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Value of miR-15b-5p combined with ultrasound imaging in early diagnosis of renal cell carcinoma

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Renal cancer is one of the most common malignancies, and its incidence is increasing year by year, second only to prostate and bladder cancers. Therefore, early screening is of great significance to prevent and improve the prognosis of patients with renal cancer. Therefore, this study intends to observe the early diagnostic value of miR-15b-5p combined with Colour ultrasound imaging in renal cell carcinoma. In this study, the clinical samples of 76 patients with renal cell carcinoma diagnosed by pathology in our hospital from April 2020 to June 2022 were retrospectively collected as the research objects. Another 100 healthy people who underwent general physical examination in our hospital during the same period were selected to detect the expression level of miR-15b-5 in serum by RT-PCR together with the 76 experimental samples. Colour ultrasound imaging was used to detect the blood flow distribution around and inside the kidney of the patients with renal cancer, and the parameters were measured and compared with the gold standard for statistical analysis. The expression level of miR-15b-5 in renal cell carcinoma group was significantly higher than that in control group. Most of them showed renal hamartoma (40.79%). Benign renal tumors were mainly characterized by type I and II blood flow, while malignant renal tumors were characterized by type III and IV blood flow. Compared with the pathological gold standard, the diagnostic accuracy of ultrasound imaging was founded to be about 81.25%. Further ROC analysis showed that ultrasound imaging combined with miR-15b-5 detection could effectively improve the early screening value of renal cell carcinoma. We observed that miR-15b-5 has a specific expression level in the serum of patients with renal cell carcinoma, and its combination with ultrasound imaging can significantly improve the early detection of renal cell carcinoma. This approach is envisaged to be highly useful for, early diagnosis, intervention and management of patients with renal cell carcinoma.

Keywords: Cancer, Kidney, Renal neoplasm, Tumor

Renal neoplasms are the second largest neoplasms of the urinary system, divided into renal parenchyma and renal pelvis. The common renal parenchymal tumors include renal cell carcinoma, wilms tumor, hamartoma, hemangioma, and renal pelvis tumors also known as renal pelvis cancer. Renal cell carcinoma is the most common one accounting for 80-90% of primary malignant tumors of the kidney[1-3]. Patients with renal tumors show comprehensive effects of primary and secondary factors. Benign renal tumors have less influence on the body, and malignant renal tumors are hidden during the early clinical stages without any discomfort. Therefore, early diagnosis of malignant renal tumors is of great significance to improve the quality of life and survival rate[4,5]. The clinical manifestations of patients with renal cancer are complex and variable. Some of these clinical manifestations are directly caused by the renal tumor itself, while others may be caused by hormones secreted by renal cancer cells or metastasis[6]. With the frequent health check-ups, most patients with kidney cancer are often diagnosed on the basis of imaging tests and then treated. There is a growing consensus that the occurrence of renal cancer is closely related to smoking, obesity, hypertension, kidney disease, occupation, drinking and other bad practices[7-9]. Therefore, it is important to screen renal cell carcinoma for early detection and timely prevention for better management of the patients.

MicroRNAs (miRNAs) are a class of highly conserved non-coding RNAs with the ability to regulate gene expression at the post-transcriptional level by binding to the 3' untranslated region (UTR) of target mRNA. Mir-15b-5p is a mature miRNA cleaved from the 5' end of miR-15b precursor. Studies have shown that the expression of Mir-15B-5p in colon

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cancer, breast cancer and prostate cancer increases, which promotes cancer cell proliferation, reduces cell apoptosis, induces tumor recurrence and metastasis, and facilitating the prognosis of patients[10-12]. Renal cancer is a typical tumor with rich blood supply, and neovascularization. This results in faster malignant tumors formation than that of the benign tumors. Colour ultrasound imaging is highly useful for detection of renal artery blood flow facilitating the evaluation of renal tumor based on themorphology and internal blood flow distribution. Thus, this approach is more conducive for the diagnosis of renal cancer[13]. Although renal cancer can be diagnosed during the early stages, it is still not consistent with the gold standard. Therefore, in order to improve the screening efficiency and detection of renal cancer, here, we explored the ability of miR-15b-5p combined with ultrasound imaging in the early stage of renal cancer.

General Information and Methods

This study retrospectively collected the clinical data of 169 patients with suspected renal cancer who were examined in our hospital from April 2020 to June 2022. Based on the clinicopathological gold standard, they were divided into renal cancer group (n=76) and control group (n=93). [But the abstract, control samples were given to be 100, please check and confirm]. The baseline data of patients in each group were compared as shown in Table 1. All were comparable (P >0.05). Seventy-six patients with renal cell carcinoma were diagnosed by pathological tissue cytology[14], and their body tolerance was found to be high. Patients with other malignant tumors and low treatment compliance were excluded from this study.

