Int J Clin Exp Med 2015;8(7):10235-10247
www.ijcem.com /ISSN:1940-5901/IJCEM0009275

Review Article

Clinicopathological and prognostic significance of high Ki-67 labeling index in hepatocellular carcinoma patients: a meta-analysis

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Received April 18, 2015; Accepted June 30, 2015; Epub July 15, 2015; Published July 30, 2015

Abstract: Background: The relationship between Ki-67 labeling index (LI) and clinical outcome in hepatocellular carcinoma (HCC) has been investigated by various studies, but no consistent result has been concluded. To define the prognostic significance of Ki-67 LI in patients with HCC, we performed a meta-analysis. Methods: We searched for literatures in the following databases: PubMed, ISI Web of Science, EMBASE, Cochrane Central Register of Controlled Trials, Science Direct, Wiley Online Library, Google Scholar, China National Knowledge Infrastructure (CNKI), Chinese VIP and WanFang Databases. Our search ended on April 6th, 2015. Data were extracted from eligible studies and the correlation between Ki-67 LI and clinicopathological features of HCC was analyzed and pooled hazard ratios (HRs) for eligible studies were calculated by STATA 11.0 (STATA Corp., College, TX). Results: In total, 54 studies involving 4996 patients were included in the current meta-analysis. The meta-analysis provided evidence that high Ki-67 LI was closely associated with histological grade, tumor size, number of tumor nodes, the status of metastasis, cirrhosis and vein invasion in HCC patients. The pooled HRs showed that high Ki-67 LI had an unfavorable impact on disease-free survival (DFS) (HR=1.626, 95% confidence interval (CI): 1.364-1.939, *P*<0.001), relapse-free survival (RFS) (HR=1.820, 95% CI: 1.215-2.725, *P*=0.004) and overall survival (OS) (HR=1.170, 95% CI: 1.102-1.243, *P*<0.001), respectively. Additionally, subgroup analysis indicated that high Ki-67 LI was related to poorer DFS, RFS and OS independent of regions, treatment strategies or statistical methods, except that no statistical significance was found on RFS (HR=2.413, 95% CI: 0.523-11.142, *P*=0.259) and OS (HR=1.998, 95% CI: 0.797-5.009, *P*=0.14) in patients with liver transplantation. Conclusions: Our meta-analysis suggests that higher Ki-67 LI confers a fast progression and poor prognosis for HCC patients.

Keywords: Ki-67, hepatocellular carcinoma (HCC), clinicopathological features, prognostic value, meta-analysis

Introduction

Hepatocellular carcinoma (HCC) is one of the most frequent malignant neoplasia with a poor prognosis and it is attributed to a high mortality in the world [1]. An estimation demonstrates that 745,500 deaths occurred among the 782,500 new liver tumor cases worldwide during 2012 [2]. In less developed countries, the increasing rates of HCC are particularly due to the infection of hepatitis B virus (HBV) and hepatitis C virus (HCV) [3]. It is well-known that surgical resection and liver transplantation (LT) are the best options to treat HCC. However, recurrence or metastasis still extremely exists in HCC patients after liver resection [4, 5]. Although a variety of factors have been shown to contribute to the development of HCC, the mechanisms are still inconclusive. Therefore, it is in urgency to identify the key factors which are of importance to the survival of patients with HCC.

Ki-67 is a nuclear protein attaching to nuclear antigens expressed in phases of the proliferation except G0, and it serves as one of the major factors related to tumor proliferation, which can be assessed by Ki-67 or MIB-1 antibody with immunohistochemistry (IHC) [6, 7]. Besides, there have been already studies demonstrating that Ki-67 LI was strongly associated with the aggressiveness of tumor, including prostate cancer, astrocytomas, gastroentero-pancreatic neuroendocrine tumors, sialoblastoma, lung

