



## Research Paper

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# Urine exosome as a biomarker for the early diagnosis of diabetic nephropathy: a meta-analysis

Accepted 24<sup>th</sup> October, 2019

## ABSTRACT

This meta-analysis aimed to evaluate the association between urinary exocytosis and the incidence of diabetic nephropathy. It was performed using Revman 5.0 statistical software by searching the Medline, China National Knowledge Infrastructure (CNKI), Wanfang and Weipu databases for studies on the relationship between urinary exosome detection and early diabetic nephropathy between 2010 and 2019. Six trials were selected for this study, and 2126 participants were included in the meta-analysis. The participants were divided into proteome and transcriptome groups according to the specified criteria. The meta-analysis showed that the content of urinary exosomes was significantly elevated in early diabetic nephropathy. The 95% confidence interval (CI) of the protein group was 208.06 (21.34–2028.28), and the 95% CI of the transcriptome group was 19.20 (9.01–1019.83). The *P* value of both groups was less than 0.01. The content of urinary exosomes increased in early diabetic nephropathy, and urinary exosomes could be used as a biomarker for the early diagnosis of diabetic nephropathy.

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**Key words:** Diabetic nephropathy, early screening, meta-analysis, urinary exocytosis.

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## INTRODUCTION

Diabetic nephropathy (DN) is one of the common chronic complications in diabetes. Its pathological basis is the pathological changes in renal microvasculature, which generally enters the terminal stage, and the progression of the disease leads to renal failure (Zhang et al., 2016). The current clinical application of urine microprotein and creatinine ratio determination is not accurate and needs a more accurate indicator for measurement. The extensive presence and convenience of access in the body have become potentially effective ways to diagnose and treat diseases (Kahlert and Kalluri, 2013). In the last decade, the number of studies on urinary exosomes has increased exponentially (Jiang et al., 2016). A large number of domestic and foreign studies have shown that renal exosomes participate in renal regeneration, repair, and renal tubular cell communication; play an important role in the progression and control of kidney disease; and have

great potential as a biomarker of kidney disease (Ke et al., 2016). This meta-analysis comprehensively analyzed a large number of studies in the last decade, evaluated the relationship between the detection of urinary exosomes and early DN, and provided a new method for the early detection.

## MATERIALS AND METHODS

### Literature retrieval

The studies on the relationship between urinary exosome detection and early DN in Medline, CNKI, Wanfang, and Weipu databases were retrieved from 2010 to January 2019. The following key words were used in the search: Exosome, Urinary Exosome, Diabetes nephropathy, early

**Table 1:** Basic information of the included literatures.

Research		Experimental group		Control group
		Simple diabetes group	Diabetic nephropathy group	
Proteome	Gupta2015 (Klein et al., 2012)	100	100	100
	Dayem 2016 (Trnka et al., 2012)	62	17	30
	Kalani 2013 (Miranda et al., 2010)	30	18	25
Transcriptome	Lai 2014 (Dayem et al., 2017)	137	190	100
	Batutta2013 (Kalani et al., 2013)	12	12	10
	Jia 2016 (Delic et al., 2016)	30	50	10
Gnome	Fawwaz 2017 (Lai et al., 2019)	64	50	0
	Mehrabzadeh 2016 (Barutta et al., 2013)	100	100	100
	Yang 2016 (Jia et al., 2016)	24	61	30
	Yadav2016 (Fawwaz et al., 2017)	199	185	180
<b>Total</b>		<b>758</b>	<b>783</b>	<b>585</b>

screening, Meta-analysis, Leucine aminopeptidase (LAP) or Dipeptidyl peptidase-4 (DPP4) and DN, Wilms tumor-1 (WT-1) and DN, Podocylaxin( PCX) and DN, microRNA (miRNA), and DN.

### Inclusion criteria

Selected documents were published in conference guides. Selected literature research methods were cohort studies or case-control studies. All the selected participants signed the informed consent form, and some were diagnosed with DN based on the clinical and laboratory tests. According to the experimental study of urinary albumin, participants were divided into early DN (urinary albumin creatinine ratio of 30–300 mg/g) and advanced DN groups. Incomplete information and studies lacking full text were excluded.

