

Systematic Review

Expression of let-7 family as a survival biomarker for lung cancer: A meta-analysis

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Abstract: **Introduction:** Lung cancer is the most common cancer among men around the world. Today, by evaluating the expression of microRNA biomarkers, cancer cells can be detected in specific tissues. However, it's still controversy that the expression of let-7 in the prognosis of patients with lung cancer is informative.

Material and Methods: A meta-analysis was performed by searching Google Scholar, PubMed, Scopus, Web of Science, IranMedex, MEDLIB, IranDoc and Scientific Information Database (SID). All data were extracted from articles comparing prognosis in patients with lung cancer having low expression of let-7 with those having high expression. Pooled hazard ratios (HRs) and corresponding 95 % confidence intervals (CIs) were calculated. Subgroup analyses were conducted for ethnicity.

Results: A total of 1,370 cases of lung cancer were involved for this meta-analysis. The HR of low let-7 expression was 1.32 (95 % CI 0.68–2.58). A subgroup analysis was performed on ethnicity; combined HR was 1.56 (95 % CI 0.52–4.62) for Asians and 1.08 (95%CI 0.42–2.74) for non-Asians.

Conclusion: There was no significant relationship between the expression of let-7 and lung cancer, let-7 might be a biomarker in Asian patients with favourable prognosis. Furthermore, with large-scale investigations, useful prognostic microRNA biomarkers in the diagnosis, treatment and follow-up could be detected.

Keyword: Lung Cancer; Let-7 Family; Expression Analysis; Hazard Ratio

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1. Introduction

Despite progress in diagnosis and treatment, lung cancer (LC) is one of the leading causes of death in the world (1). Due to age, population growth and high risk behaviours such as smoking, the incidence of respiratory cancers in the worldwide is still high. Until the early 20th century, lung cancer was a rare disease, but its prevalence increased sharply, so today it is one of the most common cancers. After breast cancer in women and prostate in men, lung cancer is the second

common cancer with a ratio of 15% of all cancers and the first cause of cancer deaths in both genders (2, 3). In addition to genetic and environmental factors, epigenetic factors are also involved in the aetiology of cancer. Recently, it has been found that microRNAs are closely related to various diseases, including lung cancer. MicroRNAs are small non-coding RNAs that regulate the expression of the gene through the destruction of mRNA molecules or by preventing their translocation (4). The Let-7 family is a group of microRNAs that act as an oncogene and tumour suppressor genes. This family has 13 members including: Let-7a-1, Let-7a-2, Let-7a-3, Let-7b, Let-7c, Let-7d, Let-7e, Let-7f-1, Let-7f-2, Let-7g, Let-7i, mir-202, and mir-98. In most cancers, the expression of Let-7 is reduced, which results in its tumour-suppressive role

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(5, 6). Let-7, in many genes, is in the fragile region associated with cancer, and the report by Boyerinas et al. has revealed the first relationship between Let-7 and human cancers (7, 8). According to the previous literatures, it has been quite clear-cut that let-7 expression is frequently reduced in lung cancer and that changes in the miRNA expression may have a prognostic impact on the survival of surgically treated lung cancer patients (9).

In this meta-analysis, the relationship between expression of let-7 and LC has been evaluated, to ultimately determine its efficacy as an invasive biomarker in detecting LC.

2. Method

2.1. Search strategy and Selection criteria:

In an effort to retrieve the original Farsi and English language papers about the expression of let-7 family and lung cancer, a systematic search was performed on international and Iranian databases including Web of Science, Proquest, Google Scholar, PubMed, Scopus, IranMedex, MEDLIB, IranDoc and Scientific Information Database (sid). All relevant papers which contained the selected key terms (expression analysis, hazard ratio(HR) and lung cancer) and published until September 2018, were included. The reference lists of the extracted articles were also checked to find other helpful sources. The selection of papers occurred firstly through the analysis of titles and abstracts/summaries. The analysis of the manuscripts followed the predetermined eligibility criteria for the inclusion criteria: (1) papers that had in the title at least a mixture of the terms outlined in the search strategy; (2) articles written in English or Farsi;

(3) papers had been published and indexed in one of the above-mentioned databases.

The elements excluded: (1) non-original studies such as Letters to the Editor, Prefaces, brief communication, Corrections/Editorials, and Monographs (2) Papers repeated in more than one database were counted just once (3) studies based on non-human research (4) lack of data information such as HR and 95% CI.

