Correlation Of NAG, NGAL, and Oxidative Parameters For Patients With Diabetic Nephropathy

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KEYWORDS

Diabetic Nephropathy, N-acetyl-β-D_glucosaminidase {NAG}, Human neutrophil gelatinase_associated lipocalin {NAGL}, TOS, TAC, Malondialdhyde, Glutathione.

ABSTRACT

Background: Diabetic nephropathy is a clinical syndrome that is present in diabetic mellitus patients and characterized by albuminuria {>200 or >300 mg\day}, a gradual glomerular filtration rate decline along with hypertension. Objective: Study the correlation between N_acetyl-ß-D-glucamindase and human neutrophil gelatinase-associated lipocalin and oxidative parameters in diabetic nephropathy patients compared with a control group and several variables by Explaining the results scientifically. Method: The blood sample was collected from forty-five diabetic nephropathy patients and forty-five controls from Merjin Medical Hospital and Al-Imam Al-Sadiq Hospital in Iraq to assess N-acetyl-β-D-glucosaminidase and human neutrophil gelatinase-associated lipocalin levels by used Enzyme-Linked immunosorbent Assay technicality (ELISA) and also to determine the oxidative parameters (total oxidant system, total antioxidant capacity, malondialdehyde and glutathione) by used spectrophotometer. Result: positive correlation for N-acetyl-β-D-glucosaminidase with total oxidant status and malondialdehyde where the correlation coefficient (r=0.893**, 0.809**) at respectively and negative correlation with total antioxidant capacity and glutathione where the (r=-0.851**, -0.465**) at respectively, and exact correlation for human neutrophil gelatinase-associated lipocalin with total oxidant status, malondialdhyde, total antioxidant capacity, glutathione. Conclusion: The assessment of NAG, NAGL, and oxidant parameters can provide crucial information for the early diagnosis, management, and therapy of these patients with diabetic nephropathy.

1. Introduction

Diabetic nephropathy (DN) is a renal disease that is frequently verified by histological lesions and is closely linked to long-term diabetes [1]. About {30-40}% of diabetic individuals get diabetic nephropathy, and {5-10}% of these patients go on to develop end-stage renal disease [2], accompany with increase the excretion of albumin in urine following acute renal damage is seen by microalbuminuria { 30-299 mg\24h } which is followed by macroalbuminuria {>300 mg\24h}, together with mild systemic hypertension that get worse over time [3]. The onset and development of DN are influenced by several pathways and mediators, such as oxidative stress, inflammation, and angiotensin II {Ang-II}, with oxidative stress being the most significant [4]. An imbalance between oxidant antioxidants leads to an excessive build-up of reactive oxygen species {ROS}, which causes the body's oxidative damage [5]. Both direct and indirect mechanisms that damage tissue cell's DNA, proteins, and lipids result in oxidative stress that induces kidney damage. This mechanism also directly causes a pathological change in the glomeruli, renal tubules, and renal interstitium, including cellular components like decreased levels of podocin, nephrin, and podocyte-related proteins [6]. Free radicals will oxidize polyunsaturated fatty acids in lipids, such as arachidonic acid and linoleic acid, which are the cellular membrane's main constituent and cause significant tissue damage. That is known as lipid peroxidation, which forms MDA as an end product [7], and individuals with chronic kidney disease will have higher levels in the serum compared to healthy control participants [8].

Antioxidants are the body's defense mechanism against oxidants' damaging effects, which

can be categorized into enzymatic and nonenzymatic. The major nonenzymatic antioxidants are glutathione {L-γ–glutamyl-Lcysteinyl-glycine, GSH} [9]; this ubiquitous thio tripeptide system protects normal cellular function and survival by interacting with reactive oxygen species {ROS} and reducing superoxide, peroxynitrite and hydroxyl radicals [10]. Glutathione acts as a direct scavenger and co-substrate for GSH peroxidization. We observed that GSH levels were significantly lower in type 2 diabetic nephropathy than control [11].Serum N-acytel-ß-D-glucamindase is an intracellular lysosomal enzyme with a significant molecular weight found in many tissues, with the kidney's proximal tubules having the highest concentration [12]. Elevations in this enzyme in the serum and urine during kidney injury precede elevations in serum creatinine, making it an excellent early indicator [13]. Human Neutrophil Gelatinase-associated Lipocalin {NGAL} is a 25 KDa secreted glycoprotein expressed by vascular endothelial cells, neutrophils, and renal tubular epithelial cells. It is a member of the lipocalin superfamily [14]. It has been discovered that NAGL excretion in serum and urine serves as an early predictor of kidney damage [15].

