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The effect of micro RNA-21 and interleukin-6 on development of heart failure: A meta-analysis of the literature

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ABSTRACT

Objective **We determined the overall effects of microRNA-21 and Interleukin-6 on development of heart failure by aggregating literature and subjecting studies meeting the inclusion and exclusion criteria to statistical meta-analysis.**

Background **MicroRNA's are short regulatory RNA's that act as negative regulators of gene expression by inhibiting mRNA translation or by promoting mRNA degradation. Experimental evidence is rapidly accumulating, which demonstrates that microRNA's constitute a new mode of governing pathophysiological mechanisms in cardiac hypertrophy and heart failure.**
Inflammatory activation with increased serum cytokine levels has been described as an important factor in the progression of the syndrome of chronic heart failure. In multifactorial analysis, elevated levels of interleukin-6 have been identified as the prognostic heart failure marker.

Materials and Methods **Original research papers were gathered from Tongji medical college library resources and online data bases. The papers were screened and coded; those meeting the inclusion and exclusion criterion were subjected to meta-analysis, using Cohen's d test to determine the effect size of each study.**

Results **In microRNA-21 study six studies with 6 effect sizes met the stringent inclusion and exclusion criteria. The overall mean effect of microRNA-21 from animal experiment on development of heart failure was highly significant (d=+1.36). The fail safe number associated with overall effect of microRNA-21 on development of heart failure in animals was 76, a high number representing the number of additional studies in which microRNA-21 has negative or no effect on development of Heart failure required to negate the overall large effect size of +1.36.**

In interleukin-6 study seven studies with 7 effect sizes met the inclusion and exclusion criteria. The mean effect of interleukin-6 on development of heart failure from human studies was highly significant (d=+1.41) as well as in animal experiments (d=+1.40). The fail safe number associated with overall effect of interleukin-6 on development of heart failure in human was 65 while in animal 26, a high number representing the number of additional studies required to negate the overall effect size of +1.4.

Conclusion **We conclude that both microRNA-21 and interleukin-6 has positive effect on the development of heart failure.**

INTRODUCTION

Definition: Heart failure is a complex syndrome, resulting from structural or functional cardiac disorder that impairs the ability of the cardiac pump to support a physiological circulation [29].

Heart failure is a worldwide problem; and has become an increasingly frequent reason for hospital admissions during the last 2 decades and clearly represents a major health problem. The most common conditions predating its onset include Hypertension and coronary heart disease, diabetes mellitus is also associated with increased risk of heart failure.

Heart failure is the fastest growing clinical cardiac disease entity in the United States, affecting 2% of the population. Furthermore underdeveloped countries urbanize and become more affluent, the rate of heart failure increase in concordance with the rates of diabetes mellitus and hypertension, a more processed diet and a more sedentarily lifestyle[31]. This was illustrated in a population in Soweto, South Africa, as the community transformed into a more urban and westernized city, an increase in diabetes mellitus and hypertension was met with an increase in rate of heart failure.

In China according to the report released by the China National Center for Cardiovascular Disease, based on the survey in 2002, there are 160 million patients with dyslipidemia, 20 million patients with diabetes mellitus, 200 million overweight and 60 million obese patients, and 350 million smokers in mainland China. The prevalence of heart failure in China is 0.9% of population (5.8million people). In which Male is 0.7%, and in female is 1%. And affects people of the age >65years (6-10%). Affecting urban (1.4%) more than rural (0.5%), moreover it affects the north (1.1%) more than the south (0.8%). Mortality from heart failure has increased six times for the last 40 years [30].

Diseases of the cardiovascular system represent the primary cause of human morbidity and mortality underscoring the need for innovative new therapies and diagnostics for heart disease [18].

Cardiovascular diseases are currently the leading cause of death and permanent disability in the developed countries and looks to set to become the leading cause of death worldwide by 2020. In 2000, cardiovascular diseases were responsible for 16.7 million deaths representing 30.9% of all causes of death. More than 40% of all causes in developed countries and more than 25% in developing countries[30].

