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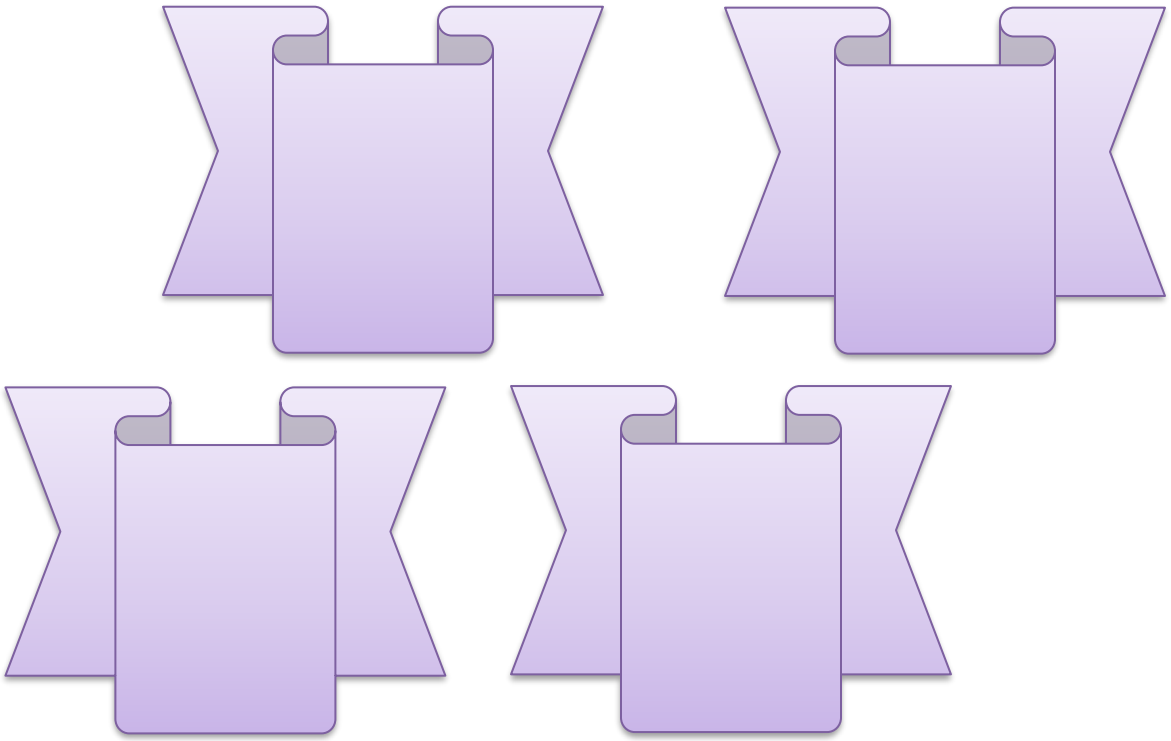

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Original Article

Assessment of Autotaxin, Oxidized LDL and β2-Microglobulin in Patients with Diabetic Nephropathy

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ABSTRACT

Background: Diabetes mellitus [DM] is a metabolic condition characterized by hyperglycemia caused by deficiencies in insulin action or secretion or both. Diabetes' persistent hyperglycemia is linked to long-term organ failure, including kidney loss of functions, leading to diabetic nephropathy [DN].

Aim of the work: This study aims to assess the value of serum autotaxin [ATX], oxidized LDL [ox LDL], and β2-microglobulin [β2M] as biomarkers for DN patients.

Patients and Methods: This cross-sectional study included 100 subjects divided into three groups. Group 1; control group [20 healthy subjects]. Patients were subdivided into two groups, group 2 [40 patients diagnosed with DN stages I, II, and III], and group 3 which involved 40 patients diagnosed with DN stages IV and V.

Results: As regards the ATX, it was significantly higher in group 3 than in group 1 and group 2 with a statistically significant difference between every 2 groups [P value > 0.05 for all]. In terms of Ox LDL and β2M, the difference between the three groups was significant statistically [P value = 0.005, and 0.002 respectively], however the difference between group 2 and group 3 was not significant [P value = 0.44, and 0.98 respectively].

Conclusion: Serum autotaxin, ox LDL, and β2M can be used as diagnostic noninvasive biomarkers in DN.

Keywords: Autotaxin; Oxidized LDL; β2-microglobulin; Diabetic Nephropathy.

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INTRODUCTION

Diabetes is the primary cause of chronic kidney disease [CKD], with the majority of diabetic nephropathy [DN] patients finally requiring renal replacement therapy [1]. Diabetic nephropathy is one of the commonest diabetic microvascular complications accounting for 20–40% of diabetic patients [2, 3].

