGFR through six monthes follow up. So that tryptophan might be regarded as a good prognostic marker for diabetic nephropathy.

Keywords: Diabetic nephropathy, Diabetes mellitus, Chronic kidney disease, Tryptophan.

INTRODUCTION

Diabetic nephropathy leads to more specific pathological structural and functional changes that are seen in the kidneys of diabetic patients which result from the effects of diabetes mellitus on the kidney. Diabetic nephropathy is mostly leading to end-stage renal disease in the United States and most developed countries such as Egypt. Diabetes accounts for 30% to 50% of cases of end-stage renal disease and this percentage was considered high related to other causes which lead to end-stage renal disease. [1]

Diabetic nephropathy is mostly leading to changes in many metabolites and tryptophan level is one of them, so kidneys are responsible for excretory and absorptive functions ,and also responsible for rapid protein synthesis and amino acid oxidation. Metabolism of biomolecules of the kidney could fluctuate in the early stages of diabetic nephropathy. [2] Tryptophan is essential non-polar aromatic protein amino acid with one side chain. Decreased tryptophan level is accompanying with deterioration of renal function . [3] In our study, we investigated tryptophan levels in diabetic patients to search for the relation between the serum tryptophan concentration and renal function degeneration so, we can consider that tryptophan is a potentially predictive marker for diabetic nephropathy.

METHODS

A cohort study which includes 60 diabetic patients. They were selected from patients who checked at outpatients and inpatients of Nephrology unit and Internal Medicine department of Zagazig University hospitals from January 2018 to August 2018 and follow up the same patients after six months. We can diagnose our patients with diabetes mellitus according to American Diabetes Association (ADA) criteria. [4] Since 2003, which based on:The use of a fasting plasma glucose (FPG) test for the diagnosis of diabetes was recommended, and the cut point separating diabetes from nondiabetes was lowered from FPG ≥140 mg/dl (7.8 mmol/l) to ≥126 mg/dl (7.0 mmol/l). Normal FPG was defined as <110 mg/dl (6.1 mmol/l).The use of HbA_{1c} (A1C) as a diagnostic test for diabetes was not recommended. The primary reason for this decision was a lack of standardized methodology resulting in varying nondiabetic reference ranges among laboratories.Although the OGTT (which consists of an FPG and 2-h PG value) was recognized as a valid way to diagnose diabetes, the

use of the test for diagnostic purposes in clinical practice was discouraged for several reasons (e.g., inconvenience, less reproducibility, greater cost). The diagnostic category of impaired glucose tolerance (IGT) was retained to describe people whose FPG was <126 mg/dl but whose 2-h PG after a 75-g oral glucose challenge was 140–199 mg/dl.The range of FPG levels between "normal" and that diagnostic for diabetes was named "impaired fasting glucose" (IFG). IFG identified people whose FPG ranged from 110 mg/dl (6.1 mmol/l) to 125 mg/dl (6.9 mmol/l). This construct was established so that there would be a fasting category analogous to IGT.

From our work we excluded patients with renal diseases other than diabetic nephropathy, Kidney transplantation, acute renal failure or rapidly progressive glomerulonephritis, liver diseases, inflammatory or malignancy diseases or active infection and critically ill patients.

These criteria are based on exclusion of other causes of nephropathy rather than diabetic nephropathy and showing the role of tryptophan in diabetic nephropathy progression.

The patients were divided into two groups:

- **Group one (DM):** include 30 diabetic patients without nephropathy.
- **Group two (DN):** include 30 diabetic patients with nephropathy.

Patients were subjected according to history taking regarding age, sex, body mass index, arterial blood pressure,and diabetes mellitus index (duration, type, medication). Full clinical examination was done. Routine investigations were carried to verify the inclusion and exclusion criteria of studied patients: complete blood count(CBC), kidney function tests(KFT), liver function tests(LFT), eGFR and Albumin/creatinine ratio (ACR) were measured to all patients. Specific investigations included Serum Tryptophan using enzyme linked immune sorbent assay (ELISA kits).The assay was carried out by human tryptophan ELISA kit by Andy Gene Biotechnology Co., LTD. After collection of the whole blood, we allowed the blood to clot by leaving it undisturbed at room temperature taking 10-20 minutes. Then we remove the clot by centrifuging at 2000-3000 rpm for 20 minutes.

