



## Recent Progress on Urinary Exosomal miRNAs in Bladder Cancer

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### Abstract

Bladder cancer (Bca) is one of the most common tumors in the urinary system. Cystoscopy biopsy is a gold standard for diagnosis of Bca in the clinic. However, cystoscopy biopsy is invasive, costly and invasive process causing pain in the patients. Because the Bca cells are closely in contact with urine, and the exosomes of the Bca can pass through the base membrane and transport the microRNA (miRNAs) to the urine, so the detection of the exosome-derived miRNAs in the urine is expected to be a new non-invasive method for diagnostics of Bca. Recently, liquid biopsy for miRNAs in urine is the recent research hotspot. In this review, we mainly introduce the feasibility of the application of the miRNAs from exosome to Bca, and its feasibility to detect Bca.

**Key words** exosomal miRNAs, urine, bladder cancer, diagnostics

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## Introduction

Bladder cancer (Bca) is one of the malignant tumors of the urinary system with high incidence rate and it is the ninth major cancer in the world, ranking 13th in the number of deaths worldwide [1-4]. Bca can be divided into non-muscle invasive bladder cancer (NMIBC, including pTa and pT1) and muscle invasive bladder cancer (MIBC, pT2 ~ pT4). Upon diagnoses for the first time, NMIBC constitutes about 70% of the Bca patients, and 25% of the patients were diagnosed as MIBC [5, 6]. Bca easily metastasizes to other organs outside of bladder but the mechanism(s) of tumor metastasis is currently unknown. In the clinic, cystoscopy is still a gold standard for the diagnosis of Bca and the follow-up evaluation for the patients with NMIBC [7, 8]. As an important media for intercellular communication, the exosomes may contain protein, lipids, mRNA, short-chain non-encoding RNA (miRNAs), and long-chain non-encoded RNA (lncRNAs) and other substances, which can regulate the signal pathway of receptor cells. There is a permanent contact between the bladder and urine, and the exosome in the Bca can transport the secreted miRNA to the urine [9, 10]. Multiple studies have shown that changes in the types and expression of the exosome-derived miRNAs in urine is closely related to the urinary carcinoma [11, 12]. In order to better understand the impact of the exosomal miRNAs in the urine on the occurrence and development of Bca, this review summarizes its application in the diagnosis, treatment, and prognosis of Bca.

## Exosome-derived miRNAs

Non-coded RNAs (ncRNAs) refers to RNA transcriptions that do not encode proteins. ncRNA is divided into two main categories: miRNA (<200 bp) and long-chain non-encoded RNA (lncRNAs, > 200 bp). miRNAs are shorter ncRNA, with an average length of about 22 nucleotides. It has long been thought to be a byproduct of transcription. The first miRNA Lin-4 was discovered by Ambors and Ruvkun in 1993. So far, there are more than 38,000 miRNAs discovered in more than 50 species. miRNAs mainly combine the 3'UTR (3'UTR)-targeted mRNA's 3'UTR (3'UTR) in alkaline base to regulate the expression of genes, thereby regulating the expression of cancer genes or cancer suppressor genes, and then participate in various growth stages of the tumor. A number of studies have shown that miRNAs is widely distributed in various organs of the human body and participate in the process of tumor angiogenesis, invading metastasis, growth differentiation, and apoptosis of Bca [13-15]. The exosome is a diameter of 30 ~ 120 nm, and a membranous microvesicles with a lipid bilayer structure. In 1983, it was first found in sheep reticulocyte by the Johnstone Group and then Johnstone named it "exosome" in 1987. As a signal transporter in tumor cells, it regulates local and tumor microenvironment in the whole body by conveying biological active factors such as protein, RNA, and DNA [16]. miRNA can be enriched in the exosome. The miRNA content in the exosome is higher than that of metrocyte. The exosomes can protect miRNA from RNA enzyme degradation compared with the plasma miRNA [17-19]. According to studies, exosome-derived miRNAs can be transported by high-density lipoprotein (HDL) or combined with Argonaute (AGO) protein to further reduce the risk of being degraded [20, 21]. Exosome-derived miRNAs can enter various body fluids including urine, this makes it possible to monitor the progression of various human tumors, including Bca by detecting exosome-derived miRNAs in body fluids [22].