Objective

Assessment of the levels of NAG and NGAL as Predictors for the Risk of Dialysis in patients with diabetic nephropathy compared with control

2. Methods and subjects

Study design

In September 2023, a case-control study design was initiated to October 2024, with 90 participants divided into forty-five patients and forty-five control, performed for patients in dialysis and kidney transplant centers in Mirjan Medical City, Al-Imam al-Sadiq Teaching Hospital City/Iraq.

The following equation calculated the sample size :

$$N = Z^2\ P\frac{(P-1))^2}{d^2}$$

Sample number is N, Z Score{1.96}, mention to the society= P, and an absolute marginal error {5%}= d, so the number of samples collected is ninety.

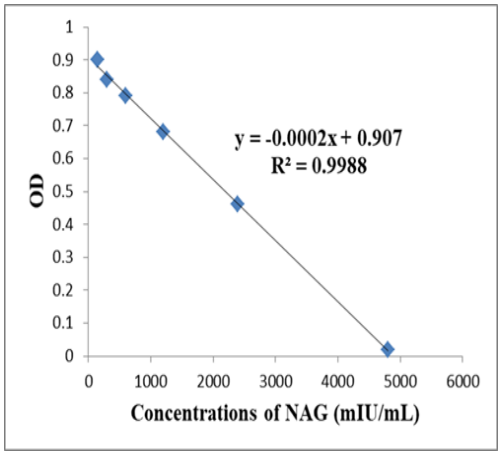
Control and Patients

Individuals who participated in this study were 45 without diabetic nephropathy, and the physician diagnosed 45 patients with diabetic nephropathy.

Procedure

Determination of Human Neutrophil Gelatinase Associated Lipocalin and Human N-acetyl- β-D-Glucosaminidase.

Assessment by ELIZA kit supplied from BT LAB applied to the in vitro for quantitative determination of serum's Human (NAG) and (NAGL) concentration. This ELISA kit used the Competitive - ELISA principle at 450 nm for Human N-acetyl- β-D-Glucosaminidase and the sandwich-ELIZA for Human Neutrophil Gelatinase- Associated Lipocalin. The NAG and NAGL standard curves were obtained using the ELISA technique (Figures 1 and 2)



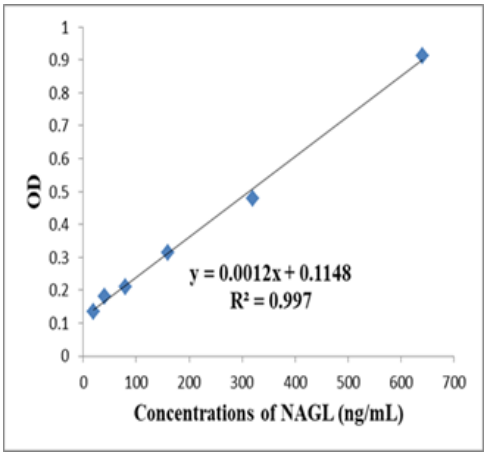


Fig.(1 and 2): Standard Curve NAG and NAGL

Determination of TOS

By oxidation, the reaction of ferrous ion O-dianisidne to ferric ion is improved by glycerol molecules found in the reaction medium. Then, the complex is measured by spectrophotometry.

Determination of malondialdhyde

Determination of MDA predicated on the color that was measured spectrophotometrically during the reaction between thiobarbituric{TBA}.

Determination of Total Antioxidant Capacity

Spectrophotometric techniques by the oxidation reaction for copper ion.

Determination of Glutathione (G The measurement of GSH depends on the reaction of DTNP to produce chromogen absorbed at 412nm.