Ethical Clearance: Approval for performing this study was obtained from Internal Medicine, Medical Biochemistry, and clinical pathology Departments at Zagazig University Hospitals after taking Institutional Review Board (IRB) approval.Written informed consent was obtained from all participants. The study was done according to The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

STATISTICAL ANALYSIS

All data were collected, tabulated and statistically analyzed using SPSS 20.0 for windows (SPSS Inc., Chicago, IL, USA) & MedCalc 13 for windows (MedCalc Software bvba, Ostend, Belgium). Data were tested for normal distribution using the Shapiro walk test. Qualitative data were represented as frequencies and relative percentages. Chi square test and fisher exact was used to calculate difference between qualitative variables as indicated. Quantitative data were expressed as mean+_SD(standad deviation) for parametric and median and range for non-parametric data. Independent T test and Mann Whitney test were used to calculate difference between quantitative variables in two groups for parametric and non-parametric variables respectively. Pearson and Spearman correlation coefficient were used for correlating normal and non-parametric variables respectively. (+) sign indicates direct correlation and (-) sign indicates inverse correlation. Values near to 1 indicate strong correlation and values near 0 indicates weak correlation. Receiver operating characteristic curve (ROC) was constructed to permit selection of threshold values for test results and comparison of different testing strategies. Value of area under a ROC curve indicates: 0.90-1=excellent, 0.80-0.90=good, 0.70-0.80=fair, 0.60-0.70=poor,and 0.50-0.60=fail and the optimal cutoff point was established at point of maximum accuracy. All statistical comparisons with significance level of p-value <0.05 indicates significant, p<0.001 indicates highly significant difference while p>0.05 indicates non-significant difference.

RESULTS

We can use receiver operating characteristic curve (ROC curve) to permit selection of threshold values for test results and comparison of different testing strategies. Areas under ROC curves and their standard errors were determined using the method of cantor,and compared using the normal distribution, with correction for correlation of observations derived from the same cases.

The demographic and clinical features of the patients by using independent T test, Chi square test and p-value test are described in (Table 1).

We found there are positive correlation between Tryptophan and eGFR in DN group. Also, there are negative correlation between Tryptophan and diabetes duration, creatinine, ACR and HbA1c in DN group. Besides, a negative correlation between Tryptophan with diabetes duration and HbA1c were found in DM group.

After six months follow up by using p-value test we found there is no difference in group 1 (DM group) according to serum creatinine, albumin creatinine ratio, blood urea, eGFR and tryptophan

level as described in (Table 2).But in group 2 (DN group) by using p-value test we found there is increase in serum creatinine and albumin creatinine ratio and decrease in eGFR and serum tryptophan compared to the previous results as described in (Table3).The best cut-off value of tryptophan as a marker of diabetic nephropathy by using Chi square test,p-value test and kappa agreement test was 39.5 ng/L with sensitivity of 83.3% and specificity of 96.7%. (Table 4

Table 1: Patients' characteristics between the group.

	DM patients (N=30)	DN patients (N=30)	P
Age (years) Mean ± SD	44.2 ± 9.48	48.01 ± 5.41	**.003**
Female n (%)	20 (66.7%)	22 (73.3%)	.206
BMI (kg/m^2) Mean ± SD	27.45 ± 2.81	29.62 ± 1.47	**.001**
Duration of DM (yrs) Mean ± SD	9.14 ± 4.45	11.61 ± 2.3	**.001**
S. Creatinine (mg/dL) Mean ± SD	0.73 ± 0.13	2.1 ± 0.12	**<0.001**
BUN (mg/dL) Mean ± SD	22.51 ± 8.35	63.76 ± 22.17	**<0.001**
eGFR (ml/min/1.73 m^2) Mean ± SD	119.13 ± 22.71	52.61 ± 7.07	**<0.001**
Albumin/creatinine ratio Mean ± SD	11.68 ± 4.69	635 ± 198.86	**<0.001**
S. Albumin (mg/dL) Mean ± SD	3.52 ± .9	3.27 ± .32	.195
Total protein (mg/dL) Mean ± SD	5.96 ± 1.08	6.06 ± .61	.666
Total bilirubin Mean ± SD	0.6 ± 0.2	.53 ± .297	.572
Direct bilirubin Mean ± SD	0.11 ± 0.13	0.11 ± 0.11	.514
AST (U/L) Mean ± SD	25.12 ± 6.12	26.6 ± 5.05	.356
ALT (U/L) Mean ± SD	25.88 ± 7.19	29.4 ± 3.64	**.036**
Hb (g/dL) Mean ± SD	12.78 ± 1.23	10.79 ± 3.24	**.001**
HbA1c (%) Mean ± SD	8.4 ± 2.15	8.19 ± 2.5	.638
FBS (mg/dL) Mean ± SD	103.52 ± 13.46	182.32 ± 43.22	**<0.001**
Tryptophan (ng/L) Mean ± SD	132.13 ± 6.27	38.3 ± 26.03	**<0.001**

Table 2: Change assessment between base data and follow up data among group1 (DM group):

	Base	Follow up after 6 months	P
Cr	0.73±0.13	0.75±0.14	0.158
ACR	11.68±4.69	11.5±3.6	0.258
Urea	22.51±8.35	21.6±7.32	0.321
e GFR	119.13±22.71	120.5±14.6	0.654
Tryptophan	132.13±6.27	131.8±15.36	0.354

No significant change.