2.2. Quality assessment and Data extraction:

This study has been performed as a meta-analysis in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement guidelines (www.prisma-statement.org). Two reviewers independently screened all potential titles, abstracts of the identified studies and if needed, full-texts for eligibility. Disagreement for inclusion was resolved by discussion and consultation with a third reviewer during study selection and data extraction. Then, each sample item was read in its entirety, and the information was recorded into a spreadsheet that included authors, year of publication, sample size, ethnicity, histotype, and statistical data. Some of the papers found were focusing the theme of Let-7 and/or LC correlated to other type of cancers, diseases or polymorphisms, considering that this article focuses expression analysis of Let-7 and LC, correlated data to other elements were not analysed. Reference lists of all included full-text manuscripts were screened for additional articles. Authors of papers were contacted to ask clarification where inadequate information was provided.

2.3. Statistical analysis:

Hazard ratio estimates LnHR and corresponding 95% CI for the association of LET-7 expression with overall sur-

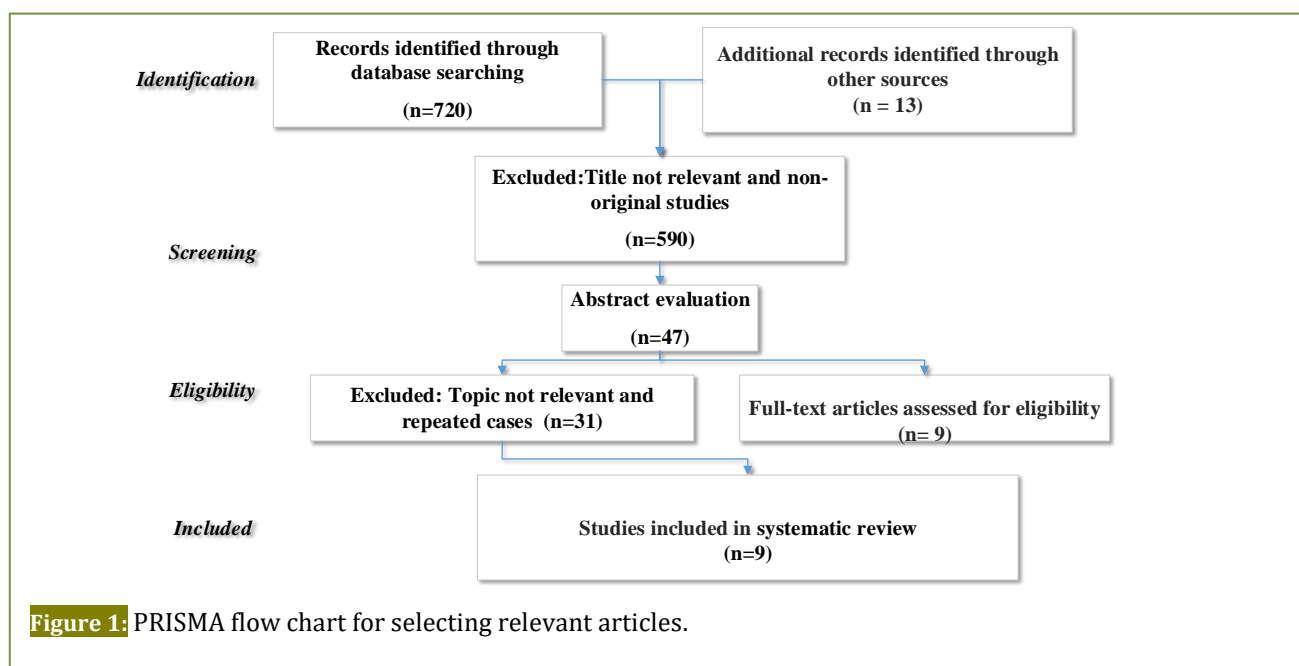


Table 1: Summary of included studies.