Thus it is justifiable to study more on heart failure particularly on expression micro RNA's which are contributing factors in its cause and interleukin-6 which may plays a great role as a prognostic indicator, furthermore the research results will facilitate to emphasize the existing knowledge regarding expression of Micro RNA-21 and interleukin-6 in relation to heart failure.

MATERIALS AND METHODS

Subjects and design

Original research papers, investigating the effects of microRNA-21 and interleukin-6 on development of heart failure, were gathered and used for this study. The papers were sought and obtained from Tongji medical college library sources and online data bases, including Nature, American heart association, and European society of cardiology, American college of physicians and American college of cardiology and Pubmed. Search terms used include *microRNA-21, effect of microRNA-21 on heart failure, microRNA-21 and myocardial hypertrophy, micro-RNA-21 and myocardial fibrosis, interleukin-6, effect of interleukin-6 on heart failure and interleukin-6 and myocardial hypertrophy*. The additional secondary sources of information include articles from aforementioned sources, internet web pages, and pertinent papers published in journeys that were not found from any of the above data bases.

Inclusion criteria:

- Animal experiments and Human clinical studies
- Journal published between January 1997 to January 2012
- Title: microRNA-21 and interleukin-6 in relation with heart failure

Exclusion criteria:

- The article that did not present new data or only presented qualitative information
- If sufficient information to compute effect sizes could not be obtained from the article
- Journals published before January 1997 and beyond January 2012
- microRNA's other than microRNA-21 and Cytokines other than interleukin-6

Data Extraction

Data were extracted for study title and year, human clinical studies and animal experiments. Data were extracted by one of the authors and verified by another author. Table 1 and 2 shows the studies used in the present meta-analysis. The overall result of each study was transformed into a standardized effect size statistic using Cohen's'd' formula.

Data analysis

Calculation of Cohen's d: effect sizes were calculated using the formulae for determining Cohen's'd'. According to wolf, Cohen's'd' may be defined as the standardized difference between the means of the experimental group and the comparison group. This definition and Cohen's classification of effect sizes were applied to each study included in our meta-analysis. According to Cohen, the values of 0.2, 0.5 and 0.8 indicate a small, medium and large average effect, respectively.

Conceptually Cohen's statistic may be expressed as follows:

$$d = \frac{X_1 - X_2}{SD_{comparison}}$$

Where;

d = is the effect size

X_1= is the mean of microRNA-21 or interleukin-6

X_2= is the mean of the comparison group

SD= is the standard deviation of the comparison group

The effect size (d) was assigned a positive or negative value depending on the outcome of the study. For example, positive values were assigned to studies whose results were positive, that is, indicated that microRNA-21 or interleukin-6 is up regulated in heart failure. Negative values were assigned to studies that showed negative effect on development of heart failure that means microRNA-21 or interleukin-6 down regulated or no change in levels in heart failure. After calculating the effect size of each study independently, the mean overall effect size was calculated by summing all the effect sizes and dividing by the total number of effect using the following formula:

$$d_{average} = \frac{\sum d}{N}$$

Where;

$d_{average}$ = is mean effect size

$\sum d$ = is the sum of the effect sizes, and

N= is total number of effect sizes calculated and used.

Calculation of failsafe number

Given the likelihood that we did not obtain every study that ever examined the effects of microRNA-21 and interleukin-6 on development of heart failure, a failsafe number (N_{fs}) was calculated. The failsafe number reveals the number of additional studies with effect sizes below a set criterion value that would have to be included in the meta-analysis in order to change the outcome of the study.

We used 0.10 as the criterion, a number that is less than the small effect size of 0.2 suggested by Cohen. The following formula was used to calculate the failsafe number:

$$N_{fs} = \frac{N(\bar{d} - d_c)}{d_c}$$

Where:

N_{fs} = is the failsafe number

N= is the number of studies in the meta-analysis

$\bar{d}$ = is the average effect size of all studies

d_c = is the criterion value

3.2 STATISTICAL ANALYSIS

Statistical analysis was performed by using **Meta-analyst software beta and SAS software**. All the data was presented as mean ±SD and all P value was sided with significant level of α=0.05. And the significance difference of micro RNA or interleukin-6

levels between case and control subjects was analyzed accordingly.

3.3 ETHICAL CONSIDERATION

Ethical principles were followed; in this research were the Tongji medical college of HUST Ethical review committee approved this research.