Statistical Analysis

All results were statistically analyzed using version 22 (SPSS) software. Data presented as (mean±standard deviation) with 95% confidence intervals and correlation tests to determine whether there was an association between the variables and probability values less than P-value= 0.05 that were considered significant statistically. The diagnostic values of NAG, NAGL, TOS, TAC, MDA, and GSH were evaluated utilizing the receiver operating characteristic curve. One can calculate the optimal cutoff based on the Youden index and ascertain the sensitivity and specificity of biochemical parameters by selecting the point closest to the top left corner of the ROC curve.

3. Results and Discussion

Table 1 shows the correlation between NAG and NAGL with oxidant-antioxidant parameters for patients with diabetic nephropathy.

Parameters	TOS	TAC	MDA	GSH

NAG	**0.893****	**-0.851****	**0.809****	**-0.465****
NAGL	**0.629****	**-0.562****	**0.565****	**-0.294****

Table 1: Correlation of NAG and NAGL with Oxidant-Antioxidant Parameters

****Correlation is significant at P-value ≤ 0.01 level (2-tailed)**

*** Correlation is significant at P-value ≤ 0.05 level (2-tailed)**

(Table 1) shows a significant correlation for NAG with TOS (r= 0.893**) and MDA(r= 0.809**) and a negative correlation for NAG with TAC(r= -0.851**) and GSH (r= -0.465*In addition, A significant correlation for TOS and MDA with NAGL where r=(0.629**) r=(0.565**) at respectively, While have been explained negative correlation for GSH and TAC with NAGL where r=(-0.294**), r=(-0.562**) at respectively.

Receiver operating characteristic {ROC}

ROC curve of NAG and NAGL.

Markers	**Specificity**	**Sensitivity**	**Area**	**P-Value**	**95% confidence interval**	
					Lower bound	**Upper bound**
NAG	**1**	**0.822**	**0.950**	**0.001**	**0.910**	**0.989**
NAGL	**0.933**	**0.889**	**0.939**	**0.001**	**0.885**	**0.992**

Table 2: shows the ROC curve result of NAG and NAGL

For NAG, values for sensitivity and specificity are (0.822, 1) respectively in patients with diabetic nephropathy, where is cutoff ((295 IU/mL); it can be considered NAG is a good marker for diagnostic the severity and stages of diabetic nephropathy patients as in (figure 3).

