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Mini–review: clinical and molecular markers in early diabetic nephropathy

Abstract

Diabetes mellitus (DM) is the sixth leading cause of death worldwide because of its complications. One of these deadly complications is diabetic nephropathy, the leading cause of end–stage renal disease in the western world. Despite the worldwide acceptance to use albumin–to–creatinine (A/C) ratio and estimated glomerular filtration rate (eGFR) in clinical settings, there is no trustable and valid biochemical marker that can sensitively detect early stages of diabetic nephropathy. Therefore the early detection of the deterioration in kidney function and the changes in kidney structure before the albumin level becomes significantly high in urine is very important for patient's life. The aim of this review is to summarize some novel clinical and molecular markers being investigated as potential candidates to fill in the gap.

Keywords: early stage, molecular markers, clinical markers, mi–RNA, urinary proteome, kidney

Volume 2 Issue 3 - 2018

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Received: April 03, 2018 | **Published:** May 14, 2018

Abbreviations: IDF, international diabetes federation; DM, diabetes mellitus; DN, diabetic nephropathy; GFR, glomerular filtration rate; ESRD, end–stage renal disease; MDRD, modification of diet in renal disease

Introduction

Diabetes Mellitus (DM) is now seen as one of the strongest enemies we have to defeat. Despite our efforts and newly developed weapons, the current estimation of total diabetes population is 425 million people in the world and unlikely, it is estimated that by 2045 around 438 million people aged 20–64 years old will be diagnosed with diabetes.[1]According to the International Diabetes Federation (IDF), Egypt is ranked as the 8th country for the number of adults with diabetes (20–79 years) and for their healthcare expenditure. According to WHO, the prevalence of type 2 diabetes in Egypt was almost tripled over the last two decades. By 2030, it is estimated that the number of Egyptians with DM will rise to 6,726,000. In terms of etiology, DM occurs as a result of either insulin deficiency or insulin resistance. Consequently, DM has two main types: type 1 and type 2. DM is characterized by hyperglycemia, which is the primary cause of most complications seen in patients such as nephropathy and endothelial dysfunction.[2,3] Diabetic nephropathy (DN) is one of the most common complications of DM. DN is a progressive renal disease caused by alterations in tubular and glomerular structure and function. These alternations include basement membrane thickening in the glomerulus and tubules, accumulation of the components of extracellular matrix, detachment of podocytes from glomerular basement membrane, hyperplasia of mesangial cells and thickening of mesangial matrix. These pathological changes are usually induced by the rise in blood glucose level.[4,5] Major advances have been made over the past few decades in diagnosing and treating patients with DN however, we are still unable to increase the survival rates among these patients.[6] DN is considered to be the leading cause of end–stage renal disease (ESRD) in the Western world representing about 50% of cases. It is characterized by albuminuria (urine albumin/creatinine ratio is >300 mg/g), and/or a glomerular filtration rate (GFR) below 60mL/min/1.73m^2.[7,8] Novel well–validated biomarkers, when used in combination with conventional biomarkers, can efficiently clarify the pathophysiology of DN and can accurately stratify DN patients based on their disease stage. This will help finally in tailoring the appropriately personalized mediations for each one of these patients. To detect the early stages of diabetic nephropathy, there are many recently–investigation biomarkers that can be tracked in blood or urine.

Discussion

Definition of biomarkers

Biomarkers could be defined as "characteristics which are objectively measured and evaluated so as to indicate pathogenic processes, normal biochemical processes, or therapeutic responses to a certain drug".[9]

Current status of DN biomarkers

Renal biopsy is known to be the gold standard for definitive and affirmed diagnosis of DN; however, because it is an invasive procedure it is now conserved for diagnosis confirmation. The diagnosis is now based on measuring the level of albumin in urine as well as on estimating the GFR. Current guidelines state that both parameters have to be measured, at least once a year, in order to diagnose, screen for or monitor DN.[10] Estimated GFR (eGFR) is calculated using creatinine levels measured in patient's serum. There are some formulae that are now available to estimate eGFR, for example, Chronic Kidney Disease Epidemiology Collaboration (CKD–EPI) tool and the Modification of Diet in Renal Disease (MDRD) study equation.[11,12]

Limitations of using eGFR and A/C ratio

There are a number of reasons why eGFR is considered to have "limited use" for early diagnosis of DN; the level of creatinine in blood is highly affected by muscle mass so the use of eGFR in obese or malnourished persons will give hesitated results.[13] Second, the estimation of GFR is considered to be less accurate so eGFR won't be able to accurately predict the early stages of DN. Finally, the accuracy of the two previously mentioned formula is low in patients with diabetes mellitus.[14] Similarly A/C ratio isn't the ideal biomarker to be measured for the early detection of DN. The following observations

will clarify the reasons; it was reported that there are some phenotypes of DN with neither microalbuminuria nor reduced eGFR.[15–17] Second, the level of albumin in urine was found to be independently related to the risk of renal or cardiovascular complications.[18] Finally, Long–term studies on patients with diabetes revealed that it isn't necessary that patients with microalbuminuria will proceed to overt DN because many of these patients, by time, became normoalbuminuric again.[19,20]