Methods

miR - 15-5 p detection

Total RNA was extracted from cells with Trizol reagent. cDNA was synthesized with miRNA using reverse transcription kit and the expression of miR-15b-5p was detected by miRNA qPCR kit. miR-15b-5p upstream 5'-ATGAACTTTCTCTCTCTCTTGG-3', downstream 5'-CAGTGCGT-GTCGTGGAGT-3'; U6 upstream 5'-CGCTTCGGCAGCACATATAC-3', downstream 5'-AACGCTTCACGAATTTGCCT-3' primers were used for RT-PCR reaction.

Table 1 — Comparison of baseline data

	Kidney cancer group (n=76)	Control groups (n=93)	(χ2/t)/P
Age (years)	56.63±8.21	54.85±9.12	1.320/0.189
Gender			0.039/0.843
Man	59 (77.63)	71 (76.34)	
Woman	17 (22.37)	22 (23.66)	
BMI (kg/m2)	23.35±1.13	23.19±1.35	0.824/0.411
Smoking	61 (80.26)	68 (73.12)	1.182/0.277
Drinking	59 (77.63)	61 (65.59)	2.945/0.086
Diabetes	55 (72.37)	68 (73.12)	0.012/0.913
Hypertension	57 (75.00)	64 (68.82)	0.786/0.375

Colour ultrasound imaging

Hitachi HV900 and Philips iU22 Colour Doppler ultrasound analyzer, probe frequency 3.5-5.0 MHz were used. The clients were placed in prone, lateral, or supine position. The size of the kidney was measured routinely, and the continuity of the renal capsule was observed, to ensure whether the collecting system was separated. Further, the origin, location, morphology, internal and marginal echo of the tumor, and its size was measured. Following this, the tumor thrombus of renal vein and inferior vena cava and retroperitoneal lymph node transformation were observed. Finally, the Colour Doppler flow imaging (CDFI) was used to detect the distribution of blood flow around and inside the tumor.

Statistical treatment

SPSS25.0 processes all research data in the article.

Results

Compare the expression of serum miR-15b-5p between the two groups

RT-PCR results showed that the expression of miR-15b-5p in renal cancer patients was (1.77±0.04), and that in the control group was (1.15±0.03), indicating that the expression of miR-15b-5p in renal cancer group was higher than that in the control group (Fig. 1).

Characteristics of blood flow distribution in patients with renal cell carcinoma diagnosed by Colour ultrasound

According to the results of ultrasound diagnosis, renal hamartoma (40.79%) was most common in the patients with renal cancer by ultrasound imaging.

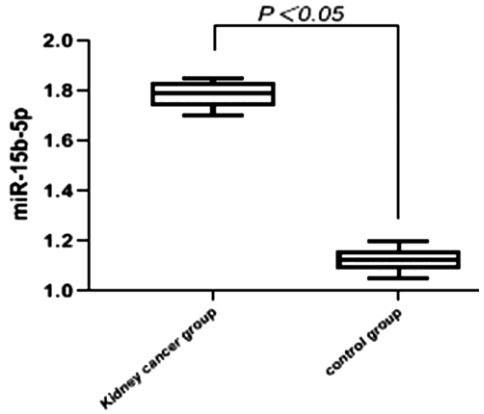

Fig. 1 — Comparison of miR-15b-5p expression between renal cell carcinoma group and control group