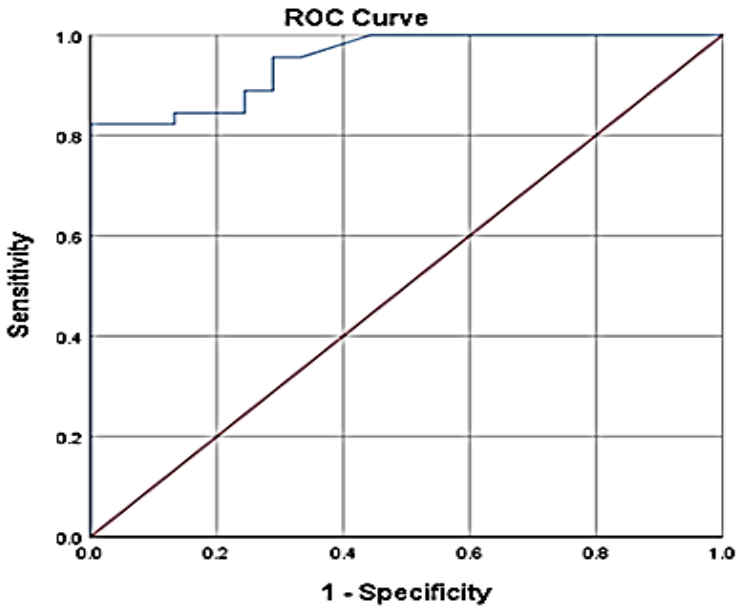


Fig 3: ROC curve for NAG

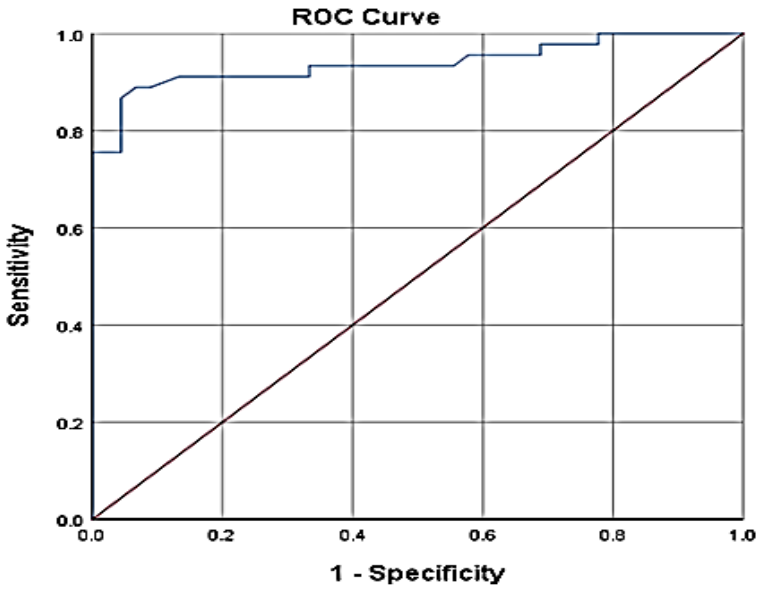


Fig 4: ROC curve for NAGL

Also, the Receiver Operating Characteristic Curve {ROC} in (figure 4) shows sensitivity and specificity of NAGL (0.889, 0.933) respectively in patients with diabetic nephropathy, where the cutoff point was(78.9167 ng\ml) and a result to that NAGL useful as early diagnostic and biomarker for the patients with diabetic nephropathy.

ROC curve of TOS and MDA

Markers	Specificity	Sensitivity	Area	P-Value	95% confidence interval	
					Lower bound	Upper bound
TOS	0.978	0.733	0.859	0.001	0.774	0.944
MDA	0.978	0.022	0.830	0.001	0.748	0.911

Table 3: AUC of TOS and MDA

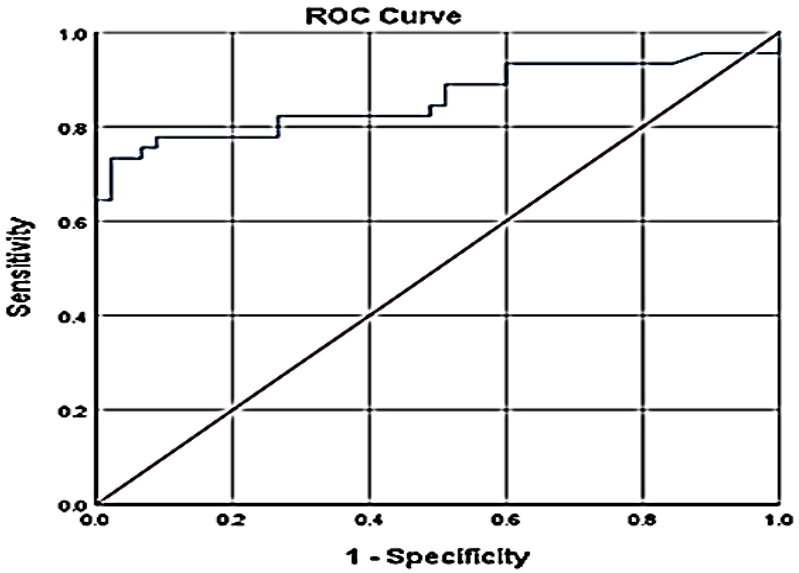


Fig. 5: ROC curve for TOS

Receiver Operating Characteristic Curve {ROC} in (Figure 5) shows the sensitivity and specificity of TOC (0.733, 0.978), respectively, in patients with diabetic nephropathy, where is the cutoff point (0.6075 µmol/l).