Novel biomarkers that can be potentially used in the early detection of DN

Because of the previously–mentioned limitations for both A/C ratio and eGFR, there are a lot of biomarkers now under investigation for the potential use as indicators for early–stage DN. The pathogenesis of DN was found to be somehow complex. Consequently, there would be multiple biomarkers that appear in blood and urine and can be tracked in both of them. This observation led to the development of some diagnostic approaches for "multimarker" analysis so that the specificity and sensitivity of detection can be increased and/or improved.[21–35]

Clinical biomarkers

By extracting and refining the literature over the last decade, it was found that most of the clinical biomarkers being investigated are proteins in nature, some of them are demonstrated in Table 1 shows examples of the recently–investigated clinical biomarkers which can be potentially used in the detection of early stages of DN or in the monitoring of DN in some situations.

Table 1 Summary of some proteins that were found to be associated with DN

Biomarker	Isolated From	Type of DM	Reference	Conclusion	Comment(S)
Cu–Zn superoxide dismutase (SOD)–1	Kidney lysate of an STZ–induced rat model of DN	NA*	[23]	The study stated that the expression of SOD–1 was reduced in streptozotocin (STZ)–induced rats in comparison to normal rats. These changes in expression appeared prior to detectable microalbuminuria	Lacking the same investigation on human samples.
Urinary epidermal growth factor (uEGF)	Human urine	Type 2 DM	[24]	Low uEGF–to–creatinine ratio was found to be associated with the decline in renal function in patients with normoalbuminuria so it can then be used as a novel early marker of DN	Small sample size
Inositol pentakisphosphate 2–kinase (IPP2K), zona occludens 3, and FAT tumor suppressor 2	Human urine	Type 1 DM	[25]	These markers have a correlation with early renal function decline so they are considered good candidates for use as sensitive biomarkers in clinical settings.	The study mentioned that validation studies are needed for these biomarkers using large populations.
CKD273 classifier	Human urine	Type 1,2 DM	[26,27]	Good et al.[26] developed a classifier which functions through categorizing patients with CKD, based on the status of 273 predetermined peptides in comparison to a healthy control, with high sensitivity and specificity. While, Zürbig et al.[27] demonstrated that previously generated CKD273 classifier can identify patients with normoalbuminuria, that will develop diabetic nephropathy during a 5–year follow–up	It is highly recommended to up–scale the application of the classifier thus it can be used routinely in clinical practice.
Chitotriosidase (CHIT1)	Human plasma	Type 2 DM	[28]	There is a concomitant increase in the plasma levels of CHIT1 with albuminuria in patients having T2D. So, CHIT1 represents a promising and noninvasive tool for the evaluation of DN	Up–scaling of the marker for potential use in assessing the risk of ESRD in clinical practice is highly recommended.
TGF–β1	Human serum	Type 2 DM	[29]	This meta–analysis of several studies concluded that elevated TGF–β level in the serum of patients with diabetes is associated with a high risk of DN.	Further research is needed to support this point.
Biomarker	Isolated From	Type of DM	Reference	Conclusion	Comment(S)

Table Continued

monocyte/ neutrophil elastase inhibitor	kidney lysate of OVE26 transgenic mouse model**(1)**	Type 1 DM	[30]	The decrease in elastase inhibitor–to–elastase ratio in the OVE26 mice causes an increase in the rate of deposition of elastin in renal tubules and the interstitium. This means that expression of elastase inhibitor is correlated to DN in diabetic mice.	Lacking the same investigation on human samples.
Ceruloplasmin, transferrin, and prostate stem cell antigen	Human urine	Type 2 DM	[31]	These proteins, plus other proteins, were significantly increased in microalbuminuric vs normoalbuminuric patients with type 2 diabetes	Small sample size
E–cadherin and urinary soluble fragment of E–cadherin (sE–cadherin)	Human urine	Type 2 DM	[32]	This protein was found to be upregulated in microalbuminuric vs DM and control patients. The sE–cadherin–to–creatinine ratio was significantly increased in microalbuminuric and macroalbuminuric patients vs normoalbuminuric and control groups	Small sample size
Haptoglobin	Human urine	Type 2 DM	[33–35]	One study has concluded that haptoglobin–to–creatinine ratio can be used as a predictor for ESRD in microalbuminuric patients.[33] However, in another study, no significant difference was found between patients with DN and those with non–complicated DM.[34] A third study found that there is no significant correlation between different genotypes of haptoglobin and risk of overt DN.[35]	Further studies are needed to alleviate this conflict.