RESULTS

Our literature search revealed several studies that examined the effects of microRNA-21 and interleukin-6, 26 studies for microRNA-21 and 22 for interleukin-6, but the final set of papers from which the effect sizes could be calculated was just 6 and 7 for microRNA-21 and interleukin-6 respectively.

Insufficient data with which to calculate effect size and/or inadequate reporting of microRNA-21/interleukin-6 were the major reasons that so many studies were not included in the meta-analysis. In addition, there were several studies in which data were summarized in the form of illustrations and graphs from which it was not possible to extrapolate the data needed to compute Cohen's d. The overall mean effect size (Cohen's d and hedge's g) plus the p-values and I^2 from the studies were as follows:

Table 1

Study	Cohen's d	Hedge's g	p-value	I^2
MicroRNA-21	1.36	1.09	0.008	68.23
Interleukin-6-animal	1.40	15	0.004	82
Interleukin-6-human	1.41	2.33	0.000	96

Indicating that both MicroRNA-21 and interleukin-6 have positive effects on the development of heart failure, I used hedge's g for more clarification of the effect measure because it has additional advantage of providing accurate estimate and stable measure with no bias. An initial test of homogeneity indicated heterogeneity across the studies with the p-values lower than 0.05 and high I^2 as indicated in the table above in Table 1, with medium heterogeneity in microRNA-21 (68.23) and high heterogeneity in both interleukin-6 studies 82 and 96 in animal and human studies respectively. Furthermore the fail safe number was calculated for both studies, and it was 76 for MicroRNA-21, meaning that 76 additional studies in which microRNA-21 had little or no effect on development of heart failure would be needed to negate the large positive effect of microRNA-21 of this meta-analysis. In interleukin-6, the fail safe number was 26 in animal experiment and 65 in human studies meaning that 26 and 65 additional studies in animal and human studies respectively in which interleukin-6 had little or no effect on development of heart failure, would be needed to negate the large positive effect of interleukin-6. The statistical significance test was done with p-values in both studies being below 0.05, P-value of 0.008 for microRNA-21 study, P-values of 0.004 and 0.000 in interleukin-6 animal and human studies as shown in Table 1, indicating a statistical significance in this meta-analysis.

Despite the above fact all the studies favors the test, indicating the distribution of their confidence interval on the right side above zero. Moreover the 95% confidence intervals in the microRNA-21 study were distributed as follows; 4 studies had medium effect at a certain confidence interval 0.5 and large effect at 1.0 confidence interval in 1 study, furthermore the 95% confidence interval for the overall microRNA-21 meta-analysis is between 0.52-0.56, and at 0.54 point estimate there is no difference with no effect as shown in figure 1, in addition to the above fact the 95% confidence intervals in the interleukin-6 human studies lie between 0.5-0.6 confidence interval, and the overall interleukin-6 human study meta-analysis is between 0.53-0.57, and at 0.55 point estimate there is no difference with no effect as shown in figure 2, furthermore the confidence interval in interleukin 6 animal study lie between 0.51-0.56, and at 0.52 point estimate there is no difference with no effect. In both

studies MicroRNA-21 and interleukin-6 animal experiments there was symmetry of the effects in the studies indicating no bias, as shown in the figures 5 and 6, despite the above fact there was asymmetry in the effect of interleukin-6 human studies indicating publication bias, although the fail safe number was 65 studies.

In summary the results indicate that both microRNA-21 and interleukin-6 have positive effect on the development of heart failure proving my hypothesis.