NO.	Author (Year)	Sample Size	Ethnicity	Follow-up years	Survival analysis	Histotype (AD/SC/other)	Stage	HR(95%CI)	P-value
1	Takamizawa (2004) (11)	143	Asian	>5 years	Multivariate Cox analysis	105AD, 25SCC, 13 other	75I-19II-49III	2.17 (1.21-3.89)	0.009
2	Yanaihara (2006) (12)	104	Mixed	>1years	Multivariate Cox analysis	65 AD - 39 SCC	65I-17II-22III,IV	3.55 (1.64-7.69)	0.001
3	Landi (2010) (13)	290	European	>5 years	Multivariate Cox analysis	165 AD - 125 SCC	---	2.22 (1.27-3.89)	NA
4	Jusufovic (2012) (14)	50	European	>1 years	Kaplan-Meier analyses	18 AD - 32 SCC	5II-26III-19IV	0.20 (0.09-0.43)	<0.0001
5	Voortman (2010) (15)	638	European, American	>3 years	Multivariate Cox analysis	218AD, 344SCC, 76 other	222I-149I-267III	0.84(0.68-1.04)	0.11
6	Yu (2008) (16)	112	Asian	2years	Multivariate Cox analysis	55AD, 50SCC, 7 other	47I-28II-37III	2.81 (1.13-7.01)	0.026
7	Xia (2010) (17)	31	Asian	>3years	Kaplan-Meier analyses	19 AD - 12 SCC	16 II -15 III	1.36(0.45-4.10)	NA
8	Zhu (2014) (18)	76	Asian	2 years	Kaplan-Meier analyses	34 AD - 36 SCC	36 I - 34 II,III,IV	0.058 (0.01~0.33)	0.001
9	Zhang (2012) (19)	105	Asian	2 years	Kaplan-Meier analyses	51 AD - 54 SCC	69 I,II-36 III, IV	1.04 (1.01-1.07)	0.004

AD, lung adenocarcinoma; SCC, squamous-cell lung carcinoma; HR, hazard ratio; NA, not available

* P<0.05, as compared to the control

vival of lung cancer. The random effects model was applied to ensure the selection of more conservative estimates. Moreover, hazard ratios (HRs) of individual studies were presented as forest plots. Chi-squared test, I² and Galbraith plot were used to assess statistical heterogeneity and heterogeneity plot. The p-values less than 0.05 suggested statistically significant heterogeneity in Chi-squared test. In order to measure publication bias, funnel plots of precision and standard error against log (HR) were developed. Begg's rank correlation and Egger's regression intercept were also carried out to evaluate publication bias. The STATA MP/14 was used in all analyses.

3. Result

Following the above-mentioned procedure (Figure 1), 720 papers were extracted at the initial phase of their work. Of these, 9 articles were finally retrieved and analysed (Table 1). A total of 1,370 cases of lung cancer were collected in this meta-analysis. The HR of low let-7 expression in lung cancer was (1.32,95 % CI 0.68-2.58), (Figure 2). Forest plot analysis indicated the heterogeneity of the let-7 expression in lung cancer (Figure 2). All nine studies reported data that allowed for the calculation of overall survival. The main information of these studies is shown in Table 1. Meanwhile, as shown in figure 3, there was enough evidence indicating the presence of publication bias; Begg's rank correlation analysis, $z = 0.00$; p -value >0.05 (Figure 3) and Egger's regression intercept (Figure 4). The method for investigating the expression of let-7 was quantitative real-time PCR. Empirically, HR of less than 1.5 is considered as a weak prognostic factor (10). The combined analysis of the nine studies showed that let-7 low expression was not associated with overall survival ($z=0.83$ $p = 0.40$), and the heterogeneity (I² statistic) was 88.6 % (Figure 2), chi-squared =70.16 (df. = 8) $p = 0.000$, and Galbraith plot showed a high heterogeneity(Figure 5). Then a subgroup analysis was performed on ethnicity; no association of let-7 expression and survival was evident (1.56,95 % CI 0.52-4.62); ($z=0.81$, $p = 0.42$) and (1.08,95 % CI 0.42-2.74); ($z=0.17$ $p = 0.86$) for Asians (Figure 6) and non-Asian (Figure 7) patients, respectively.

4. Discussion

More research is needed to clarify the regulatory mechanisms for the biogenesis of microRNAs and their role in cancer. Diagnosis of target microRNA and the study of their molecular interactions in signalling pathways will

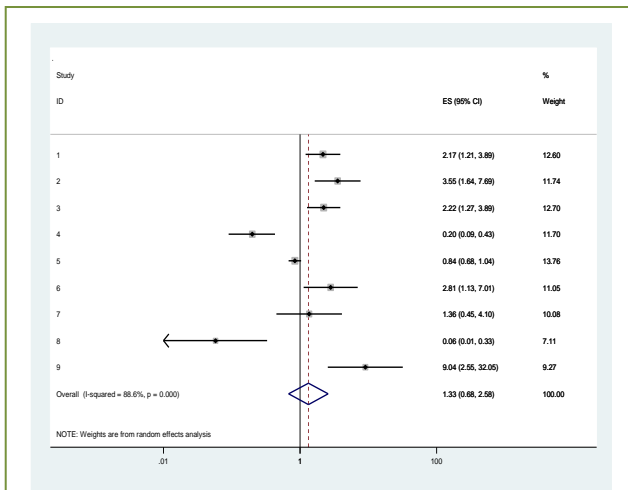


Figure 2: Forest plot of the relationship between expression of let-7 and lung cancer (overall survival) included in the meta-analysis.