*NA= not available **(1)**The OVE26 mouse is a model of type 1 DM, with a deficient production of insulin, as a result of transgenic overexpression of calmodulin in pancreatic β cells.[36]

Molecular biomarkers

Micro RNAs associated with DN

Major pathophysiological alternations of DN include expansion of the glomerular mesangium, podocyte dysfunction; which results in proteinuria, and also glomerular basement membrane thickening and tubulointerstitial fibrosis caused by the accumulation of extracellular matrix (ECM) proteins.[37,38] MiRNAs are short non–coding RNAs that consist of about 20–22 nucleotides. They were found to play an important role in mammalian gene expression through suppressing translation process, inducing mRNA degradation and consequently silencing the gene.[39,40] Different miRNA were found to be involved in the development and progression of the pathophysiological alternations associated with DN. This means that we can detect certain types of miRNAs which are specific for the tissues exhibiting alternations in their structure and/or function as a result of hyperglycemia–related complications. Number of miRNAs were found in literature that were well–studied by researchers and were summarized in Table 2.

Table 2 miR–377, miRNA–216a, miR–21and miR–192 are examples of the recently–nvestigated mi RNAs that can be used in the detection of early stages of DN

Biomarker	Isolated From	Type of DM	Study	Conclusion	Our comment(S)
miR–377	Human urine	Type 1 DM	[41]	In this recent clinical study, it was found that urinary excretion of miRNA 377 was significantly higher in microalbuminuric patients than normoalbuminuric patients and healthy controls. These results suggest that miR–377 can be used as an early biomarker for nephropathy in pediatric type 1 diabetes	The next step is to measure miR–377 on a large population and to develop a validated protocol that can be used in the clinical practice for the assessment of DN cases.
miRNA–216a	Human urine	Type 1 DM	[41]	MiR–216a was negatively correlated to HbA1C and urinary albumin–to–creatinine ratio (UACR). These results suggest that miR–216a can be used as an early biomarker for nephropathy in pediatric type 1 diabetes	We recommend to measure this marker on larger sample size and to develop a validated protocol that can be used in the clinical practice for the assessment of DN cases.
Biomarker	**Isolated From**	**Type of DM**	**Study**	**Conclusion**	**Our comment(S)**

Table Continued

miR-21	Cultured mesangial cells from diabetic db/db mice	NA*	42–43	The over expression of miR-21 inhibited proliferation of mesangial cells in diabetic db/db mice. miR-21 expression was found to be down regulated in early DN in vitro and in vivo suggesting that it can be used as an early biomarker of DN.	We recommend measuring the level of miRNA–21 in human urine and relating its level to UACR.
miR–192	Kato et al.[44] used renal cortex of WT STZ–diabetic and db/db mice. Ma et al. isolated the mi RNA from human urine	NA*	44–46	MiR–192 levels were increased significantly in glomeruli isolated from streptozotocin–injected diabetic mice as well as in diabetic db/db mice in comparison to control mice without diabetes.[44] Another study proved that the expression of miR–192 was negatively correlated with UACR.[45] It was suggested by another study that inhibition of miR–192 expression may be an approach to slow down the pathogenesis of DN.[46]	The scope now should be focused on the development of a validated protocol which can be used in the clinical practice for the assessment of DN cases and alleviate the contradictory correlation with UACR test.

*NA= not available

Conclusion

Urinary proteomics has a wide applicability in differentiating individuals with normoalbuminuria and risk factors for developing DN from those with stable renal function. Urinary proteomics also can be used to detect patients with a decline in renal function despite being normoalbuminuric.[23,24,28] It seems that Urinary epidermal growth factor (uEGF), inositol pentakisphosphate 2–kinase (IPP2K), zona occludens 3, FAT tumor suppressor 2 and Cu–Zn superoxide dismutase (SOD)–1, specifically, have a significant association with the early stages of DN thus they are potential biomarkers for early diabetic nephropathy.[23-25] It was shown in the mentioned studies that the expression level of mi RNAs in tissues, especially those with pathological alternations in their structure and/or function can be detected and correlated to the pathophysiology of these alternations. When these alternations result from hyperglycemia–induced complications, the changes in miRNA levels can be then correlated to the pathophysiology of the disease. We recommend that the future research should focus on tracking miR–377, miRNA–216a and miR–21 in human urine in phase 3 clinical trials recruiting large DN populations and also focus on the development of validated protocols for using these biomarkers in the detection of early DN in clinical practice.

Acknowledgments

None.

Conflict of interest

Author declares that there is no conflict of interest.

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Citation: Reda M, Amin HK. Mini–review: clinical and molecular markers in early diabetic nephropathy. *MOJ Drug Des Develop Ther.* 2018;2(3):88–92. DOI: 10.15406/mojddt.2018.02.00034