Autotaxine is a soluble protein belonging to the ecto nucleotide pyrophosphate pyro-phosphatase phosphodiesterase [ENPP] family. ATX catalyzes a lysophospholipase D-activity that converts lysophosphatidylcholine [LPC] to lysophosphatidic acid [LPA] [4]. Many different bodily fluids, such as blood plasma, serum, urine, seminal fluids, and cerebrospinal fluid, contain high concentrations of ATX. Its concentrations and activities in clinical samples can be utilized to diagnose a variety of illnesses [5].

Plasma lipid peroxidation products and ox LDL increase in uremic individuals. Nephrotic or nephritic patients have higher levels preceding renal failure [6].

β2M is a low-molecular-weight, non-glycosylated protein that is secreted at a steady rate, filtered by the glomerulus, absorbed by the proximal tubules, and then catabolized. Because of this, it might be a good biomarker for kidney dysfunction [7].

So, this study aims to assess the value of serum ATX, ox LDL and β2M as biomarkers in patients with DN.

PATIENTS AND METHODS

This cross-sectional study included 100 subjects divided into three groups. Group 1; control group [20 healthy subjects]. Patients were subdivided into two groups, group 2 [40 patients diagnosed with DN stages I, II, and III], and group 3 which involved 40 patients diagnosed with DN stages IV and V. Data were collected from December 2020 to April 2022 at Al-Azhar university hospital. All subjects participated in this study after informed consent and ethical approval from our institution were obtained. We excluded any case with malignancy, pregnancy, Infection, or Liver diseases.

Every patient in this study was subjected to complete medical history and comprehensive physical examination. Five ml of venous blood were collected from every patient and then left to be clotted, then it was collected and stored at -80° C till the time of assay.

The following laboratory investigations were done for all participants: Serum creatinine, Blood urea, Urine albumin, Urine creatinine, and random blood glucose [RBG] level. On semiautomated spectrophotometry Clinidiag system, Belgium, GFR estimation for patients was calculated using the following equation: eGFR [mL/min/1.73 m^2] = 194× serum creatinine-1.094 × Age-0.287 [If female, ×0.739] [8]. Serum ATX [Cat.No E4178hu], ox LDL [Cat.No E1542Hu], and β2M were estimated by enzyme-linked immunosorbent assay [ELISA] technique Abcam, USA.

Statistical Analysis: The statistical analysis was done using IBM® SPSS® Statistics Version 22 for Windows. Continuous data were displayed as mean ± SD. Qualitative data were presented in the form of numbers and percentages. ANOVA test was used to compare continuous data between three study groups followed by post hoc analysis to compare every 2 groups. For qualitative data, the Chi-square test was used. The level of significance was set at 0.05.

RESULTS

As regards the ATX, it was significantly higher in group 3 than in group 1 and group 2 with a statistically significant difference between every 2 groups [P value > 0.05 for all]. In terms of Ox LDL and B2M, the difference between the three groups was significant statistically [P value = 0.005, and 0.002 respectively], however the difference between group 2 and group 3 was not significant [P value = 0.44, and 0.98 respectively] [table 1].

Correlation analysis between the ATX, Ox LDL, and B2M revealed a statistically significant positive correlation between the ATX, Ox LDL [r = 0.24, P = 0.001], Also there was a strong positive correlation between the ATX and B2M [r = 0.85, P = 0.001], and between the Ox LDL and B2M [r = 0.87, P = 0.001]. A statistically significant positive correlation was found between the ATX, Ox LDL, B2M, creatinine, and GFR [P value = o.001 for all], However, this correlation with the GFR was negative [P value = 0.001 for all] [table 2].

We have used the receiver-operating characteristics [ROC] curve to predict DN with

serum ATX. The area under the curve [AUC] of serum ATX was found 1.0. The best cut-off point of serum ATX for DN was found 1.4 mg/l. At this cut-off point, the sensitivity of serum ATX was 100% and the specificity was 100% [Figure 4].

The sensitivity and specificity of serum β2M for detecting diabetic nephropathy were calculated in this study. The diagnostic performance of serum β2M microglobulin was determined to have an area under the curve [AUC] of 0.998.

The optimal serum β2M cutoff for detecting DN was determined to be 5.0 g/ml. The serum 2M cutoff has a sensitivity of 97.5% and a specificity of 100% at this concentration [Figure 5].

The diagnostic accuracy of serum ox LDL was calculated to be 0.96 with an area under the curve [AUC] of 0.96. The optimal serum ox LDL cutoff for detecting DN was determined to be 4.35 g/ml. Serum ox LDL's sensitivity was 97.5% and specificity was 92.5% at this cutoff [Figure 6].