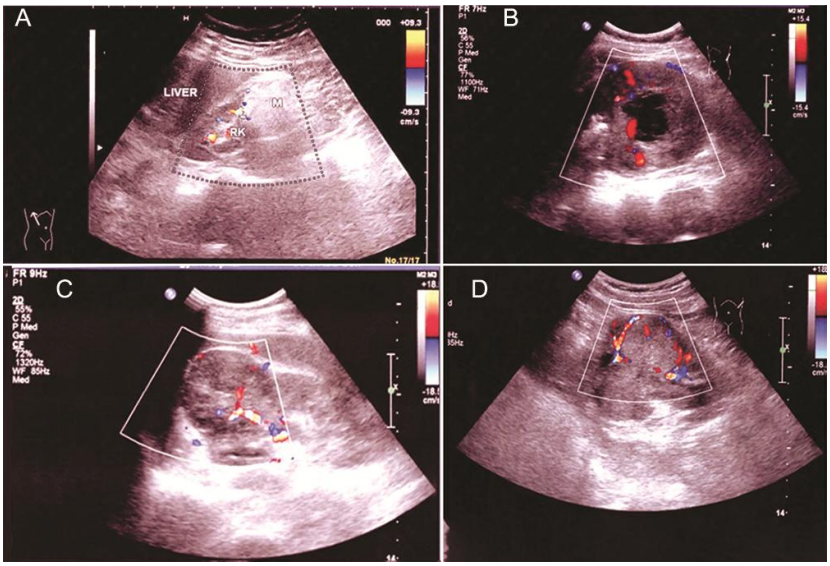

Fig. 2 — Ultrasound image of blood flow signals of different renal tumors. "A, B, C, D" correspond to "I, II, III, IV" blood flow signals, respectively.

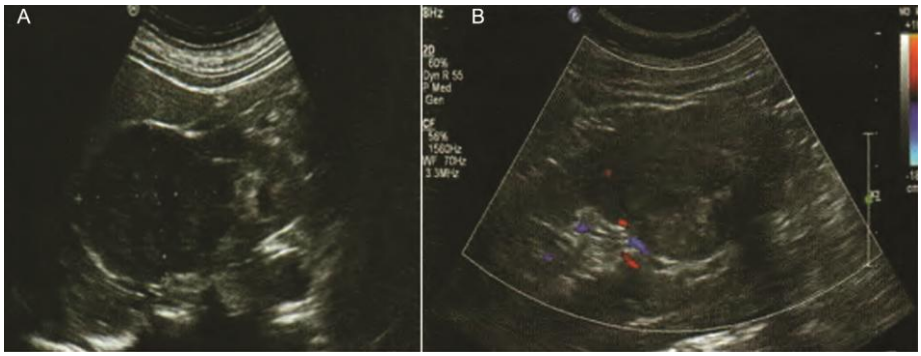

Fig. 3 — Ultrasound image showing abundant blood flow signals in renal tumor programs, which are mostly "ring" or "semi-ring", but rarely "strip" or "star".

(Fig. 2 & 3). Renal benign tumors were mainly characterized by type I and II blood flow, while renal malignant tumors were mainly characterized by type III and IV blood flow (Table 2).

Comparison of colour ultrasound imaging diagnosis with gold standard

The pathological gold standard showed that there were 48 malignant tumors and 28 benign tumors in 76 patients with renal cell carcinoma. The ultrasound imaging results showed 39 malignant tumors whereas 9 cases were wrongly diagnosed, as shown in Table 3.

ROC analysis of value of miR-15b-5p combined with colour ultrasound imaging in early screening of renal cell carcinoma

Table 4 shows the screening efficiency of single index and combined index for early renal cancer, and Figure 4 is the ROC diagram for predicting each index. These results indicate that miR-15b-5p combined with Colour ultrasound imaging has a high value in early detection of renal cell carcinoma.

Table 2 — Diagnostic results of Colour ultrasound imagin

Tumor types	n	Blood flow classification			
		I	II	III	IV
CCRCC	21 (27.63)	2	4	6	9
Papillary cell carcinoma	16 (21.05)	0	6	4	6
Carcinoma of the renal pelvis	6 (7.89)	0	0	2	4
Wilms cell carcinoma	2 (2.63)	0	0	0	2
Cystic renal cancer	0 (0.00)	0	0	0	0
Hamartoma of kidney	31 (40.79)	18	8	4	1
Hemangioma of kidney	0 (0.00)	0	0	0	0
Total	76 (100.00)	20	18	16	22

Table 3 — Comparison of Colour ultrasound diagnosis and gold standard

Results of ultrasound imaging	The gold standard		
	Malignant	Benign	Total
Malignant	39	12	51
Benign	9	16	25
Total	48	28	76

Table 4 — Diagnostic efficiency table

	95% CI	Sensitivity (%)	Specificity (%)	AUC	Cut-off value
Joint diagnosis	0.799~0.938	85.20	88.50	0.869	--
miR-15b-5p	0.682~0.852	71.40	77.50	0.767	>1.52
Ultrasound imaging	0.712~0.878	78.70	80.30	0.795	--

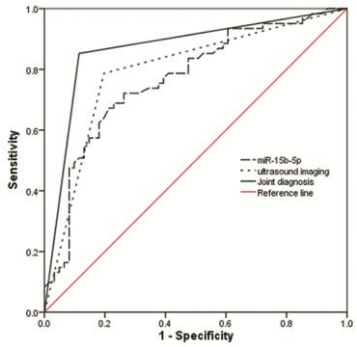

Fig. 4 — ROC diagram for predicting single index and combined index for early renal cancer.