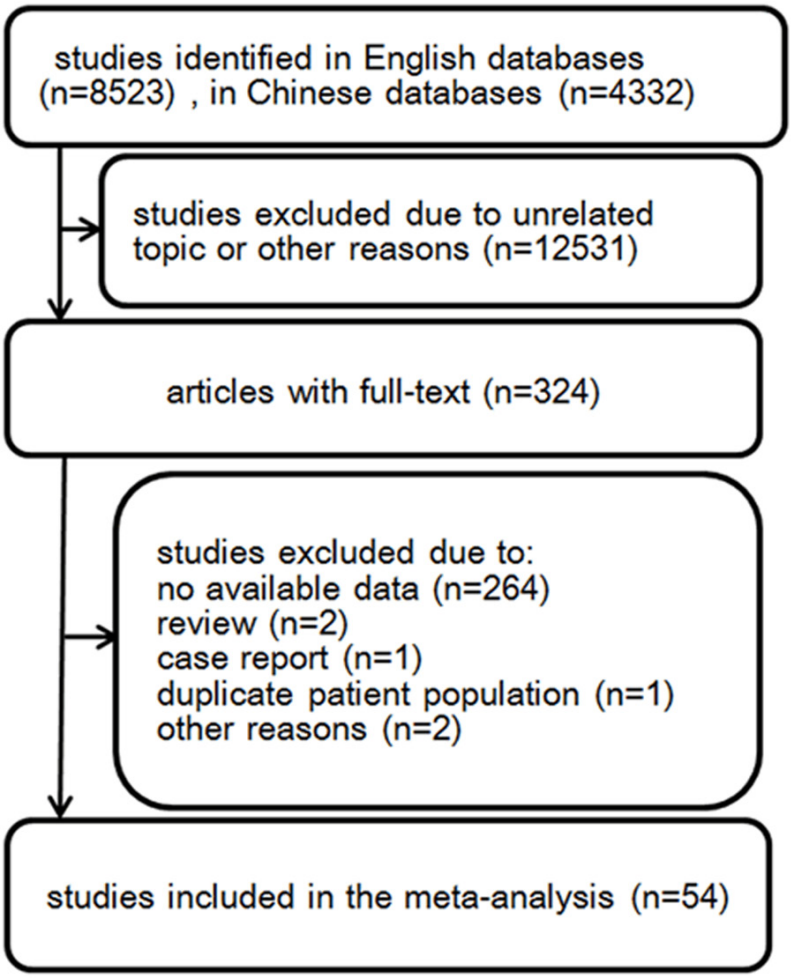

Figure 1. Flow chart of study selection in this meta-analysis.

cancer, breast cancer and pituitary adenomas [8-14]. In addition, several meta-analyses conclude that high Ki-67 LI could predict poor prognosis in patients with cervical cancer, gliomas, lymphoma, breast cancer, and lung cancer [15-19]. However, no meta-analysis has been available on the relationship between Ki-67 LI and deterioration and prognosis of HCC. Although it was reported that Ki-67 could be an independent marker for worse prognosis in patients with liver cancer, the results were contradictory [20-24]. Therefore, we conducted the current meta-analysis to identify whether Ki-67 LI had significant influence on the progression and prognosis of HCC.

Materials and methods

Search strategy

An electronic literature search was conducted to collect studies which evaluated Ki-67 LI in HCC, in English databases of PubMed, ISI Web of Science, EMBASE, Cochrane Central Register of Controlled Trials, Science Direct, Wiley Online Library, Google Scholar and Chinese databases of CNKI, VIP, and WanFang. The keywords were used as following in various combinations: (1) 'HCC' OR 'hepatocellular' OR 'liver' OR 'hepatic'; (2) 'cancer' OR 'tumor' OR 'neoplas*' OR 'malignancy' OR 'carcinoma'; (3) 'Ki67' OR 'Ki-67' OR 'MIB-1' OR 'proliferation index' OR 'PI' OR 'proliferation activity' OR 'mitotic index' OR 'mitotic count' OR 'proliferation marker' OR 'labeling index' OR 'LI'; (4) 'prognos*' OR 'surviv*' OR 'follow-up' OR 'mortality' OR 'predict' OR 'outcome'. Our search ended on April 6th, 2015.

Study eligibility

Firstly, the studies which contained Ki-67 LI in HCC were selected to search for full articles. Then, we picked out articles with inclusion criteria as below: (1) the samples for the studies must be human liver tissue and serum, but not animals or cell lines; (2) the results for the studies must include the correlation between Ki-67 LI and clinicopathological parameters, survival or prognosis; (3) the studies must provide the value of HR or it could be obtained from the survival curve or primary data in the article; (4) the article types were not reviews or case reports. To avoid the same cohort of patients, if there were same patient population in different articles, only the study with the largest patient size was included.

Data extraction

Two authors extracted the information cautiously from the eligible articles and the third author checked the data finally. The following data were extracted: first author, publication date, country, the number of patients, histologi-

Table 1. Characteristics of eligible studies included in the Meta-analysis for DFS and RFS

First Author	Year	Region	Patients N (M/F)	Treatments	Antibody	Cut-off	C	HR (95% CI)	Outcome
Murakami K	2015	Asian	136 (104/32)	hepatectomy	anti-Ki-67	20%	1/300	1.33 (0.71-2.51)	DFS
Jang KY	2012	Asian	154 (132/22)	hepatectomy	MIB-1	10%	NR	1.21 (0.81-1.82)	DFS
Pang XF	2011	Asian	231 (197/34)	hepatectomy	anti-Ki-67	5%	NR	1. 58 (1.04-2.41)	DFS
Kitamura K	2011	Asian	63 (46/17)	hepatectomy	anti-Ki-67	42%	1/100	0.89 (0.31-2.58)	DFS
Mitsuhashi N	2007	Asian	37 (29/8)	hepatectomy	anti-Ki-67	10%	1/400	2.55 (1.04-6.34)	DFS
Watanabe J	2004	Asian	33 (23/10)	hepatectomy	anti-Ki-67	NR	10%	1.92 (0.68-5.41)	DFS
Wang NF	2003	Asian	51 (46/5)	hepatectomy	anti-Ki-67	10%	1/150	2.02 (0.86-4.76)	DFS
Aoki T	2003	Asian	143 (107/36)	hepatectomy	anti-Ki-67	20%	1/50	1.63 (1.11-2.39)	DFS
Han SH	2000	Asian	85 (79/6)	hepatectomy	MIB-1	20%	NR	3.50 (1.8-6.78)	DFS
Ito Y	2000	Asian	85 (NR)	hepatectomy	MIB-1	20%	1/50	2.13 (0.96-4.79)	DFS
Ito Y	1999	Asian	76 (65/11)	hepatectomy	MIB-1	20%	1/50	1.34 (0.54-6.74)	DFS
King KL	1997	Asian	67 (54/13)	hepatectomy	anti-Ki-67	10%	NR	0.96 (0.3-3.11)	DFS
Ng IO	1995	Asian	72 (65/7)	hepatectomy	MIB-1	20%	1/50	1.99 (0.92-4.33)	DFS
Srivastava S	2015	Asian	179 (142/37)	hepatectomy	MIB-1	5%	1/50	1.80 (1.05-3.11)	RFS
Zheng SS	2014	Asian	152 (140/12)	hepatectomy	NR	20%	NR	1.67 (1.07-2.09)	RFS
Pang XF	2012	Asian	290 (242/48)	hepatectomy	anti-Ki-67	5%	NR	2.21 (1.02-4.77)	RFS
Srivastava S	2012	Asian	121 (104/17)	hepatectomy	NR	NR	NR	1.50 (0.49-4.56)	RFS
Aktas S	2011	Asian	50 (44/6)	LT	NR	10%	NR	1.05 (1.03-1.08)	RFS
Guzman G	2005	America	20 (13/7)	LT	MIB-1	10%	1/200	2.88 (0.35-51.36)	RFS
Nakanishi K	2004	Asian	135 (103/32)	hepatectomy	MIB-1	50%	1/200	8.63 (1.11-66.78)	RFS
Fiorentino M	2004	European	83 (68/15)	LT	MIB-1	10%	1/50	11.68 (1.31-104.41)	RFS
Cui J	2004	Asian	41 (36/5)	hepatectomy	anti-Ki-67	NR	NR	8.93 (0.73-111.11)	RFS