## The biological role of exosome-derived miRNAs in Bca

The exosome-derived miRNAs in the urine have promoted the progress of Bca through various mechanisms, while some of them

are inhibited factors of Bca.

**Figure 1** shows that a diagram for exosome in urine in the patients of bladder cancer.

## Exosome-derived miRNAs to promote Bca progression

There are studies suggesting that some miRNAs in fibroblasts in tumors can be up or down regulated to affect the tumor micro-environment, to promote invasion, metastasis and epithelial transformation (EMT) in tumor cells. EMT can promote the invasion of MIBC [23]. The high expression of circPRMT5 in Bca is positively correlated with low survival rates. Chen et al. [24] further explored its mechanism of action and found that circPRMT5 can reduce the inhibition function of miR-30c by infiltrating miR-30c in vivo and in vitro, thus promoting EMT of Bca cells. RAB27A/B protein in the bladder metastasis increases compared with the expression of in situ cancer, which increases the exosome secretion of metastatic cancer, causing the miR-23B, miR-224 and other inhibitory molecules to inhibit the metastasis of the Bca, the formation of tumor blood vessels, and the lung metastasis of the Bca. miRNAs discharged from cells, promoted the invasion and metastasis of Bca [25]. Some studies have shown that the low expression of miR-29c and miR-200c in NMIBC is related to the progress of tumor, and the miR-146A-5P in urine is highly expressed in high-grade Bca [26]. Therefore, miRNAs in urine plays an important role in the metastasis of Bca.

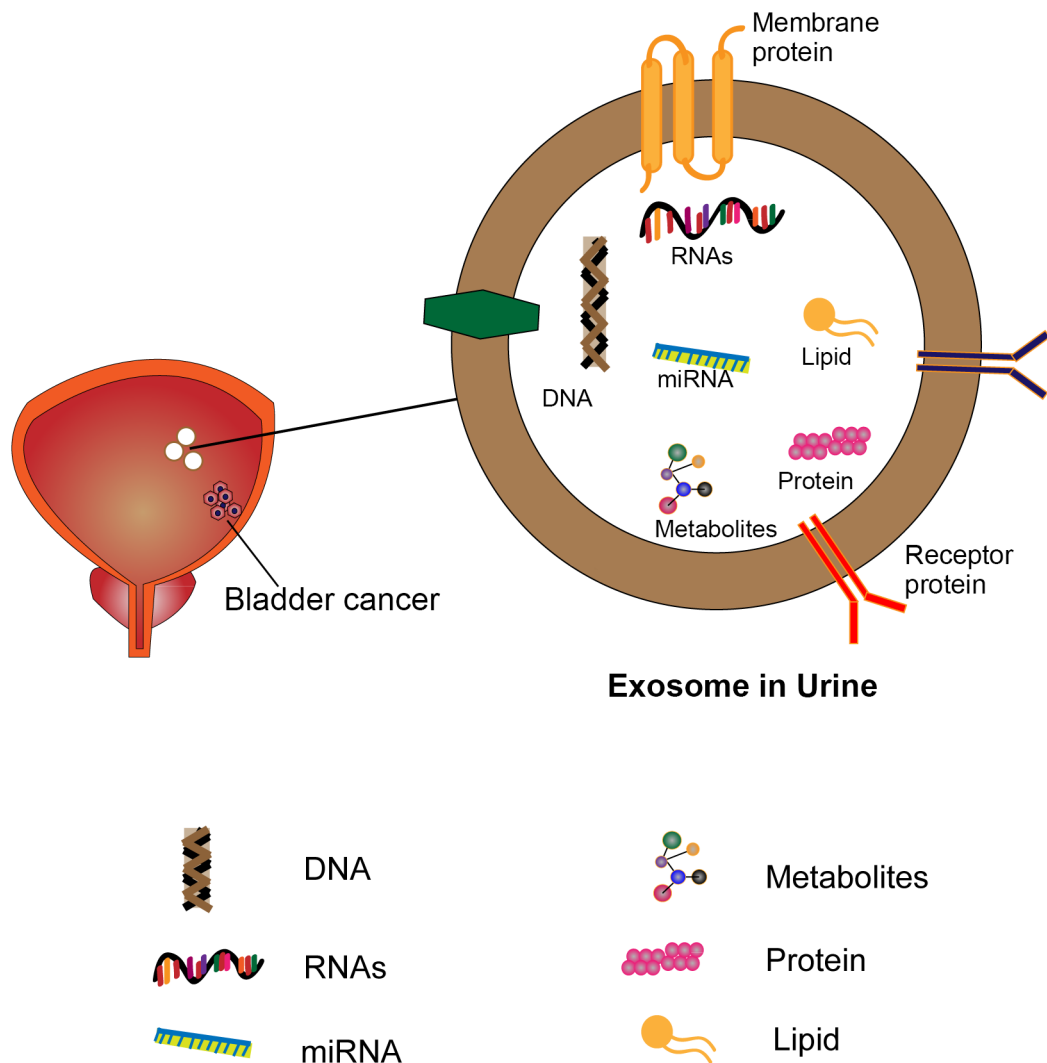
## Exosome-derived miRNAs to inhibit Bca progression