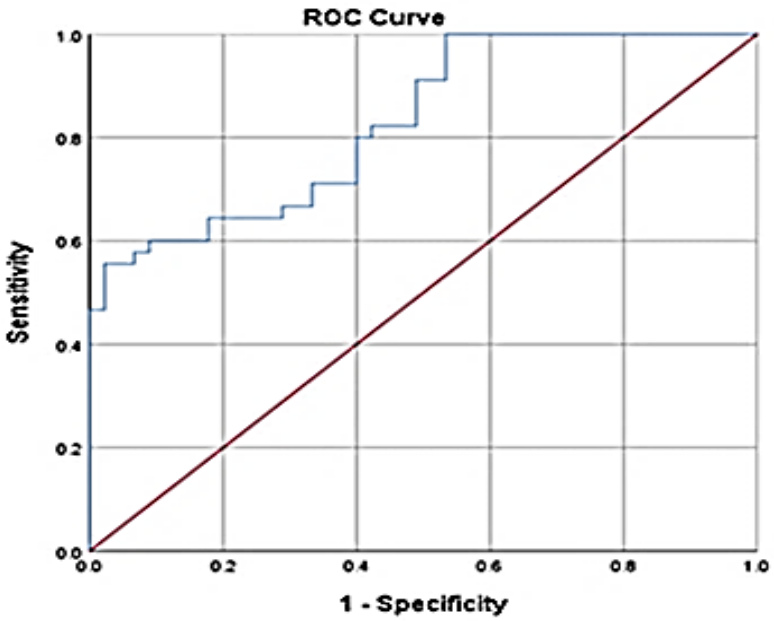


Fig. 6: ROC curve for MDA

(Figure 6) shows receiver operating characteristic Curve {ROC} of MDA sensitivity= 0.022 and specificity= 0.978 in patients with diabetic nephropathy, where the cutoff point (0.37 µmol/l) that indicates TOS and MDA are considered a good diagnostic and biomarker for the patients with diabetic nephropathy**.**

ROC curve of TAC and GSH

Markers	Specificity	Sensitivity	Area	P-Value	95% Confidence Interval	
					Lower bound	Upper bound

TAC	**0.956**	**0.644**	**0.770**	**0.001**	**0.668**	**0.872**
GSH	**0.6**	**0.889**	**0.950**	**0.001**	**0.910**	**0.989**

Table 4: AUC of TAC and GSH

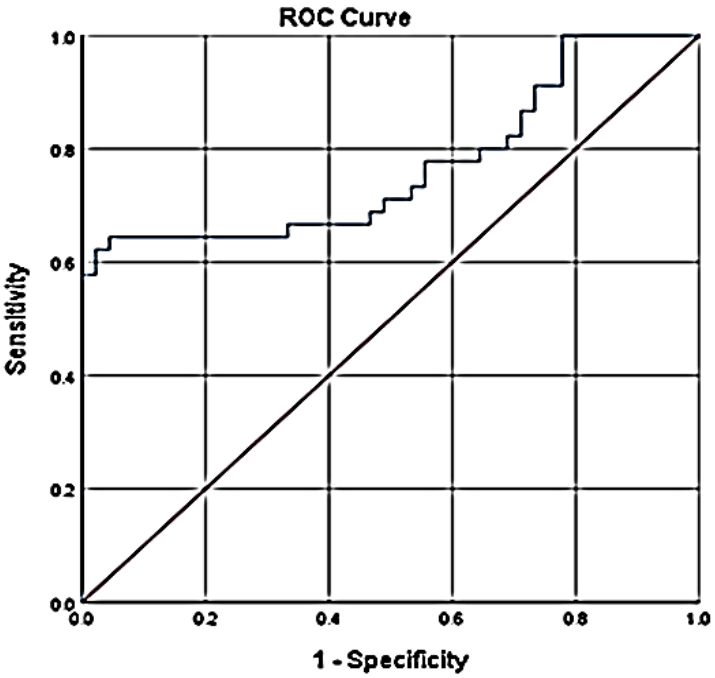


Fig.7: ROC curve for TAC

Receiver Operating Characteristic Curve {ROC} appear sensitivity and specificity of TAC (0.644, 0.956) respectively in patients with diabetic nephropathy, where is cutoff point (1.4850 µmol/l) as occurs in (Figure 7).