### Statistical analysis

This meta-analysis was performed using Revman 5.3 software. The relationship between urinary exocytosis levels and early-onset DN was assessed using pooled OR values and 95% CI. Heterogeneity tests were performed on the selected studies using *P* values. A *P* value more than 0.05 indicated that the results of each study were homogenous, and the fixed-effects model was used. If the *P* value was less than 0.05, the random-effects model was used.

## RESULTS

### Characteristics of the eligible studies

According to the selection and exclusion criteria, 10 studies

were screened out, and 2126 participants were included in this meta-analysis. The grouping of participants is shown in Table 1.

### Meta-analysis

Three studies examined the levels of protein components in urine exosomes of patients with DN. In the heterogeneity test, the *P* value was more than 0.05, and the fixed-effects model was used. The results showed a significant proportion of urinary exosome protein components in early DN (95% CI 21.34–2028.28, *P* < 0.01, Figure 1).

### Analysis of the detection level of the transcriptome in early DN

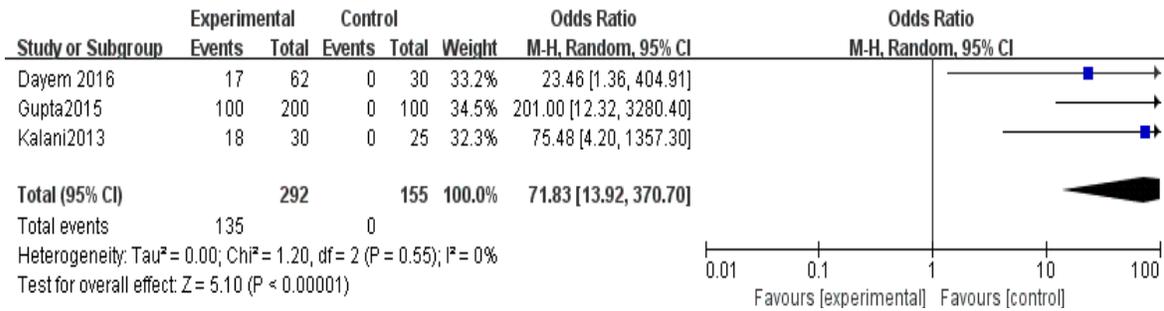
Three studies evaluated the levels of transcripts of urinary exosomes in DN. In the heterogeneity test, the *P* value was more than 0.05, and the fixed-effects model was used. The results showed that the ratio of transcripts of urinary exosomes was significant in early DN (95% CI 6.63–271.55, *P* < 0.01, Figure 2).

### Analysis of the detection level of exosomes in early DN

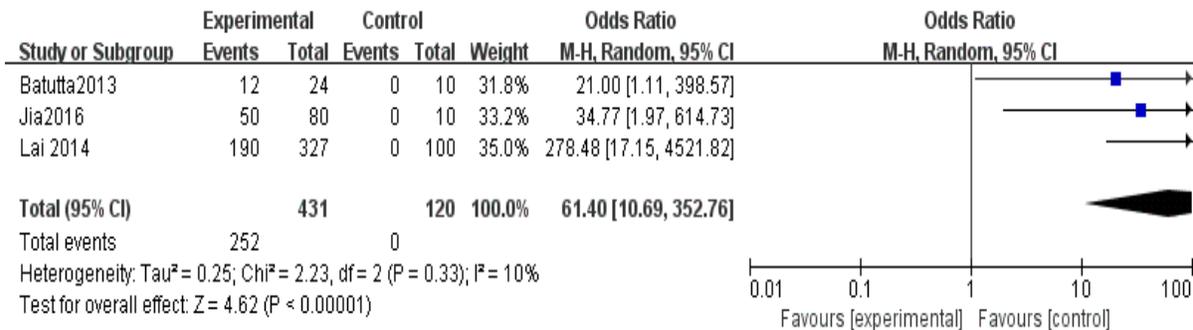
Three studies investigated the levels of urinary exosomes in DN. In the heterogeneity test, the *P* value was less than 0.05, and the random-effects model was used. The results showed a significant proportion of urinary exosomes in early DN (95% CI 0.36–1019.83, *P* = 0.14, Figure 3).