DFS: disease-free survival; RFS: relapse-free survival; N: number; M/F: male/female; C: concentration; HR: hazard ratio; CI: confidence interval; NR: not report; LT: liver transplantation.

cal grade, tumor size, clinical tumor stage, number of tumor nodes, metastasis, cirrhosis, vein invasion, treatment strategies, antibody and its concentration, cutoff value, statistics method and data to evaluate the prognosis between Ki-67 LI and clinical outcome of HCC.

Statistical methods

The relationship between Ki-67 LI and clinicopathological parameters was analyzed by two-sided χ^2 test (Chi squared test; Chi^2). HR value was used to describe the intensity of relationship between Ki-67 LI and clinical outcome of HCC. If HR and 95% CI were offered in the articles, these data were extracted directly to calculate the pooled HR. Otherwise, HR and 95% CI were estimated by primary data or Kaplan-Meier survival curves with the software SPSS20.0 and Engauge Digitizer Version4.1 and the method reported by Parmar et al. [25], respectively. High Ki-67 LI indicated worse outcome if there was an HR>1 observed, and it would be regarded as statistically significant if the 95% CI of pooled HR did not overlap 1, with $P<0.05$.

Statistical heterogeneity was assessed with χ^2 test (Chi squared test; Chi^2) and inconsistency (I^2) [26], with a P value of <0.05 or $I^2>50\%$ taken to reflect a significant heterogeneity. HR value and 95% CI were pooled with fixed-effects model (Mantel-Haenszel method) if there was no significant heterogeneity. Otherwise, the random-effects model (DerSimonian and Laid method) was used. Finally, publication bias was assessed, if the meta-analysis including 10 or more studies. Begg's test was used to assess the possibility of publication bias. The above statistical analysis was performed by using the software STATA11.0 (STATA Corp., College, TX) and $P<0.05$ was considered to be statistical significance.

Results

Study selection and characteristics

There were 8523 studies identified in English databases and 4332 studies identified in Chinese databases with the search strategies, respectively. **Figure 1** showed the detailed selection procedure, and full articles of 324

Table 2. Characteristics of eligible studies included in the Meta-analysis for OS