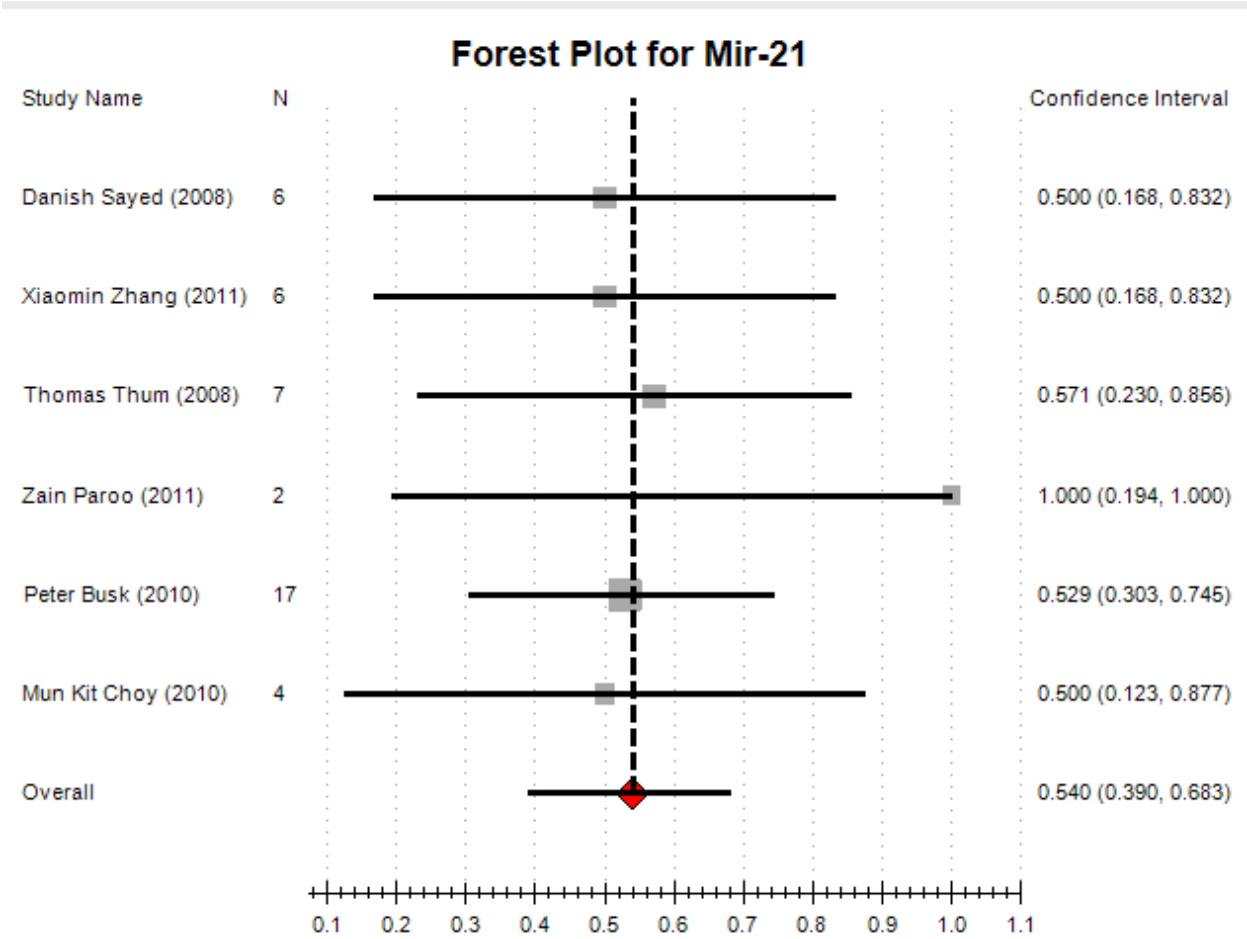

Figure 1 Showing how the 95% confidence intervals of microRNA-21 study are distributed differently within the forest plot. Indicating how the study is significant as there all above zero