### Publication bias analysis

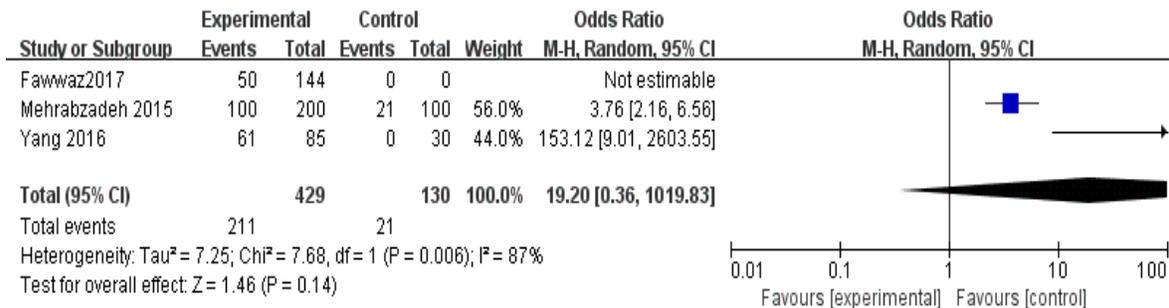
The Revman5.3 software was used to perform this meta-



**Figure 1:** Proteome.



**Figure 2:** Transcriptome.



**Figure 3:** Genome.

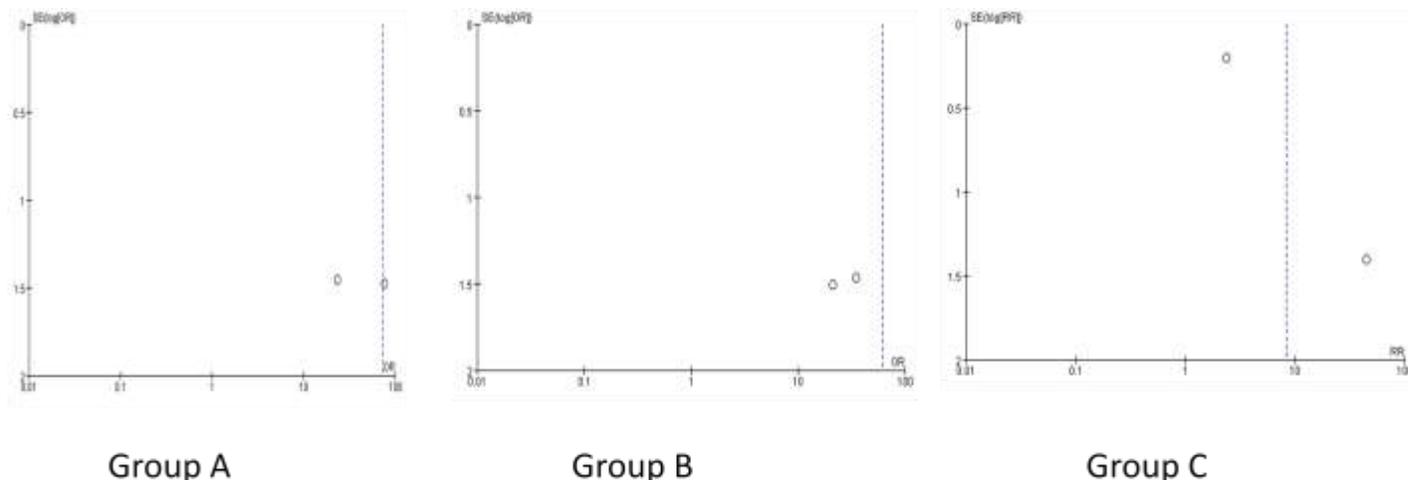
analysis on the selected studies and to draw a funnel plot. The studies on the proteome and transcriptome were basically symmetrically distributed, with no publication bias. An asymmetry was found in the distribution of genes between races (Figure 4).

## DISCUSSION

The prevalence of diabetes has increased every year. The proportion of kidney damage has also significantly increased, eventually leading to uremia (Klein et al., 2012). In the early kidney damage, the development of the disease can be obviously delayed, the damage of the kidney can be

alleviated, and the quality of life of the patient can be improved if further prevention and treatment are carried out. Experimental studies on exosomes have gradually matured in the last decade. Both blood and urine can secrete exosomes. The exosomes are divided into two groups: proteome and transcriptome (Trnka et al., 2012). Urine exosomes are used for a noninvasive examination, and their excretion can directly reflect the degree of kidney disease progression, thus having a unique advantage for detecting DN (Miranda et al., 2010).