miRNAs can target specific genes through endogenous blocking the function of tumor suppressor gene, regulating the genes expression related with the progression of Bca, to promote down-regulation of target genes or inhibit their translation. Xu et al. [27] used immunohistochemical method to detect BCL-2 and MCL-1 expression in 28 cases of carcinoma and adjacent tissue. Further studies have found that the exosome-derived miR-29C can trigger the apoptosis of BIU-87 by reducing the expression of Bcl-2 and MCL-1, thereby restraining the progress of Bca. In addition, research by Sun et al. [28] found that Cyclin J (CCNJ) of cell cycle regulation gene is a new target for miR-205, and it proves that miR-205 can inhibit the proliferation, migration and invasion of Bca cells. Segersten et al. [29] found that there is significant difference in miR-130A-3p expression levels between Bca progressors and controls group. The study by Zhu et al. [30] found that circEHBPI promotes lymphangiogenesis and lymphatic metastasis of bladder cancer via miR-130a-3p/TGFbetaR1/VEGF-D signaling. miR-9-3p, an exosome-derived miRNAs from osteamonal mesenchymal stem cells (BMSCS), can reduce ESM1 to inhibit the progression of Bca. Therefore, increased levels of miR-9-3p in Bca can reduce the invasion and metastasis of Bca cells and promote apoptosis. The exosome-derived miR-9-3p is expected to be a new target for Bca [31]. The above research shows that miRNAs can increase or lower its expression, directly or indirectly control gene expression to play an important role in the progress of Bca.

## The significance of exosome-derived miRNAs in the urine in the diagnosis and prognosis of Bca

### Exosome-derived miRNAs can be used as Bca biomarker

Several studies have shown that exosome-derived miRNAs from the urine can be used as biomarkers in the diagnosis and prognosis of Bca. Andreu et al. [32] used miRNA probe technology to identify 23 up-regulated miRNAs and 3 down-regulated miRNAs in the exosomes in the urine of patients with high-grade Bca, and further studies found that miR-375 was a biomarker for high-grade



**Figure 1. Exosome in urine in the patients of bladder cancer.**

Bca and miR-146a was a biomarker for low-grade Bca. Hanke et al. found significant increase in the expression levels of miRNA-96 and miRNA-183 in urine in patients with Bca. However, the expression levels of these two miRNAs in the urine of the Bca patients after surgery were significantly decreased. In addition, miRNA-96 in combination with urine cytology can increase the sensitivity of urine cytology from 43.6% to 78.2%, therefore miRNA-96 can be used as a biomarker diagnosis of Bca [33-36]. Yasui et al. [37] used nanotechnology to discover a large number of urinary exosome-derived miRNAs associated with Bca: miR-558 can promote tumor growth, miR-424-3p can participate in tumor metastasis, miR-520c-3p can inhibit tumor progression, miR-378a-5p can inhibit cell proliferation and induce cell apoptosis, and miR-520b can inhibit cell invasion and migration. The above studies have proved that the exosome in urine can be used for miRNA analysis of Bca patients, and the exosome-derived miRNAs in urine can be used as a non-invasive diagnostic tool for Bca patients. It is expected to be the Bca biomarker.