Table [1]: Comparison between the studied groups regarding the ATX level

	Group 1 [n = 20]	Group 2 [n = 40]	Group 3 [n = 40]	P value	Post hoc test
ATX [mg/L]	0.76±0.12	0.87±0.128	1.73±0.13	**0.001**	**P1 = 0.002** **P2 = 0.001** **P3 = 0.001**
Ox LDL [U/L]	67.7±17.4	82.37±15.1	87.2±14.2	**0.005**	**P1 = 0.001** **P2 = 0.001** P3 = 0.44
β2M [ug/ml]	2.2 ± 0.45	2.7±0.35	2.7±0.49	**0.002**	**P1 = 0.002** **P2 = 0.004** P3 = 0.98

Table [2]: Correlation between ATX, Ox LDL, and B2M in all studied groups

	ATX	
	r	**P value**
Ox LDL	0.24	**0.001**
β2M	0.85	**0.001**
Creatinine	0.88	**0.001**
GFR	-0.82	**0.001**
	Ox LDL	
	r	**P value**
β2M	0.87	**0.001**
Creatinine	0.81	**0.001**
GFR	-0.84	**0.001**
	B2M	
	r	**P value**
Creatinine	0.88	**0.001**
GFR	-0.84	**0.001**

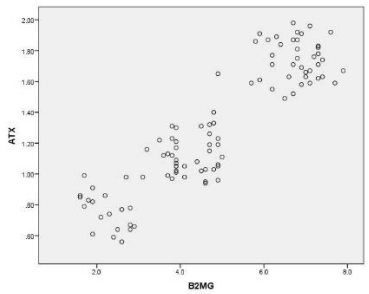

Figure [1]: The correlation between ATX, ox LDL, and β2M in all studied groups.

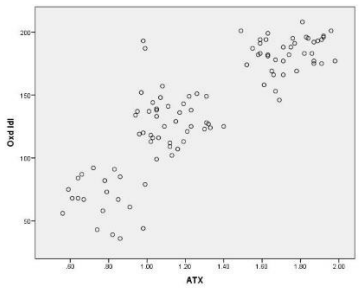

Figure [2]: The correlation between ATX and ox LDL in all studied groups**.**

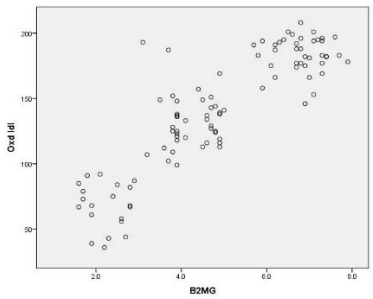

Figure [3]: The correlation between β2M and ox LDL in all studied groups**.**

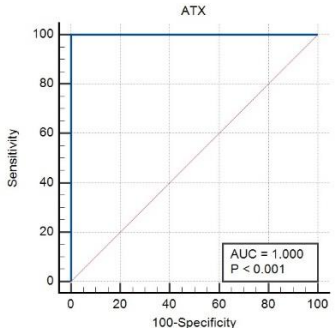

Figure [4]: ROC curve of ATX [Control excluded] GFR as a reference. AUROC = 0.998 p-value = 0.0001

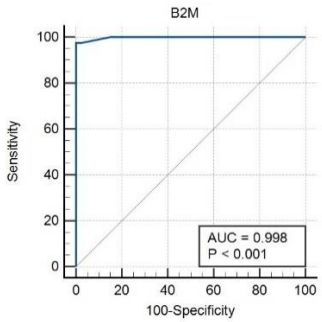

Figure [5]: ROC curve of β2M [Control excluded] GFR as a reference. AUROC = 0.998 p-value = 0.0001

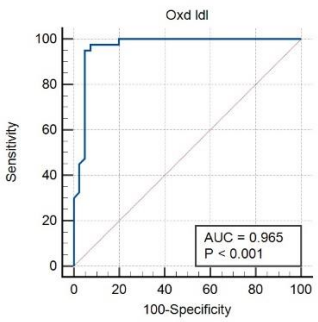

Figure [6]: ROC curve of ox-LDL [Control excluded] GFR as a reference. AUROC = 0.998 p-value = 0.0001

DISCUSSION

DN is a disabling condition, and several biomarkers have been studied for diagnosing and following up this condition, we have studied serum ATX levels, β2M, and ox-LDL in DN patients comparing them with diabetic and normal control groups.

We also examine the studied biomarkers against the current widely used biomarkers for diagnosing and following up CKD [serum creatinine levels and eGFR] to assess the validity of these biomarkers to diagnose different stages of nephropathy.