This meta-analysis selected six experimental studies based on the inclusion criteria. Three groups of proteins and transcriptome and four genomes were included. Dayem et al studied the relationship between podocyte



**Figure 4:** Funnel plot test for publication bias of proteome (A) and transcriptome (B).

surface marker protein PCX and DN. The urine was analyzed in the diabetes group, the DN group, other nephropathy groups, and the healthy control group. Only PCX was positive in the DN group. Another study (Kalani et al., 2013) experimented with urinary exosome-LAP and exosome-DPP4 in the early- and late-stage DN groups and the healthy control group. The results showed a significant increase in advanced DN, which could be used as a marker for the progression of DN. The study by Kalani et al. (2013) showed the diabetic and DN groups according to the level of proteinuria. In the DN group, Wilms' tumor-1 protein was found to be associated with decreased renal function and expected to be a biomarker for early DN. Various studies analyzed the expression profile of miRNAs, involving more than 20 kinds of mi-RNAs (Kalani et al., 2013). Many types of miRNAs exist with high sensitivity and specificity (Delic et al., 2016). A previous study (Lai et al., 2019), involving the early DN and healthy control groups found that mi-RNA320c positively correlated with urinary albumin creatinine in early DN, with the highest expression and the best specificity. Barutta et al. (2013) found that the levels of mi-RNA145 and mi-RNA130a were elevated in patients with diabetes mellitus having urinary microprotein, and the levels of mi-RNA155 and mi-RNA424 decreased. In the study by Jia et al. (2016), another mi-RNA192 was found to have significant specificity. In particular, mi-RNA192 showed a downward trend when it progressed to the stage of advanced DN. Genomic experiments were carried in different gene classes, including angiotensin-converting enzyme, engulfment and cell motility 1 (ELMO 1) gene, methylenetetrahydrofolate reductase protein-coding gene, and carnosine dipeptidase 1. The latter is a protective gene most common in the normal controls, and its heterogeneity was not included in the meta-analysis. The first three have an increasing trend with the progression of DN and have a great significance in DN and simple type 2 diabetes (Fawwaz et al., 2017; Gupta et al., 2015; Mehrabzadeh et al.,

2016; Xie et al., 2003; Yang et al., 2016).

Meta-analysis is a method for a systematic literature analysis. The clinical findings are well summarized by comparing and analyzing objective, systematic, and comprehensive studies (Su et al., 2015). This meta-analysis observed the relationship between the level of urinary exosomes and the incidence of DN. The results showed that the content of urinary exosomes was significantly elevated in early DN. The 95% CI of the protein group was 208.06 [21.34–2028.28], and the 95% CI of the transcriptome was 42.43 [6.63–271.55]. The *P* values of the two groups were more than 0.01. Exosomes are membranous vesicles. Both normal and diseased cells secrete exosomes, but with significant differences in the signal molecules they load (Zocco et al., 2014). Therefore, when DN occurs, the invasive renal puncture can determine the extent of kidney damage, and the level of urinary exosomes help understand the progression of DN. Hence, exosomes are currently used for the early screening of diseases (Sun et al., 2013).

In summary, the content of urinary exosomes increases in early DN. However, only few studies have evaluated urinary exosomes and early DN, leading to some publication biases in the present meta-analysis. Urinary exosomes are expected to become the main force from the origin of exosomes to the diagnosis of diseases to the treatment of diseases. In the future clinical diagnostic tests, the quantitative monitoring of urinary exocytosis in patients with diabetes using relevant kits, combined with clinical symptom analysis, is expected to enable the early screening and timely treatment of DN to improve the prognosis and quality of life of patients.

#### LIMITATIONS

Data on physical activity and sedentary lifestyles were limited in this study. These may be indirect measures of

sunshine exposure.

## Conclusion

The content of urinary exosomes increases in early DN, and urinary exosomes can be used as a biomarker for the early diagnosis of DN.

## ACKNOWLEDGMENTS

This study was supported by the National Natural Science Foundation of China (31571168, 30870908, and 81571246), the National Public Fund for Study Abroad (20173059), and the Kangda College Fund (201813980YJ05).

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