**Table 1** shows that a summary on exosomal miRNAs from different tissue or body fluid in bladder cancer patients.

*Multi-miRNA joint test is more diagnostic significance*

As mentioned earlier, the exosome-derived miRNAs in urine can be used as a non-invasive diagnosis tool for Bca patients [38, 39]. A large number of studies suggested that there is a higher diagnostic significance than a single miRNA detection for multi-miRNAs joint testing [40, 41]. For example, Chen et al. [42] combined 74 miRNAs to analyze Bca patients and healthy patients and found that 33 miRNAs were up-regulated and 41 down-regulated, among which let-7miR, mir-1268, miR-196a, miR-1, miR-100, miR-101 and miR-143 were more specific. Miah et al. [43-44] divided volunteers into Bca patients (68 cases) and age-matched control group (53 cases), and analyzed 15 types of miRNAs, and found that the sensitivity and specificity of miR-1224-3p in the diagnosis of Bca were 77% and 83%, respectively. The diagnostic sensitivity of miR-1224-3p combined with miR-15b and miR-135b could reach 94%. The above studies shows that there is a stronger application prospect in multi-miRNA combination detect analyses for the prognosis and diagnosis of Bca compared to a single

**Table 1. Exosomal miRNAs in Bladder Cancer Patients.**

Tumor biomarkers	Expression levels	Exosome source	Reference
miRNA-23B	Decreased	blood	[25]
miRNA-224	Decreased	blood	[25]
miR-29c	Decreased	tissue	[26]
miR-200c	Decreased	tissue	[26]
miR-146A-5P	Increased	urine	[26]
miR-205	Decreased	blood	[28]
miR-130A-3p	Increased	blood	[29]
miR-9-3p	Decreased	tissue	[31]
miRNA-96	Increased	urine	[33]
miRNA-183	Increased	urine	[33]
miR-558	Increased	urine	[37]
miR-424-3p	Increased	urine	[37]
miR-520c-3p	Decreased	urine	[37]
miR-378a-5p	Decreased	urine	[37]
miR-520b	Decreased	urine	[37]
mir-1268	Increased	urine	[42]
miR-196a	Increased	urine	[42]
miR-1	Increased	urine	[42]
miR-100	Increased	urine	[42]
miR-101	Increased	urine	[42]
miR-143	Increased	urine	[42]
miR-1224-3p	Increased	tissue	[43, 44]
miR-15b	Increased	tissue	[43, 44]
miR-135b	Increased	tissue	[43, 44]
miR-30A-3P	Increased	urine	[45]
miR-99A-5P	Increased	urine	[45]
miR-137-3-P	Increased	urine	[45]
miR-141-3-P	Increased	urine	[45]
miR-205-5p	Increased	urine	[45]
miR-21-5P	Increased	urine	[46]
miR-106B-3P	Increased	urine	[46]
miR-486-5P	Increased	urine	[46]
miR-151A-3P	Increased	urine	[46]
miR-3P	Increased	urine	[46]

**Table 1. Exosomal miRNAs in Bladder Cancer Patients (Continued).**

Tumor biomarkers	Expression levels	Exosome source	Reference
miR-185-5P	Increased	urine	[46]
miR-242-5P	Increased	urine	[46]
miR-200C-3P	Increased	urine	[46]
miR-30C-25P	Decreased	urine	[46]
miR-10B-5P	Decreased	urine	[46]
miR-451A	Increased	urine	[46]
miR-25-3P	Increased	urine	[46]
miR-71-5P	Increased	urine	[46]
miR-30A-5P	Decreased	urine	[46]
miR-23b	Increased	urine	[47, 48]
miR-921	Increased	urine	[47, 48]
miR-29c	Increased	urine	[50]
miR-141	Increased	blood	[51]
miR-639	Increased	blood	[51]
miR-210	Decreased	urine	[58]
miR-96	Decreased	urine	[58]

miRNA detect analyses.