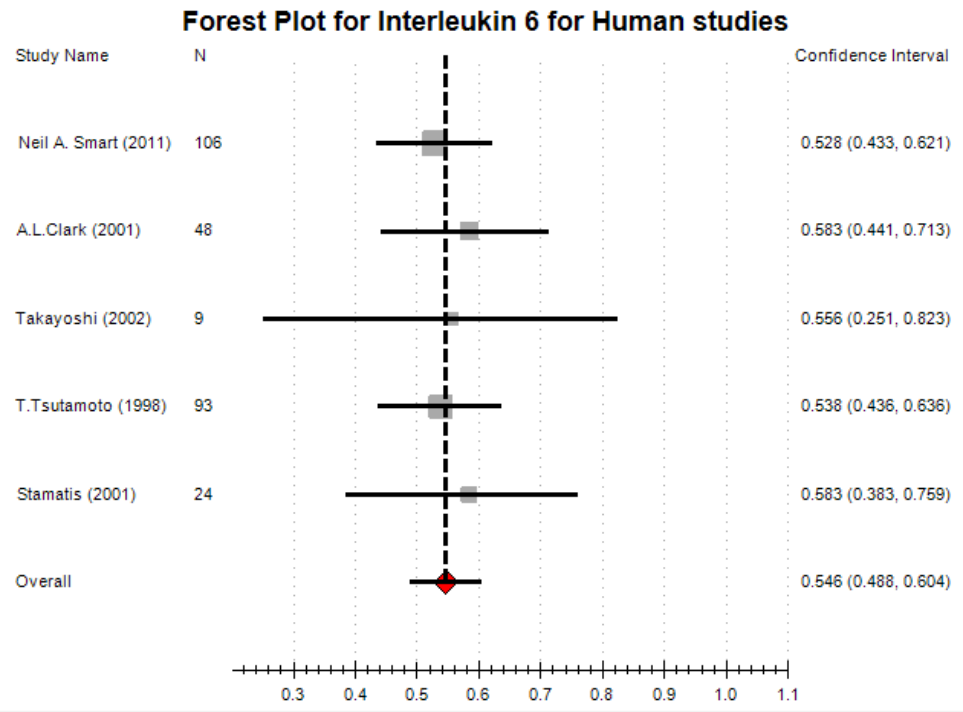

Figure 2 Showing how the 95% confidence intervals of interleukin-6 human study are distributed differently within the forest plot. Indicating how the study is significant as there all above zero

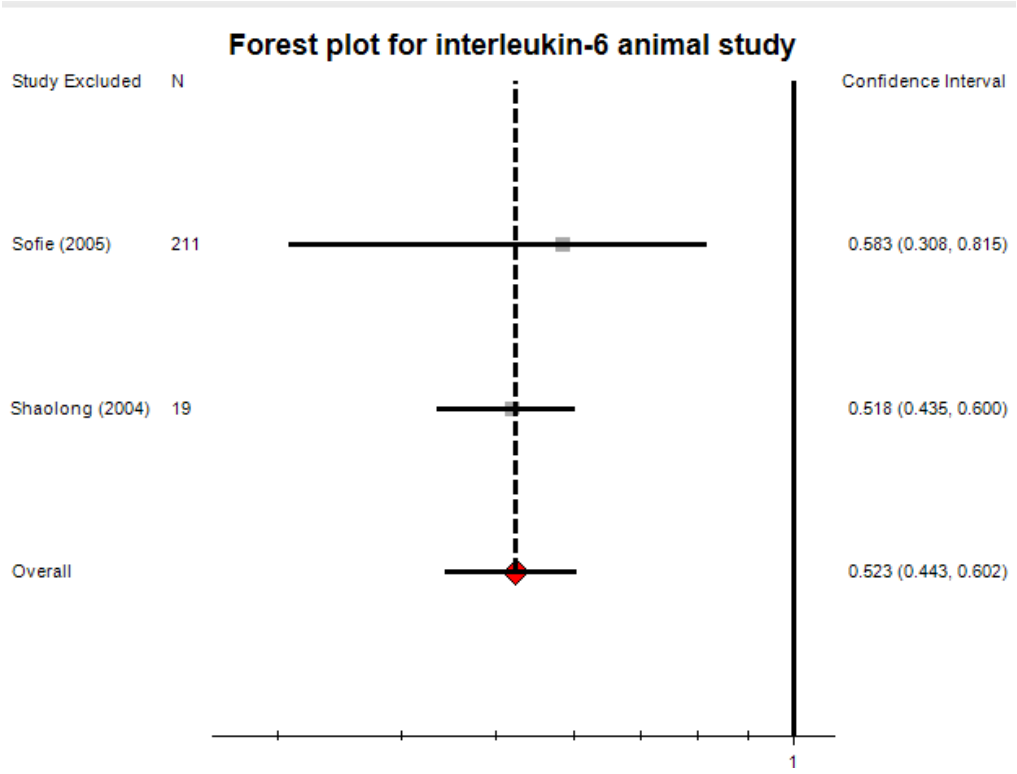

Figure 3 Showing how the 95% confidence intervals of interleukin-6 human study are distributed differently within the forest plot. Indicating how the study is significant as there all above zero