First Author	Year	Region	Patients N (M/F)	Treatments	Antibody	Cut-off	C	HR (95% CI)
Srivastava S	2015	Asian	179 (142/37)	hepatectomy	MIB-1	5%	1/50	2.08 (0.89-4.84)
Murakami K	2015	Asian	136 (104/32)	hepatectomy	anti-Ki-67	20%	1/300	1.72 (0.90-3.32)
Wang YL	2014	Asian	80 (76/4)	hepatectomy	anti-Ki-67	10%	1/200	1.12 (0.39-3.23)
Chen HW	2014	Asian	103 (86/17)	hepatectomy	anti-Ki-67	NR	1/100	4.55 (1.08-20.00)
Jiang DW	2014	Asian	98 (73/25)	hepatectomy	anti-Ki-67	50%	1/100	3.28 (1.56-6.89)
Huang XD	2014	Asian	108 (78/30)	hepatectomy	anti-Ki-67	47%	1/400	0.59 (0.34-1.03)
Hu BY	2014	Asian	103 (69/34)	hepatectomy	anti-Ki-67	50%	1/100	7.10 (2.27-22.24)
Huang XD	2014	Asian	102 (83/19)	hepatectomy	anti-Ki-67	25%	1/400	1.40 (0.69-2.84)
Chen HW	2014	Asian	103 (86/17)	hepatectomy	anti-Ki-67	NR	1/100	4.6 (1.13-19.08)
Huang XD	2013	Asian	57 (44/13)	hepatectomy	anti-Ki-67	NR	1/100	2.35 (1.02-5.45)
Lu CH	2013	Asian	81 (62/19)	hepatectomy	anti-Ki-67	NR	1/100	2.57 (1.25-5.26)
Liu GL	2013	Asian	72 (57/15)	hepatectomy	anti-Ki-67	39%	1/100	2.07 (1.10-3.91)
Lu CH	2013	Asian	96 (NR)	hepatectomy	anti-Ki-67	41%	1/100	2.47 (1.04-5.90)
Chen LT	2013	Asian	92 (80/12)	hepatectomy	MIB-1	NR	1/50	1.00 (0.97-1.03)
Zhang L	2012	Asian	92 (74/18)	hepatectomy	anti-Ki-67	5%	NR	4.50 (2.62-7.75)
Jang KY	2012	Asian	154 (132/22)	hepatectomy	MIB-1	10%	NR	0.98 (0.59-1.62)
Sofocleous CT	2012	American	63 (31/32)	RFA	NR	NR	NR	2.11 (1.05-4.25)
Srivastava S	2012	Asian	121 (104/17)	hepatectomy	NR	NR	NR	3.16 (1.11-8.99)
Geng M	2012	Asian	82 (NR)	hepatectomy	MIB-1	10%	1/100	0.84 (0.51-1.39)
Chen HW	2012	Asian	101 (84/17)	hepatectomy	anti-Ki-67	22%	1/100	4.12 (1.83-9.30)
Schmilovitz-Weiss H	2011	Asian	61 (43/18)	LT	anti-Ki-67	10%	1/100	1.03 (1.01-1.06)
Cao XL	2011	Asian	80 (58/22)	hepatectomy	anti-Ki-67	23%	1/100	1.02 (1.0-1.06)
Kitamura K	2011	Asian	63 (46/17)	hepatectomy	anti-Ki-67	42%	1/100	3.94 (0.67-23.72)
He S	2011	Asian	45 (38/7)	hepatectomy	anti-Ki-67	42%	1/100	1.06 (1.03-1.09)
Sun SG	2010	Asian	255 (215/40)	hepatectomy	anti-Ki-67	25%	1/100	1.81 (1.19-2.77)
Ke Q	2009	Asian	43 (38/5)	hepatectomy	anti-Ki-67	24%	1/100	1.03 (1.01-1.05)
Chen XG	2008	Asian	50 (48/2)	LT	anti-Ki-67	10%	1/50	3.08 (1.28-7.45)
Stroescu C	2008	European	47 (40/7)	hepatectomy	MIB-1	50%	NR	5.64 (1.18-26.82)
Zhang YR	2007	Asian	83 (74/9)	hepatectomy	anti-Ki-67	10%	NR	1.42 (0.60-3.36)
Mitsuhashi N	2007	Asian	37 (29/8)	hepatectomy	anti-Ki-67	10%	1/400	0.92 (0.37-2.30)
He P	2006	Asian	93 (81/12)	hepatectomy	anti-Ki-67	10%	1/40	2.36 (1.27-4.39)
Guo J	2006	Asian	105 (91/14)	hepatectomy	anti-Ki-67	10%	NR	1.33 (0.71-2.50)
Yang SF	2006	Asian	69 (52/17)	hepatectomy	anti-Ki-67	50%	1/75	11.74 (1.34-102.87)
Wang SN	2006	Asian	66 (50/16)	hepatectomy	anti-Ki-67	50%	1/75	1.64 (0.49-5.49)
Schmitt-Graeff A	2004	European	162 (128/34)	hepatectomy	MIB-1	13.10%	1/50	1.75 (1.09-2.81)
Morinaga S	2004	Asian	40 (27/13)	hepatectomy	anti-Ki-67	7.53%	1/25	6.18 (1.17-32.73)
Watanabe J	2004	Asian	33 (23/10)	hepatectomy	anti-Ki-67	10%	NR	2.95 (0.88-9.94)
Fiorentino M	2004	European	83 (68/15)	LT	MIB-1	10%	1/50	3.33 (1.01-7.00)
Matsuda Y	2003	Asian	40 (NR)	hepatectomy	Ki-S5	20%	1/25	7.52 (1.44-39.38)
Nolte M	1998	European	20 (NR)	hepatectomy	MIB-1	20%	1/100	3.02 (0.92-9.91)
King KL	1997	Asian	67 (54/13)	hepatectomy	anti-Ki-67	10%	NR	1.17 (0.29-4.78)

OS: overall survival; N: number; M/F: male/female; C: concentration; HR: hazard ratio; CI: confidence interval; NR: not report; RFA: radiofrequency ablation; LT: liver transplantation.

studies should be read after the first screening. Of these 324 studies, 270 were excluded due to no available data to estimate HR value or other reasons, as shown in **Figure 1**. In total, 54 studies (including 13 studies from Chinese databases) published from 1995 to 2015, involving 4996 patients were included in the meta-analysis [20-22, 24, 27-76].