Table 3: Change assessment between base data and follow up data among group2 (DN group):

	Base	Follow up after 6 months	P
Cr	2.1±0.12	2.9±0.15	0.0002**
ACR	635±198.86	769.5±254.6	0.00**
Urea	63.76±22.17	65.6±21.36	0.087
e GFR	52.61±7.07	44.65±4.9	0.038*
Tryptophan	38.3±26.03	31.3±12.36	0.021*

Cr and ACR significantly increase but Tryptophan and eGFR significantly decrease.

Table 4: Association and agreement between tryptophan level cut off 39.5 and detection of nephropathy in both groups:

			Group		Total	X^2	P	Kappa agreement
			DM	DN				
Tryptophan	>39.5	N	**29**	**5**	**34**	**39.09**	**0.00****	**0.8**
		%	**96.7%**	**16.7%**	**56.7%**			
	<39.5	N	**1**	**25**	**26**			
		%	**3.3%**	**83.3%**	**43.3%**			
Total		N	**30**	**30**	**60**			
		%	**100.0%**	**100.0%**	**100.0%**			

Sensitivity83.3% vs. specificity96.7%.

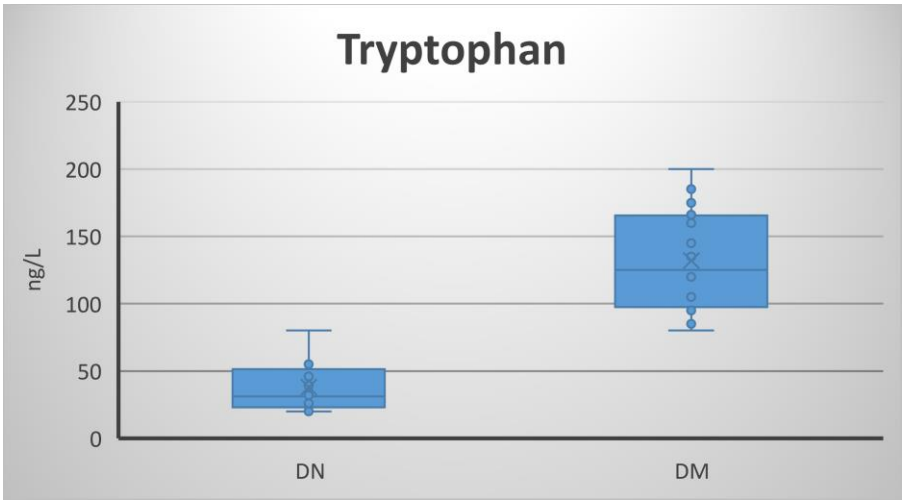

Figure1: Tryptophan levels of the two studied groups.

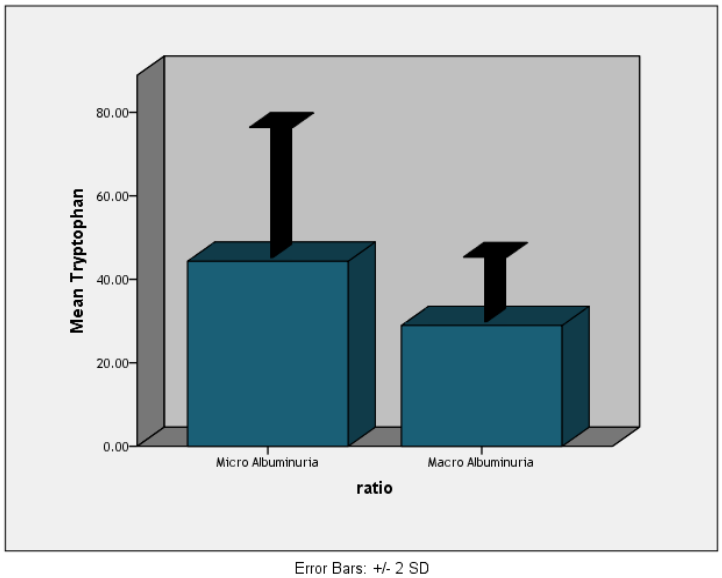

Figure 2: Albuminuria levels regard tryptophan among group2 (DN group)