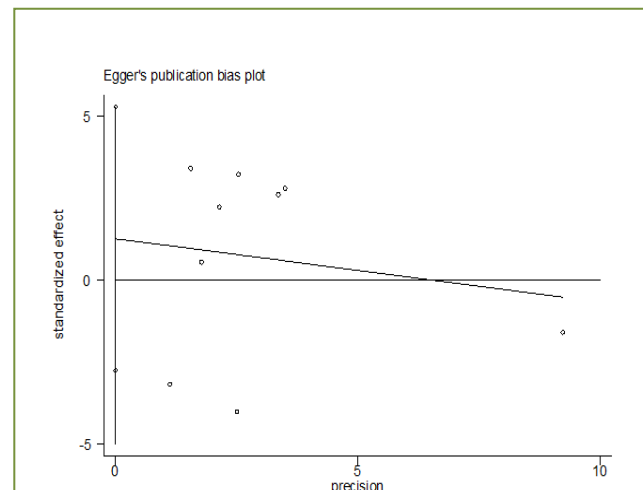


Figure 4: Egger's plot with 95%CI for publication bias testing.

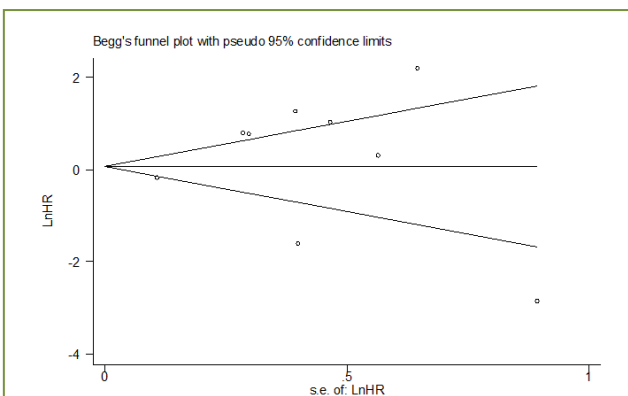


Figure 3: Begg's funnel plot with 95%CI for publication bias testing.

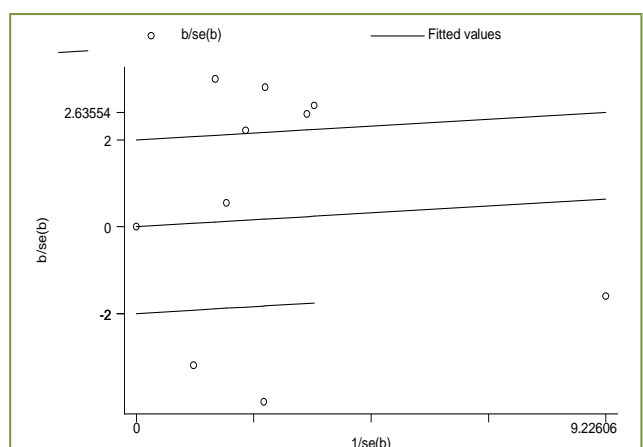


Figure 5: Galbraith plot for heterogeneity (galbr).

help to better understand the nature of cancer. Considering the fact that the most common methods for screening cancer in the early stages are not able to diagnose the disease, identification of tumour microRNAs that spread through blood during gradual progression of the disease is a key to early diagnosis of cancer. In addition, microRNAs are also used to treat cancer, and making effective changes to these molecules can affect their targets (4, 20). In a study by Lee et al, the effect of Let-7g on tumour inhibition has been proven. Its expression in lung cancer cells results in a decrease in the expression of HMGA2 oncogene and reduction of cell proliferation (21). Boyerinas et al. demonstrated the role of the Let-7 as an oncogene and by using the mouse lung cancer model, confirmed the tumour suppressor function of Let-7 with Ras gene inactivation. In humans, a significant reduction in the expression of the Let-7 family has been reported in many cancers such as; colon, stomach, and melanoma, in the lung cancer, the re-