Out of the 54 studies included in the meta-analysis, all had the sufficient information for HR extraction, including 13 studies evaluable for DFS, 9 studies for RFS and 41 studies for OS. There were 47 studies carried out in Asia, 5 studies in Europe and 2 studies in America. The number of patients included in studies ranged from 20 to 290. The only technique to detect

Table 3. The association of Ki-67 LI with clinicopathological parameters of HCC patients

Parameters	Study (N)	High Ki-67 LI (N)	Total Patients (N)	Rate	χ^2	*P*
Histological grade	20					
I-II/H-M*		452	1124	40.2%	122.10	<0.001
III-IV/P*		504	762	66.1%		
Tumor size	21					
≤5		418	928	45.0%	18.20	<0.001
>5		429	774	55.4%		
Clinic TNM stage	7					
I-II		138	318	43.4%	3.57	0.059
III-IV		89	170	52.4%		
Tumor nodes	10					
single		314	745	42.1%	28.61	<0.001
multiple		185	307	60.3%		
Metastasis	17					
No		626	1298	48.2%	11.81	0.001
Yes		164	275	59.6%		
Cirrhosis	20					
No		273	565	48.3%	12.73	<0.001
Yes		664	1156	57.4%		
Vein invasion	13					
No		411	917	44.8%	67.33	<0.001
Yes		269	386	69.7%		

LI: labeling index; HCC: hepatocellular carcinoma; N: number; LI: labeling index; H-M*: High-Moderate; P*: Poor; TNM: Tumor Node Metastasis.

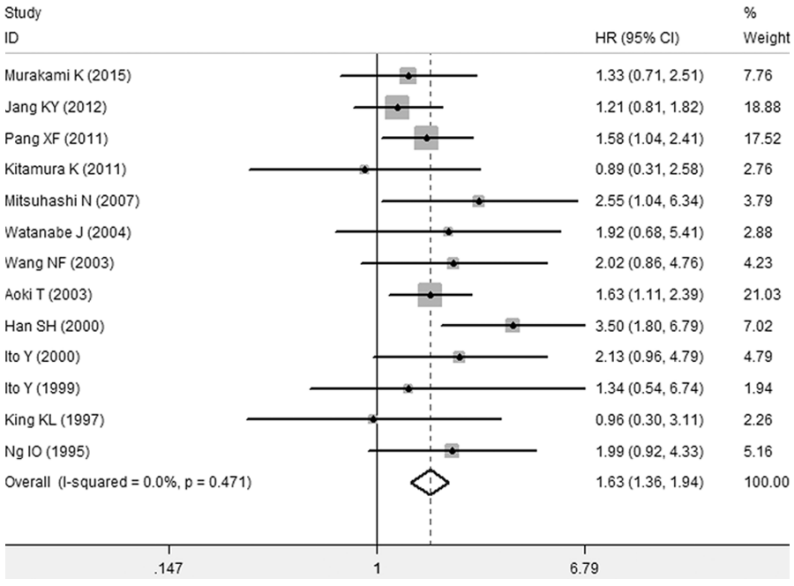

Figure 2. Forest plot of the pooled HR from the fix-effects model for DFS.

Ki-67 LI was immunohistochemistry (IHC), but different antibodies were used: anti-Ki-67 in 35 studies, MIB-1 in 14 studies, anti-Ki-S5 in one study and it was not reported in 4 studies. Meanwhile, different concentrations of antibodies were used in different studies. For the treatment of HCC, out of the 54 studies, hepatectomy was used in 45 studies, liver transplantation in 5 studies and CT-guided radiofrequency ablation (RFA) in one study. Considering the selected studies, HR value was extracted directly in 39 studies, while HRs of 14 studies were extracted from Kaplan-Meier survival curves and one was estimated by primary data with SPSS20.0. **Tables 1** and **2** outlined the main characteristics of the studies included in this meta-analysis.

Relationship between high Ki-67 LI and clinicopathological parameters in patients with HCC

To gain further information into the role of Ki-67 LI as a predictive marker for HCC progression, the relationship between high Ki-67 LI and clinicopathological parameters in patients with HCC was investigated by χ^2 test. As summarized in **Table 3**, we found a strong correlation of high Ki-67 LI with histological grade (χ^2=122.10, *P*<0.001), tumor size (χ^2=18.20, *P*<0.001), the number of tumor nodes (χ^2=28.61, *P*<0.001), metastasis (χ^2=11.81, *P*=0.001), cirrhosis (χ^2=12.73, *P*<0.001) and vein invasion (χ^2=67.33, *P*<0.001). High Ki-67 LI indicated the potential deterioration of HCC, as presented by poor histological differentiation, large tumor size, multiple tumor nodes, tumor metastasis, cirrhosis and vein invasion.

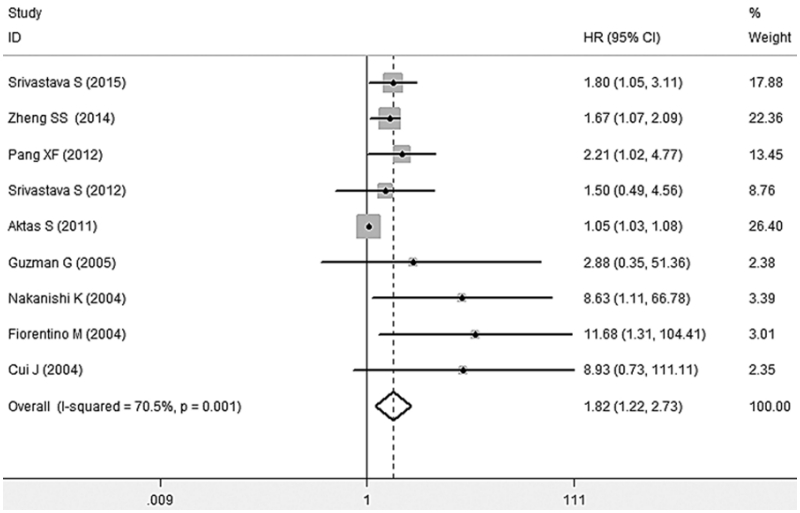

Figure 3. Forest plot of the pooled HR from the random-effects model for RFS.