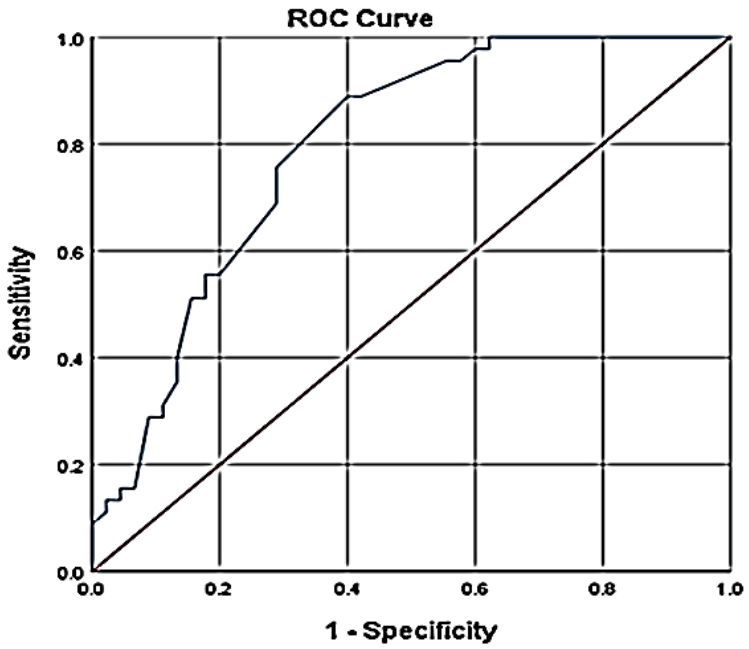


Fig. 8: ROC curve for GSH

(Figure 8) shows the sensitivity and specificity of GSH (0.889, 0.6) respectively in patients with diabetic nephropathy, where the cutoff point (23.875 µmol/l), the result is considered a good diagnostic marker for the patients with diabetic nephropathy Cells undergo continuous oxidation and reduction activities, but damage is typically prevented by several protective enzymatic and nonenzymatic anti-oxidative systems that maintain the equilibrium of these processes. Oxidative stress arises when equilibrium shifts towards greater oxidation [16]. Changing the structural and functional characteristics of different proteins and lipids leads to cellular malfunction, injury, or adverse impacts on cellular homeostasis[17]. Lipid peroxidation produces MDA, and chronic kidney disease (CKD) patients had greater serum MDA levels than healthy participants Patients with CKD have diminished total antioxidant capacity [18]. GSH is a primary nonenzymatic antioxidant in practically all live cells; plasma GSH was considerably lower in nephropathy patients than controls [8]

4. Conclusion

A critical factor in the development and course of DN is oxidative stress; several molecules and pathways are essential in DN's oxidative stress induction, so identification of oxidative stress biomarkers advances our knowledge of how DN develops and advances toward ESRD. Also, there is a correlation between oxidative parameters and increased levels of NAG and NAGL, and both these biomarkers are essential to detect the onset or progression of DN as early as possible. So, NGAL and NAG can be indicators for early diagnosis, staging, and progression of DN.

Limitations of this study

Difficulty in diabetic nephropathy samples collection according to required criteria.

Author's contribution

Conceptualization: Ali M. A. Al-Kufaishi

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Formal Analysis: Ali M. A. Al-Kufaishi

Funding acquisition: Alya'a W.Hussein

Investigation: Alya'a W.Hussein

Methodology: Noor J.T. Al-Musawi

Project administration: Ali M. A. Al-Kufaishi

Resources: Alya'a W.Hussein

Software: Alya'a W.Hussein

Supervision: Ali M.A.Al-Kufaishi

Validation: Ali M.A.Al-Kufaishi

Visualization: Alya'a W.Hussein

Writing-Original draft: Alya'a W.Hussein

Writing- review & editing: Ali M. A. Al-Kufaishi

Ethical consideration

The study was conducted after consent from the patients and the Iraqi Ministry of Health, and the authors completely observed ethical issues (including fabrication, double publication, and plagiarism).

Conflicts of Interest

NO competing interest has been declared by the authors.

Support\ Funding: Handle

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