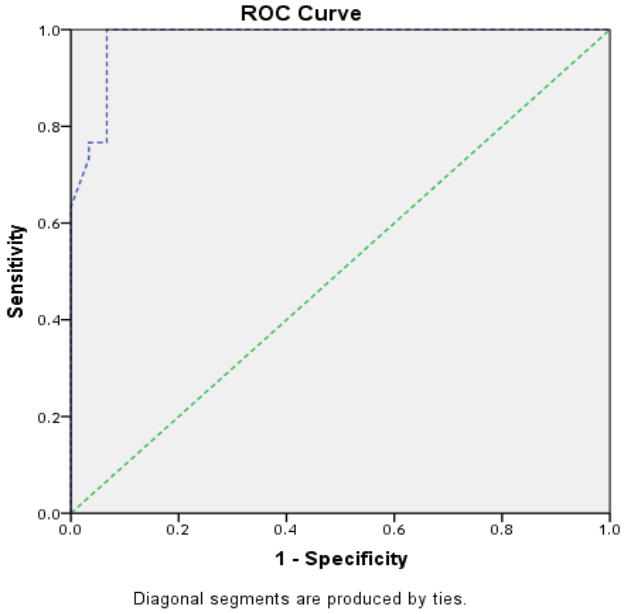

Figure 3: ROC Curve for detection of nephropathy in group2 (DNgroup).

DISCUSSION

Diabetic nephropathy is one of the most common diseases in the world which mostly lead to end stage renal disease. Also, the most common cause of death in diabetic nephropathy is cardiovascular disease. eGFR is mostly decreased in chronic kidney disease patients. [5]The most effective method is to diagnose diabetic nephropathy in earlier stages to predict and prevent progression of diabetic nephropathy. [6] In our study, diabetic nephropathy patients found to have lower hemoglobin level and eGFR and higher creatinine and albumin/creatinine ratio compared to diabetic patients.Chou et al. [6] noted that an increase in albumin/creatinine ratio and a decrease in hemoglobin level will lead to chronic kidney disease progression, and will indicate to diabetic nephropathy more severity. We found that tryptophan strongly correlated positive with eGFR while it correlated negative with serum creatinine and albumin/creatinine ratio.In our study, we found that tryptophan is associated with eGFR change as it decreased in patients with diabetic nephropathy. Chou et al. [6] reported a similar finding. In Solini et al. [7] study, tryptophan was found to be directly related to eGFR.Tryptophan is associated with rapid decline in eGFR after adjusted to more factors including sex, age, duration of diabetes, glycated hemoglobin, hemoglobin, and albumin/creatinine ratio.In Chou et al. [6] study is comparing between the metabolites and decline in eGFR, they found that tryptophan is the only metabolite that show significant difference.

In chronic kidney disease patients, serum tryptophan levels are decreased, and other metabolites of the kynurenine pathways are increased. [8]Diabetic nephropathy is pathophysiologically an interaction between (metabolic, inflammatory and hemodynamic changes). [9]The most energy pathway-related metabolites including tryptophan are diabetic nephropathy biomarkers. [10]Garibotto et al. [11] and Rhee et al. [12] studies have showing an decrease in serum branched amino acids concentrations and tryptophan in more studies, and in advanced chronic kidney disease patients. Decreased tryptophan level will clarify renal function deteriorations. Increased oxidation and chlorination of residues of tryptophan in hexamer which is non-collagenous of diabetes, patients will suggest that tryptophan is involved in renal oxidation stress and will increase the degradation of proteolytics. [3] A metabolite of tryptophan (indoxyle sulfate), plays an important role in regression of renal function. After digestion, dietary tryptophan will change to indole by microbiota in the colon, and metabolized in the liver to indoxyl sulfate. [13]Indoxyl sulfate accumulation will occurre in chronic kidney disease patients and decrease the secretory function. Ahigh level of indoxyl sulfate concentration will lead to increase in the oxidative stress by activation of nuclear factor-jB. [14]Many uremic toxins and metabolites which are derived from tryptophan will promote the oxidative stress,the activation of the leukocytes, and the inflammation of endothelial and vascular smooth muscle cells, which will lead to cardiovascular disease in patients with chronic kidney disease. [15]To detect optimal cut-point of Tryptophan on predicting progression of diabetic nephropathy, we found that cut-off value of 39.5 ng/L will show a sensitivity of 83.3% and a specificity of 96.7%.Chou et al. [6] reported when used the

analysis of receiver operating characteristic curve, Tryptophan is showing cut-off value of 42.20 µmol/L with a sensitivity of 55.6% and a specificity of 87% to predict the progression of diabetic nephropathy.

CONCLUSION

Our study revealed that low level of tryptophan is accompanying with deterioration of kidney functions and chronic kidney disease progression.so, we consider tryptophan level as aprognostic marker for diabetic nephropathy.

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