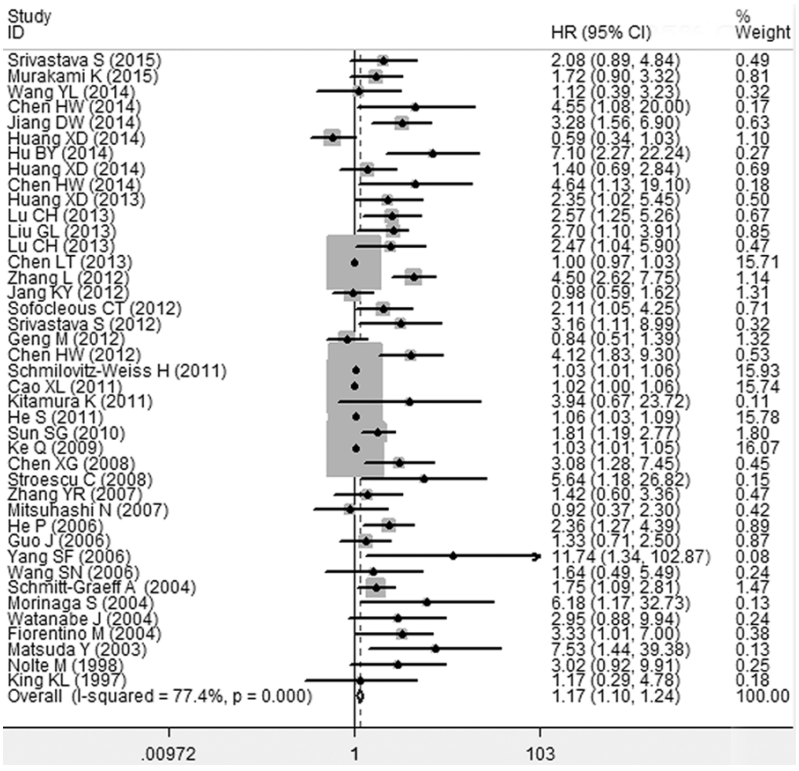

Figure 4. Forest plot of the pooled HR from the random-effects model for OS.

Meta-analysis

The main meta-analysis results of DFS, RFS and OS were showed in **Figures 2**-**4**, respectively. The pooled HR from the figures showed that worse DFS (HR=1.626, 95% CI: 1.364-1.939, *P*<0.001), RFS (HR=1.820, 95% CI: 1.215-2.725, *P*=0.004) and OS (HR=1.170, 95% CI: 1.102-1.243, *P*<0.001) were observed among HCC patients with high Ki-67 LI. Simultaneously, subgroup analysis was performed by region, treatment strategies and statistical method for RFS and OS, but not DFS because all the patients were Asian and treated with hepatectomy. All the analyzed data was summarized in **Table 4**. In Asian group with high Ki-67 LI, the pooled HR for RFS and OS were 1.667 (95% CI: 1.127-2.467, *P*=0.011) and 1.136 (95% CI: 1.072-1.203, *P*<0.001), respectively. Meanwhile, the pooled HR in non-Asian for RFS and OS was 6.351 (95% CI: 1.225-32.918, *P*<0.001) and 2.186 (95% CI: 1.558-3.069, *P*< 0.001), respectively. As for different treatment strategies, in the subgroup of hepatectomy, significant relationships were found between high Ki-67 LI and RFS (HR=1.819, 95% CI: 1.407-2.350, *P*<0.001) and OS (HR=1.212, 95% CI: 1.127-1.303, *P*<0.001). However, the result indicated that no statistical significance was found in the subgroup of liver transplantation for RFS (HR=2.413, 95% CI: 0.523-11.142, *P*=0.259) and OS (HR=1.998, 95% CI: 0.797-5.009, *P*=0.140). For the subgroup stratified by statistical methods, worse RFS (HR=1.807, 95% CI: 1.195-2.731, *P*=0.005) and OS (HR=1.147, 95% CI: 1.080-1.219, *P*<0.001) was found in the subgroup HR (M), but not in the subgroup of HR (U) for OS (HR=1.000, 95% CI: 0.970-1.030, *P*=0.996). In the subgroup providing survival curve, the pooled HR was 1.439 (95% CI: 1.107-1.869, *P*<0.001).