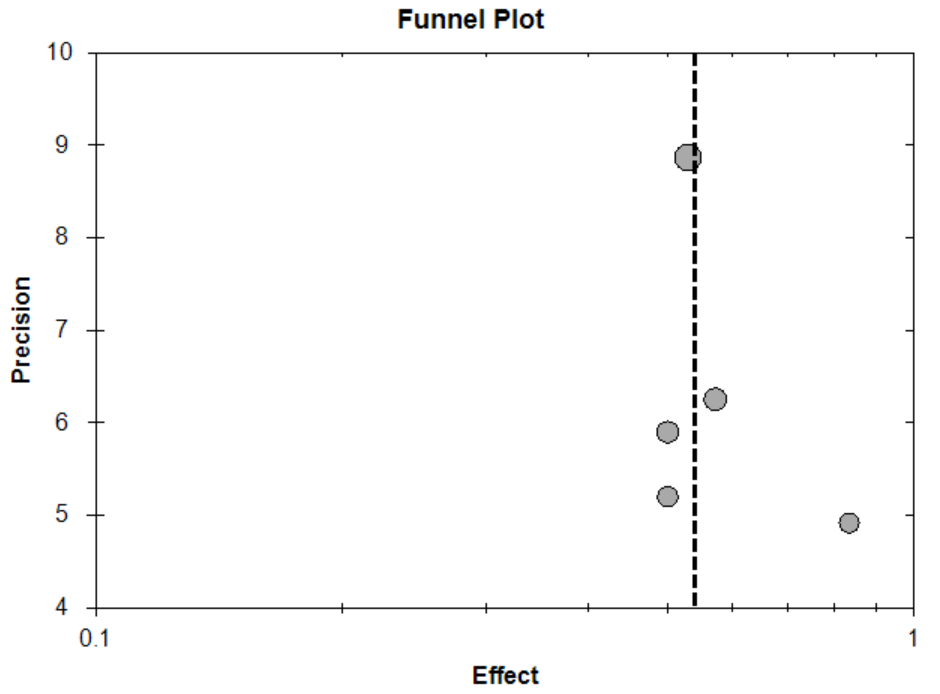

Figure 4 A funnel plot of studies with the positive effect of microRNA-21 symmetrical, suggesting the absence of bias