#### *miRNA spectroscopy application in Bca grade*

Compared with NMIBC, MIBC has a unique molecular characteristics. Some miRNAs are closely related to the invasion and migration of Bca, so their expressions in MIBC have significantly increased, so that miRNA can predict the staging of Bca by detecting miRNA. Baumgart et al. [45] analyzed the miRNA of MIBC, and found 63 specific miRNAs, of which 35 miRNAs was raised and 28 were lowered. Compared with NMIBC patients, there is altered expression in miR-141-3P, miR-146B-5P, and miR-200A-3P for MIBC patients. 6 miRNAs in the MIBC tissue and invasive cell lines are different from the expression of non-invasive cell lines. Another study by Baumgart et al. [45] compared NMIBC with MIBC where, 37 miRNAs show different expression through gene chip technology, among which 29 miRNAs expression levels are decreased and 8 miRNAs expression levels are increased. Further qPCR verification of exosome-derived miRNA for MIBC cells, it was found that there were 5 expression differences of miRNAs (miR-30A-3P, miR-99A-5P, miR-137-3-P, miR-141-3-P, and miR-205-5p) verified ( $P < 0.05$ ), while there are no difference in miR-27B-3P ( $P = 0.776$ ), miR-145-5P ( $P = 0.864$ ), miR-200A-3P ( $P = 0.456$ ) in the urine of patients with Bca. Pardini et al. [46] found a miRNA spectrum which is capable of stratifying different Bca subtypes through next-generation sequencing analysis. Compared with the health control group, miR-205-5P among the G1/G2 patients of NMIBC is raised, and miR-21-5P, miR-106B-3P, miR-486-5P, miR-151A-3P, miR-3P, miR-3P, miR-3P, miR-3P, miR-151A-3P 200C-3P, miR-185-5P, miR-242-5P expression levels were increased, miR-30C-25P, miR-

10B-5P expression levels were lowered; compared with the control group, miR-205-5P, miR-iR-5P, miR-451A, miR-25-3P, miR-71-5P expressions are higher, while miR-30A-5P expressions are lowered. Therefore, NGS technology can be used to analyze the miRNA in the urine of Bca, so as to perform staging and grading of patients with Bca. The above studies indicate that the expression levels of miRNAs in different grades are different in patients with Bca. Quantitative analysis of exosome-derived miRNAs in urine and analysis of up-regulated or down-regulated miRNAs can be used to stage and grade patients with Bca and adjust the treatment protocol.

#### **Application of exosome-derived miRNAs in the treatment of Bca**

In the process of tumor progression, by regulating the secretion of miRNA, the development of Bca can achieve the purpose of treatment [42]. Inhibition of miRNA secretion has been selected as a mechanism for coordinated tumor metastasis cascade activation mediated by exosomes. Ostenfeld et al. [47-48] found that silencing RAB27A or RAB27B, a member of the extracellular RAB family, can inhibit tumor secretion of miR-23b and miR-921, thus reducing tumor cell invasion. At the same time, Kosaka et al. [49] using neutral sphingomyelinase 2 (neutral sphingomyelinase 2, nSMase2) can promote the characteristics of the secretion of exosome-derived miRNAs, which is able to inhibit metastases of tumors. Xu et al. [50] found that miRP29c can be wrapped in the exosome, and Bca cell line are constructed with miRP29c is highly expressed. Transfection of miRNA-29c in Bca can increase the apoptosis of Bca and decrease the expression levels of anti-apoptotic genes such as BCLP2 and mcl1, which plays a role in

directly killing Bca cells. These studies suggest the promising application prospects of exosome-derived miRNAs in the treatment of Bca.

### **Feasibility analysis of exosome-derived miRNAs in the urine on detection of Bca**

Bca is susceptible to metastasis with a high recurrence rate. The gold standard for clinical diagnosis of Bca is an invasive and costly cystoscopy biopsy. Examination of exosome in the urine is expected to be a new non-invasive method for diagnosis of Bca. Here we discussed the possibility for the examination of exosome in the urine on detection of Bca.

#### *Urine is easy to collect and contact with Bca tissue directly*

Bca and urine have been exposed for a long time, and the exosomes in Bca can pass through the base membrane and transport the miRNAs to urine [8]. Urine test is a clinical routine examination and is easy to obtain. The urine collection method is simple, non-invasive, and the patients are with good compliance. Compared with blood test, urine excludes interference from other systems. The urine contains the exosome secreted by glomerular podocytes and renal tubular cells in urinary tract [50]. In 2012, Scheffer et al. [51] found that there were 22 raised miRNAs expression levels in the serum of Bca patients. Studies have found that although the levels of miR-141 and miR-639 in serum increase but it lacks specificity in the diagnosis. Therefore, miRNAs examination in urine can be an ideal liquid for analyses of miRNAs of urinary tumor biomarkers such as Bca.