Discussion

Renal cell carcinoma, also known as renal cell carcinoma, is the most common parenchymal malignant tumor in clinical practice, accounting for more than 80% of the adult renal malignant tumors. It can occur at any age and mainly affects middle-aged and elderly people, with a slightly higher incidence rate in males than in females[15]. Renal clear cell carcinoma is the most common pathological type, which seriously affects the physical and mental health of the patients. Therefore, early detection and diagnosis of renal cell carcinoma must be strengthened to provide a basis for the formulation of subsequent treatment plans[16].

Ultrasound is the simple and most commonly used method to detect renal tumors. Renal contrast-enhanced ultrasound is helpful to distinguish benign and malignant renal tumors. It is suitable for the differential diagnosis of renal tumors in patients with chronic renal failure or iodine allergy who are not suitable for enhanced CT scanning, as well as patients with complex renal cysts, and has a wide range of clinical application value[17]. In this study, analysis of ultrasound images of (76-28) 48 patients with renal cancer, showed that the lesions of most patients were "annular" or "semi-annular", and the internal echo of the lesions was mainly divided into four types. This included high, medium, low and mixed echo, while the internal composition of the tumor was the main reason for the difference in echo[18]. Tumor cells composition also affected the echo showing another reason. If the normal cells and tumor cells are completely separate, kidney ultrasound image is clearer. Thus, ultrasonic image of diagnosis and treatment of renal cancer plays an essential role. Through ultrasonic image, one can directly observe tumor blood vessels and vascular distribution. In addition, blood flow signals and velocity within the tumor can be observed, which otherwise is not possible by other instruments. This, this approach offers a major advantage to the ultrasound imaging detection[19,20].

Employing this approach,, one can see the other benign renal tumor with I, II type blood flow given priority. The kidney malignant tumor is given priority with III, IV type blood flow, suggesting that the rapid rate of malignant tumor growth, often lead to continuous formation of tumor blood vessels. The characteristics of benign tumors are the growth speed which is very slow and usually does not lead to the formation of tumor blood vessels. From the observation of most cases of renal cancer, it is concluded that the blood flow in the normal renal parenchyma is different from the blood flow in the lesion, so the accurate diagnosis of renal cancer is a crucial step[21]. In addition, as one of the most commonly detected types of renal cancer, misdiagnosis between the two types often occurs in clinical practice, mainly because of the formation of hyperechogenic renal cancer lesions. Usually, the echo size of renal hamartoma in ultrasound images is hyperechogenic, and hence the two are often confused. Although ultrasound imaging has a certain effect on the diagnosis of renal cancer, it cannot be completely consistent with the gold standard. Therefore, this study uncovers whether it can further improve the early screening rate of renal cancer by combining serum miR-15b-5p.

The results show that the expression of miR-15b-5p in patients with renal cell carcinoma is significantly higher than that in the control group. It may be noted that the occurrence and development of tumor is a complex process, which is usually accompanied by changes in the expression level of miRNA, which is a potential marker in the process of tumor development, diagnosis and prognosis[22]. The elevated expression level of miR-15b-5p in the development of various cancers has been confirmed by other studies, but its expression in renal cancer has not been elucidated by most studies[23,24]. This

study showed that the serum miR-15b-5p in patients with renal cancer also showed a significant upward trend, indicating that the increased expression of miR-15b-5p was closely related to the development of renal cancer. The author analyzed the possible pathogenesis of miR-15b-5p by referring to most of the literature. As a class of miRNA, miRNA has been confirmed to be a regulator of PI3K/ Akt/mTOR signaling pathway. This signaling pathway plays an important role in cell apoptosis, migration, proliferation, survival and other biological functions. Thus, it can be speculated that miR-15b-5p also plays a role in promoting the progression of renal cancer through the PI3K/ Akt/mTOR signaling pathway[25]. The last ROC result of this study showed that the combination of miR-15b-5p and ultrasound imaging could significantly improve the screening value of Mir-15B-5P for early renal cancer.

Conclusion

Improved early diagnosis of renal cell carcinoma with the combination of miR-15b-5p and ultrasound imaging suggests potential application in treatment. Although this pilot study has achieved some research results, the small sample size due to time limitation makes it not representative. In the next study, the sample size can be expanded to improve the representativeness of the study. In addition, the relationship between miR-15b-5p and the pathogenesis of renal cell carcinoma was concluded by combining with other clinical literatures. Whether specific miR-15b-5p can affect the occurrence and development of renal cell carcinoma through PI3K/ Akt/mTOR signaling pathway needs further studies to confirm.

Data availability

The simulation experiment data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

Authors declare no competing interests.

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