duction of expression of Let-7g has been inversely related to survival. On the other hand, Let-7a injections into mice with lung cancer lead to a decrease in tumour growth, and so this gene can be considered as a therapeutic potential for the treatment of lung cancer (22, 23). In 2014, Xia et al investigate the low expression of Let-7 with various types of cancers and illustrated that low expression of let-7 was significantly associated with poor prognosis in tumours (6). In another study, Lamichhane et al performed a meta-analysis (n = 1,239) and reported a significant low expression of let-7 with poor prognosis and overall survival (1.94, 95% CI: 0.87-4.32) in NSCLC (9). At present, there is not enough data to confirm the informative biomarkers of microRNAs in lung cancer. As a result, despite the many advances in the genetic and epigenetic aspects of lung cancer, there are still no accurate biomarkers that can be used to prognosis and predict the results of chemotherapy and survival of patients (24, 25). In addition, there are several limitations for this meta-analysis: Non-English and

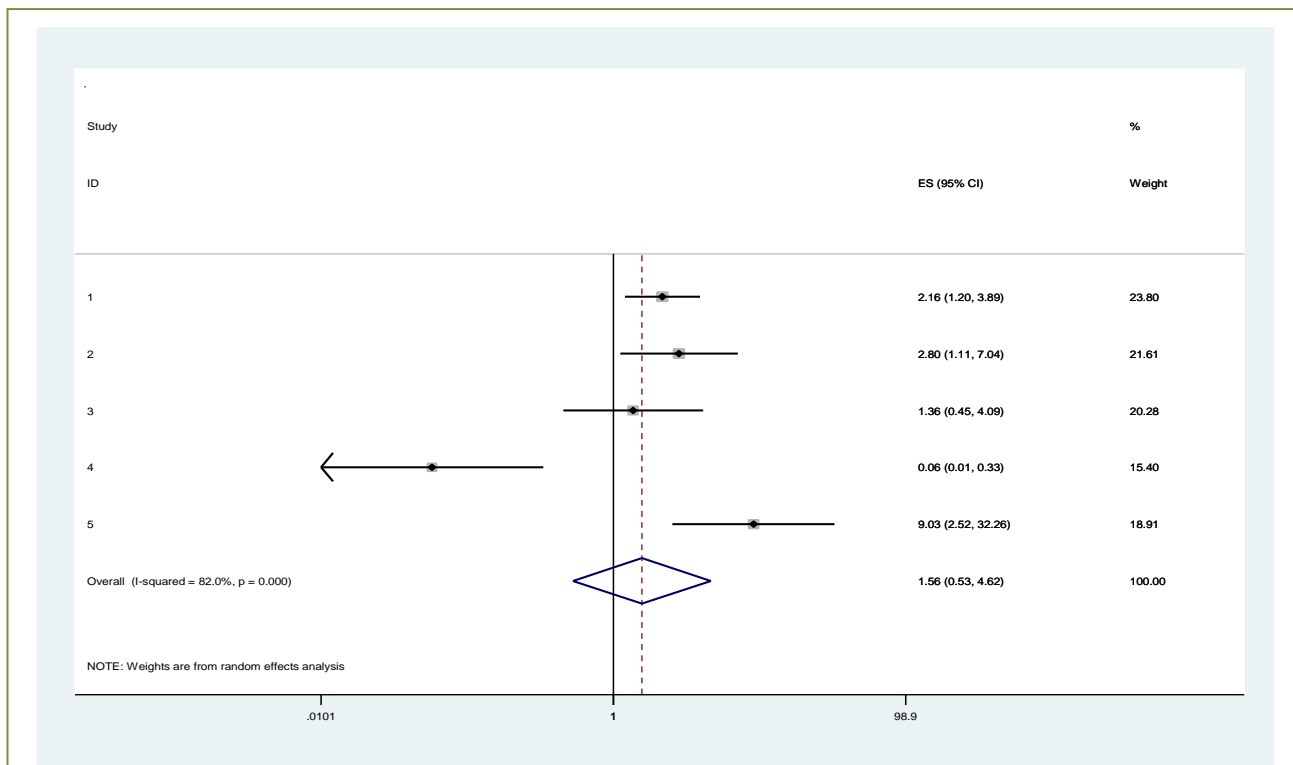


Figure 6: Forest plot of survival data from Asia.