Test of heterogeneity

Significant heterogeneity was found in the relationship between high Ki-67 LI and RFS

Table 4. Meta-analysis: HR value for subgroups analysis in HCC patients

Group	Study (N)	Fix effect HR (95% CI)	*P*	Heterogeneity test		Random effect HR (95% CI)	*P*
				I^2	*P*		
Disease-free survival	13	1.626 (1.364-1.939)	<0.001	0%	0.471	-	-
Relapse-free survival	9	1.055 (1.031-1.080)	<0.001	70.5	0.001	1.820 (1.215-2.725)	0.004
Region							
Asian	7	1.055 (1.030-1.080)	<0.001	72.50%	0.001	1.667 (1.127-2.467)	0.011
Non-Asian	2	6.351 (1.225-32.918)	0.028	0%	0.408	-	-
Treatment							
Hepatectomy	6	1.819 (1.407-2.350)	<0.001	0%	0.497	-	-
LT	3	1.050 (1.026-1.076)	<0.001	62.10%	0.071	2.413 (0.523-11.142)	0.259
RFA	0	-	-	-	-	-	-
Method							
HR (M)	8	1.055 (1.031-1.080)	<0.001	73.60%	<0.001	1.807 (1.195-2.731)	0.005
HR (U)	0	-	-	-	-	-	-
Survival curve	1	-	-	-	-	-	-
Estimate	0	-	-	-	-	-	-
Overall survival	41	1.034 (1.022-1.045)	<0.001	77.40%	<0.001	1.17 (1.102-1.243)	<0.001
Region							
Asian	36	1.033 (1.021-1.045)	<0.001	77.40%	<0.001	1.136 (1.072-1.203)	<0.001
Non-Asian	5	2.18 6 (1.558-3.069)	<0.001	0%	0.512	-	-
Treatment							
Hepatectomy	37	1.034 (1.021-1.047)	<0.001	77.70%	<0.001	1.212 (1.127-1.303)	<0.001
LT	3	1.032 (1.007-1.057)	0.012	82.70%	0.003	1.998 (0.797-5.009)	0.140
RFA	1	-	-	-	-	-	-
Method							
HR (M)	30	1.038 (1.026-1.051)	<0.001	81.0%	<0.001	1.203 (1.120-1.291)	<0.001
HR (U)	2	1.000 (0.970-1.030)	0.996	0.0%	0.428	-	-
Survival curve	8	1.656 (1.220-2.249)	0.001	0.1%	0.262	-	-
Estimate	1	-	-	-	-	-	-

HR: hazard ratio; HCC: hepatocellular carcinoma; CI: confidence interval; LT: liver transplantation; RFA: radiofrequency ablation; HR (M): HR (multivariate analysis); HR (U): HR (univariate analysis).

(I^2=70.5, *P*=0.001) and OS (I^2=77.40%, *P*<0.001). In subgroup analysis, significant heterogeneity existed in the subgroup of Asian for RFS (I^2=72.50%, *P*=0.001) and HR for RFS (I^2=73.60%, *P*<0.001); Asian for OS (I^2=77.40%, *P*<0.001), hepatectomy for OS (I^2=77.70%, *P*<0.001), liver transplantation for OS (I^2=82.70%, *P*=0.003) and HR for OS (I^2=81.00%, *P*<0.001), while there was no significant heterogeneity in other subgroups. All the heterogeneity test results were summarized in **Table 4**.

Publication bias

Begg's test was used to assess the publication bias of meta-analysis. Thirteen studies evaluating DFS of patients with HCC yielded a *P*=0.855 with Begg's test, and the funnel plot was showed on **Figure 5**. For OS, the result of Begg's test indicated absence of publication bias (*P*=0.124) and the funnel plot was showed on **Figure 6**. We did not undertake publication bias for RFS because the number of study was less than ten. After assessing Begg's test, no significant publication bias was found in our meta-analysis.

Discussion

The proliferation status of tumor cells is an important parameter to reflect its biological characteristics, and it affects the prognosis and efficiency of treatment of tumor directly. As a biomarker with high sensitivity and specificity [77], Ki-67 is widely used as a proliferative and

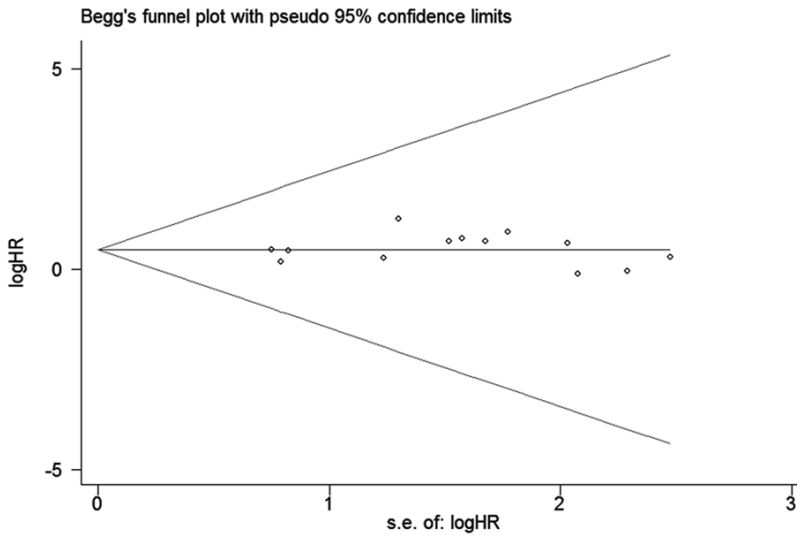

Figure 5. Funnel plot of eligible studies for DFS in HCC.