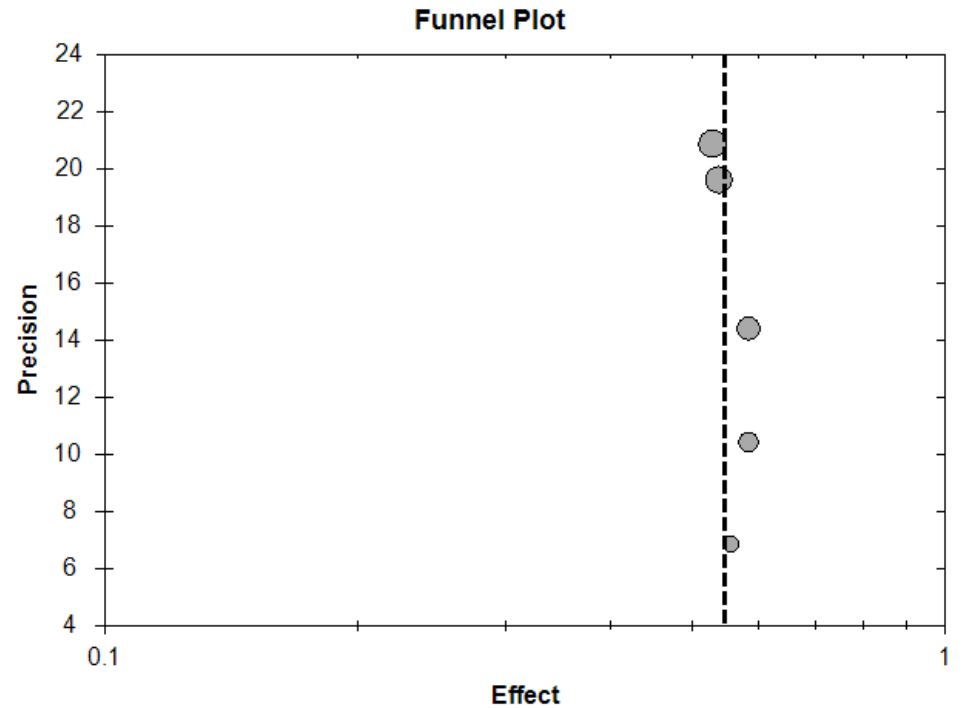

Figure 5 A funnel plot of interleukin-6 human studies with the positive effects asymmetrical, suggesting publication bias

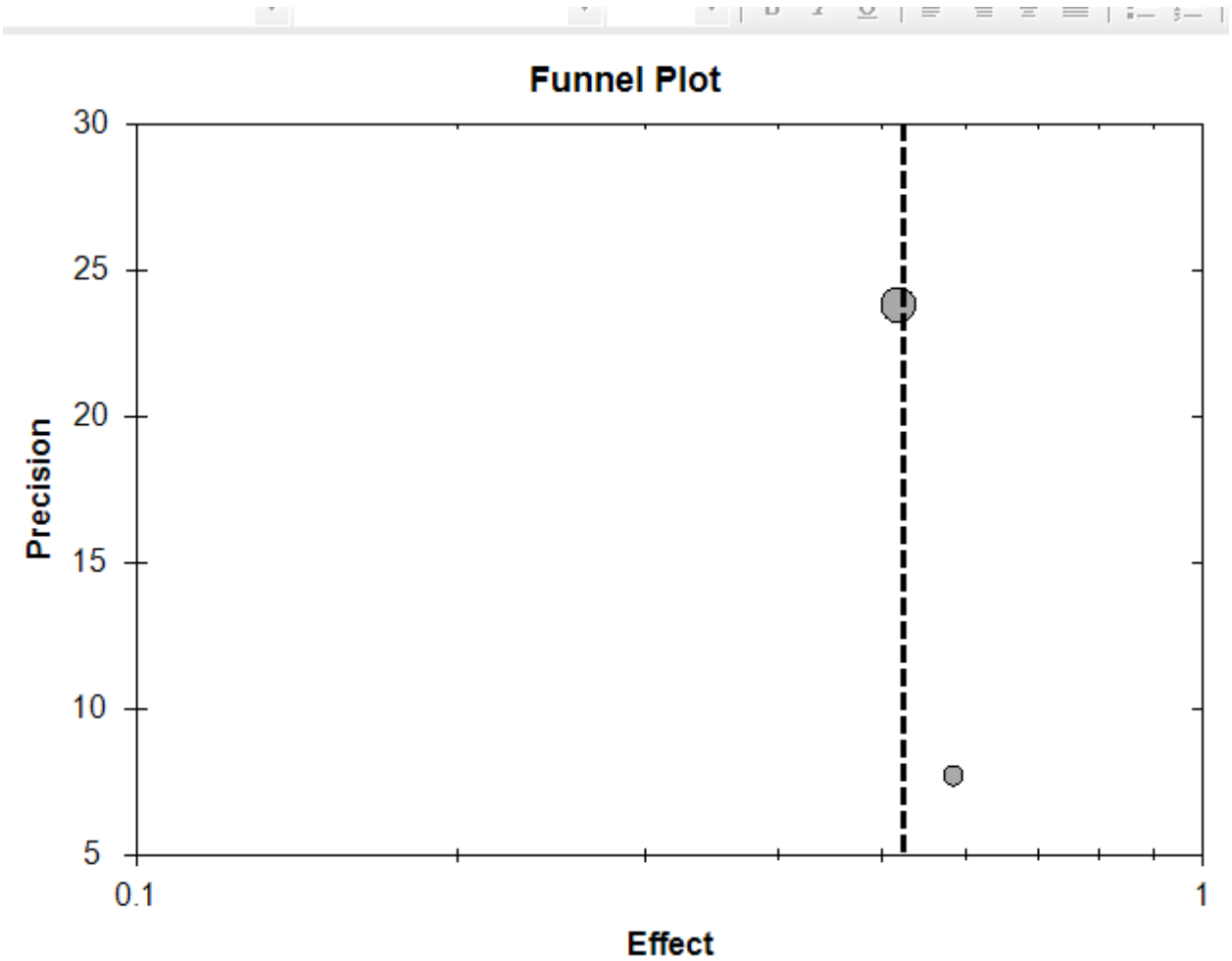

Figure 6 A funnel plot of interleukin-6 animal studies with the positive effects symmetrical, suggesting absence of bias