In the present study serum ATX level, we found that there was a significant difference between the control and the two patient groups, indicating that serum ATX levels are elevated in diabetic patients with DN so, it may be used as an early diagnostic biomarker with other clinical findings in type 2 diabetic patients with DN.

Also, there was a significant difference between patient group 2 and patient group 3, this difference suggests that the serum ATX levels may be used as a predictor for prognosis and outcome in patients with DN. A similar study was done by **Shimizu *et al.*** [9], found that, serum ATX levels were associated with advanced diabetic kidney lesions and can lead to kidney damage and consequently play a role in the pathogenesis of DN, but it does not accelerate its progression directly and serum ATX levels cannot be used as a predictor indicator for the renal outcome.

In contrast, **Nakamura *et al.*** [10] found in their study, serum ATX levels remain unchanged even in a treated case with nephrotic syndrome. He explained this contrast may be due to the probability of the measured serum ATX levels don't reflect the exact serum ATX activity.

In the current study, we have assessed the correlation between serum ATX levels against serum creatinine and eGFR levels, and we have found a significant positive correlation between serum ATX levels and serum creatinine levels, while the correlation was significantly negative between serum ATX and eGFR levels.

We have used the ROC curve to determine whether or not serum ATX is a good predictor of DN. Serum levels of ATX were found to have an AUC of 1.0. The optimal serum ATX cutoff for detecting DN was shown to be 1.4 mg/l. Serum ATX was perfectly sensitive [100%] and specific [100%].

The serum β2M was tested for its ability to predict DN in patients with type 2 diabetes. Patient groups 2 and 3 were significantly different from the control group [p 0.00002 and p 0.00004, respectively], while there was no significant difference between groups 2 and 3 [p = 0.97]. This means that while a high blood β2M level can be utilized to identify early kidney damage and diminished functions [GFR], it cannot be used as an independent predictor of renal prognosis and outcome.

β2M is a low molecular weight protein that is generally removed by the kidneys at a rate that is equivalent to GFR. After this process, the protein is reabsorbed and catabolized in the tubules, and serum levels are inversely linked to GFR [11]. For measuring GFR, serum creatinine is the most commonly used biomarker [12].

According to **Liabeuf *et al.*** [13], study on the relationship between β2M and various stages of renal disease, they found that there is an association between serum β2M levels and various stages of CKD, which is similar to our findings. Also, **Inker *et al.*** [14], reported that β2M is a newly discovered marker filtered by the kidney and its serum level can be used for GFR calculation. It was reported that serum β2M is an early predictor of renal function [15].

It was reported that people with a high level of serum β2M are more likely to have DN than those with a low level of β2M and good renal function [16].

In comparison to other biomarkers, an increase in serum β2M was found to be highly specific for DN; furthermore, it is not affected by gender, muscle mass, or medications [17].

In diabetic individuals with DN, β2M is a marker of interstitial injury; nevertheless, it does not aid in the prediction of ESRD [18].

The sensitivity and specificity of serum β2M for detecting diabetic nephropathy were calculated in this study. The diagnostic performance of serum β2M microglobulin was determined to have an area under the curve [AUC] of 0.998.

The optimal serum β2M cutoff for detecting DN was determined to be 5.0 g/ml. The serum β2M cutoff has a sensitivity of 97.5% and a specificity of 100% at this concentration.

Serum ox LDL was also measured in this investigation, with significant differences between groups 1 and 2 [p = 0.00102] and between groups 2 and 3 [p = 0.00001], but no significant differences between groups 2 and 3 [p = 0.44]. It may be a valuable diagnostic biomarker in early-stage DN due to the elevated levels of ox LDL in patient groups indicating the presence of oxidative stress; however, it is not predictive of renal progression or outcome. Other studies showed that dyslipidemia is associated with the development and progression of diabetic kidney disease [19].

Ox LDL is now obviously evident responsible for kidney injury by a mechanism similar to that of lipid peroxides toxicity to the vascular endothelium [20]. Circulatory ox LDL may be a predictor for the progression of kidney diseases in diabetic patients [21].

The diagnostic accuracy of serum ox LDL was calculated to be 0.96 with an area under the curve [AUC] of 0.96. The optimal serum ox LDL cutoff for detecting DN was determined to be 4.35 g/ml. Serum ox LDL's sensitivity was 97.5% and specificity was 92.5% at this cutoff.

Conclusion: Serum ATX, ox LDL, and β2M can be used as diagnostic biomarkers in DN.

Conflict of Interest and Financial Disclosure: None.

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