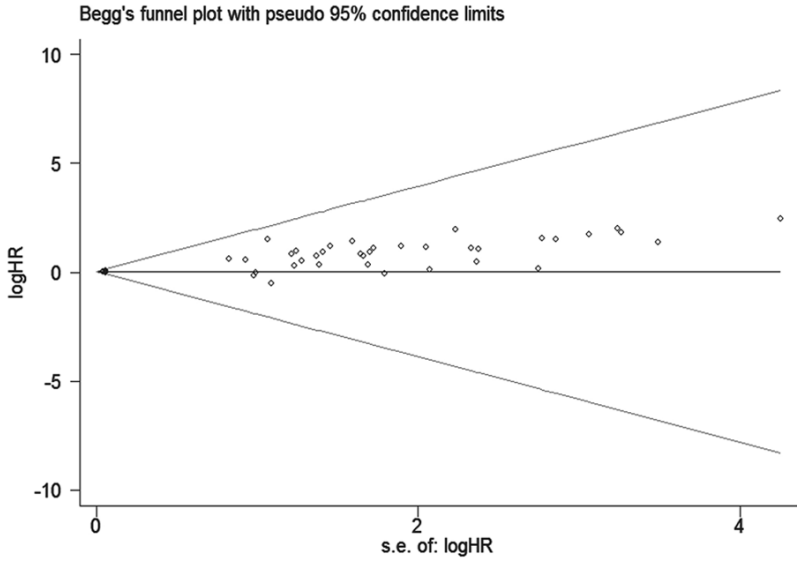

Figure 6. Funnel plot of eligible studies for OS in HCC.

prognostic factor in HCC. Currently, the relationships between Ki-67 LI and clinicopathological features, as well as prognostic significance in HCC, have been investigated, but results remained controversial [13-17]. Besides, the power of individual studies was limited due to small sample size, which may have generated a fluctuated estimation [78]. To date, no meta-analysis has been undertaken to evaluate Ki-67 as a predictive and prognostic marker for HCC. Therefore, it is required to perform a relative meta-analysis by combining these data to eventually reach an integrated conclusion.

In our meta-analysis, we enrolled 54 studies concerning high Ki-67 LI on HCC clinicopathological features and patient DFS, RFS and OS. The results showed that high Ki-67 LI was significantly associated with advanced stage of HCC, including poor differentiation, large tumor, and more tumor nodes, with metastasis, cirrhosis and vein invasion. In addition, the pooled HR of our meta-analysis, including 54 studies involving 4996 patients, indicated that Ki-67 is likely to be a poor prognostic factor in patients with HCC. In subgroup analysis, our meta-analysis indicated that high Ki-67 LI was related to poorer DFS, RFS and OS in all different regions, altered treatments and distinct statistical methods, except that no statistical significance was found on RFS and OS in patients with liver transplantation. Our meta-analysis identified a discovery that patients treated with liver transplantation would have an uncertain RFS and OS in HCC with high Ki-67 LI. However, small amount of studies with patients which were treated with liver transplantation were included. To further validate the result, more clinical studies are required.

However, several limitations existed in our meta-analysis. Firstly, significant heterogeneity was found among studies for RFS and OS, and heterogeneity is a potential factor to impact the results of meta-analyses [79]. Although we performed subgroup analysis according to region, treatment and statistical method, the cause of the heterogeneity from the study was still unclear. To deal with the potential heterogeneity issue, the random effect model was performed. Secondly, the accurate HR value should be estimated by multivariate analysis. In our meta-analysis, the HR value from only 36 stud-

ies was estimated by multivariate analysis. If the result of multivariate analysis was not reported, we accepted the result estimated by univariate analysis. Otherwise, we calculated it from survival curve or primary data. Therefore, the HR information calculated by statistical software unavoidably developed a decrease of reliability. Thirdly, there were different antibodies, concentration of antibodies and cut-offs to define the immunohistochemical positivity among distinct reports. Without a unified standard, there could be controversial result of positive expression. Publication bias on the meta-analysis should be considered though there was no significant publication bias between studies included. It is well known to all that positive results are more likely to be accepted by journals but not for negative results. If negative studies with a large number of patients have been missed, it may have minimized publication bias, which lacks of reliability. Furthermore, Chinese and English studies were included in our meta-analysis, which probably introduced publication bias.

Despite some limitations, the current meta-analysis demonstrates the intense association between high Ki-67 LI and tumor deterioration, also poor DFS, RFS and OS in patients with HCC. Our meta-analysis reveals that Ki-67 is a biomarker for clinical deterioration and poor prognosis in HCC. Hence, the detection of Ki-67 in clinic will be beneficial to the treatment and prognostic evaluation for HCC patients. However, to further validate the current result, more prospective clinical studies are required to investigate the prognostic value of Ki-67 LI in HCC.

Acknowledgements

The study was supported partly by the Fund of Guangxi Zhuang Autonomous Region University Student Innovative Plan (No. 201410598003), Guangxi Provincial Health Bureau Scientific Research Project (Z2014054), Youth Science Foundation of Guangxi Medical University (GXMUYSF201311), Guangxi University Science and Technology Research Projects (LX20-14075), and the Fund of National Natural Science Foundation of China (NSFC 81360327). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Disclosure of conflict of interest

None.

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