DISCUSSION

Overall, our findings show that both microRNA-21 and interleukin-6 have a positive effect in development of heart failure. This finding is consistent with several reports that indicate that both microRNA-21 and interleukin-6 contribute to the development of heart failure by fueling myocardial fibrosis, a major change occurring during heart failure.

In this systematic review of the effect of both MicroRNA-21 and interleukin-6 in development of heart failure had an excellent effect sizes, (Cohen's d and hedges g) with Cohen's d of 1.36 and hedge's g of 1.09 in microRNA-21 studies, and Cohen's d of 1.40, 1.41 and hedge's g of 15, 2.33 in interleukin-6 animal and human studies respectively. Our findings suggest that both microRNA-21 and interleukin-6 results can be conclusively to have positive effects in development of heart failure with their large effect sizes.

In microRNA-21 only 6 animal experiments were included in this meta-analysis because there were no quantitative data and details to extract in human studies thus microRNA-21 human studies were not included in this meta-analysis. In interleukin-6 there were 5-human studies and 2 animal studies included in this meta-analysis.

Our review has limitations we only included English language studies. The funnel plot for the interleukin-6 human study was asymmetrical which suggest publication bias may be present. Nevertheless,

because the fail-safe number was 65 studies we feel confident about our findings because it highly unlikely that there are 65 additional unpublished studies with no significant findings (no effect).

Perhaps the most important bias of meta-analysis is the expectancy effect, Cotton and Cook (1982) recommended early on that the investigators of meta-analysis explicitly state their personal view with regards to the outcome in order to acknowledge and possibly avoid expectancy effect. At the outset of our review, we were rather critical toward the effect of both microRNA-21 and interleukin-6 in development of heart failure. We expressed our personal view and we were fully prepared to report non-significant or only small effect of both microRNA-21 and interleukin-6. We were surprised to find these effects to be rather robust and strong, therefore believe that the expectancy bias was unlikely to be significant contributor to the results, which generally support the effects of MicroRNA-21 and Interleukin-6 in development of heart failure.

In conclusion, currently available literature demonstrates that both microRNA-21 and interleukin-6 have positive effect in development of heart failure. And therefore this might be a potential breakthrough in search for the treatment of heart failure, furthermore even in determination of patients prognosis. I believe there still some more researches needed to be done in this field particularly in trying to target or block MicroRNA-21 and/or Interleukin-6 in heart failure patients, more emphasis should be performed in microRNA-21 human studies in the future.

Current literature still reveals the importance of microRNA-21 in heart failure, in a study of the role for microRNA-21 in atrial profibrillatory fibrotic remodeling associated with experimental post-infarction heart failure by Cardin S, Guasch E, Luo X, et al. published in Pub-med in October 2012 where they revealed myocardial infarction induced heart failure leads to atrial remodeling in rats, and the knockdown of atrial microRNA-21 suppresses atrial fibrosis implicating microRNA-21 as a potential target for molecular intervention designed to prevent Atrial fibrosis., despite the above fact there was another study by Qin Y, Yu Y, et al. which revealed over expression of microRNA-21 following cardiac ischemia-reperfusion injury decreased infarcted area and reduced cell apoptosis in border area, which improved heart function, hemodynamic status and inhibited left ventricular remodeling in rats published in 2012 in international medical science journal this one favoring the over-expression of microRNA-21 in ischemia reperfusion injury.

In summary the results of this statistical meta-analysis mandate the following conclusions:

- microRNA-21 has a positive effect in development of heart failure
- interleukin-6 has a positive effect in development of heart failure

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