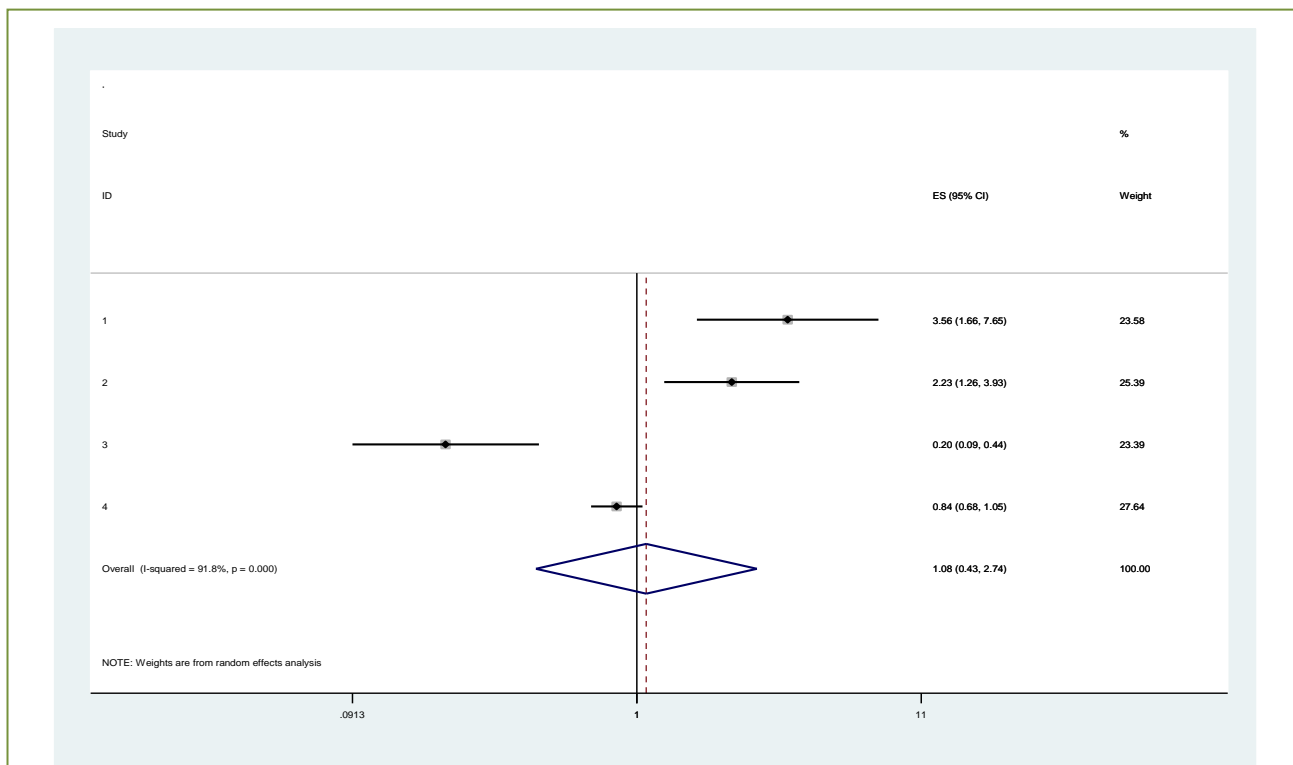


Figure 7: Forest plot of survival data from non-Asia.

Farsi articles and that did not have important survival information (eg, HR, CI or SE) were deleted and the number of studies available were limited. As previously mentioned, there are statistical heterogeneous articles for a variety of reasons, including differences in patient

clinic pathological characteristics, geographical distribution and different assay method. In summary, this article is not entirely complete due to heterogeneity, bias, and limitations, but the purpose of this article was to re-

view the LET-7 as a prognostic biomarker for lung cancer. Taken together, expression of let-7 was not significantly related to poor prognosis in patients with lung cancer.

5. Conclusion:

In general, microRNAs can be an important tool in early diagnosis and prognosis. It is also possible to use these molecules to design and treat the cancers. Considering that in our study there was no meaningful relationship between the expression of let-7 and lung cancer, it is hoped that in the future, with large-scale and standard studies and more sample size, an informative biomarker designed to help determine the type of treatment and the prognosis of cancer.

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7. Conflict of interest:

The authors declare that there is no conflict of interests associated with the manuscript.

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9. Author's contributions:

Reza Hashemi; Supervision, Faezeh Azizi & Fereshteh Aliakbari & Reza Hosseini; Data gathering, Faezeh Azizi & Zahra Zolghadr; Statical analysis, Faezeh Azizi; Manuscript writing.

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