#### *A large number of undegraded miRNAs exists in urine*

The exosome volume in the urine is large and it cannot cross the glomerular filtration membrane in the kidneys. Most of them are directly secreted by the cells of the extrarenal urogenic tract. The protein miRNAs in the exosomes secreted by different tissues are also different and with its individual specificity. Cheng et al. [52-54] extracted and analyses these RNAs in urine, urine cell precipitation, cell-free urine, urinary supernatant without exosomes. They found that only urine has a large number of intact miRNAs in the exosome in above several specimens. Although a large number of miRNAs was detected in the precipitation of urine cells, while most of them have been degraded. The application of high-throughput depth sequencing method detected 184 exosome-derived miRNAs in 3 different subjects, while only 7 of them were shared with free-cell urine. Therefore, it can be concluded that there are a lot of RNases in the urinary system, naked miRNAs in urine are more easily degraded, and exosomes can protect miRNAs from RNase degradation. The research by Yun et al. [55] found that in urine, miRNAs has not changed significantly after several freezing, thawing cycles, and long-term preservation at room temperature, indicating that miRNAs is more stable in urine. Therefore, the study on exosome-derived miRNAs is more meaningful.

#### *Exosome-derived miRNAs in urine is a suitable choice to detect Bca*

The close correlation of miRNAs and tumor has been confirmed in the literature that it has participated in the development and metastasis of tumors and other diseases. Tumor-related exosomes miRNAs can regulate the interaction between tumor cells and tumor microenvironment by affecting the tumor-promoting process of target cells. The exosomes secreted by MIBC cells has a specific miRNA expression pattern [56]. Matsuzaki et al.

[57] used qRT-PCR to find that miR-21-5p was highly expressed in exosomes of Bca cells with negative urine cytology, and the specificity and sensitivity of exosomes-derived miR-21-5p were higher than those of urine ablative cytology. These results indicate that miR-21-5p in exosomes can be used for the detection of Bca with cytological negative urine shedding. At the same time, Eissa et al. [58] identified miR-96 and miR-210 in Bca patients with a cystoscopy test negative. Sapre et al. [59-60] used 12 miRNAs as the diagnosis method of Bca. Sapre et al. [59-60] used 12 miRNAs to diagnose Bca and reduced the cystoscopy rate by 30% by increasing specificity and sensitivity, indicating that miRNA is expected to replace cystoscopy as a new noninvasive method for detecting Bca in the near future. Armstrong et al. [61] analyzed the miRNA molecular spectrum of four biological specimens: miRNA molecular spectrum of four biological specimens sources: Bca tumors, urine (free cells and exosome source), blood and plasma rich in white blood cells (circulation and exosome sources).

Studies have shown that the exosome-derived and rich white blood cell source miRNA spectrum in urine is highly related to the miRNA of Bca, and it may have the huge potential of diagnosis of Bca as a source of biomarkers for miRNA. The circulating miRNA, which has no cell plasma exosome, has no correlation with the bladder tumor miRNAs. It may not be a good choice for the biomarkers of Bca. The above studies have shown that the absence of exosome in urine is a suitable choice to detect Bca.

### **Conclusions**

miRNAs accounts for about 70% of the nucleic acid components in the exosome. miRNAs exist in a large amount of urine exosome and is not easily degraded. Urine is easily collected and is representative due to long-term contact with Bca cells. Therefore, exosome-derived miRNAs in urine plays an important role in the development, diagnosis, prognosis, and treatment of Bca. It has not been transformed into conventional clinical practice, and it is applied to the clinic and still requires large-scale clinical trials and larger sample analysis.

At the same time, there are many ways to separate and purify the exosome, the existing methods make it hard to achieve the purity required for clinical applications, and it is easy to get false negative or false results. Better technology to increase the division and purity of exosomes, and increase the yield of exosome in urine, to achieve more effective clinical applications. In the near future, through multi-disciplinary cooperation, in-depth analysis of scientists, and clinical research of large samples, the samples in urine can replace the cystoscopy test to become a new non-invasive biomarker in the diagnosis and treatment of Bca.

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### **Ethical policy**

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study. Approval from institutional ethical committee was taken.

### **Availability of data and materials**

All data generated or analysed during this study are included in this publication.

### Author contributions

MHD designed the study and was responsible for the writing of the original draft. KYZ edited and approved the final manuscript.

### Competing interests

All authors declare no competing interests.

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