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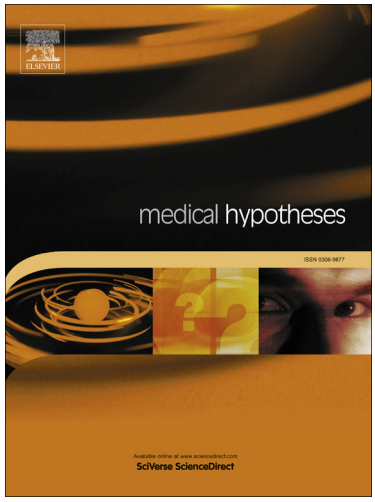

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Role of Leptin as a Biomarker for Early Detection of Renal Cell Carcinoma? No Evidence from a Systematic Review and Meta-analysis

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Abstract

Renal cell carcinoma (RCC) is the commonest from of renal neoplasm. Although surgery is a successful curative treatment for localized RCC, most patients are diagnosed with advanced or metastatic RCC, which has poor prognosis. RCC is classified by stage and grade using tissue samples. Whilst these provide good prognostic information, they are not very useful for early detection. Proteins that are dysregulated in patient's serum can be a valuable alternative and less invasive biomarker for early detection of the disease. For this reason, a hypothesis was formed that leptin is a possible biomarker for early detection and prognostication of RCC. The literature has disparate results on the usefulness of leptin as a biomarker for the early detection of RCC. Hence, a systematic review and a meta-analysis was carried out to investigate whether serum leptin could be a reliable diagnostic and prognostic factor in RCC patients. Literature on the available cohort and case-control studies on serum leptin in RCC was searched in electronic databases and included to evaluate this adipokine in the progression of RCC. The relevant studies were evaluated for the diagnostic and prognostic value of leptin in RCC patients. Overall, only 6 original research studies matched selection criteria and were included for meta-analysis. This study was hypothesised that; leptin might be a useful biomarker for early detection and prognostication of RCC. However, the data were presented in this study did not support our hypothesis. Serum leptin levels in RCC patients do not strongly associate with the development or progression of RCC, thus cannot act as a biomarker for early detection in RCC in patients. Extending our hypothesis further to include levels of obesity and RCC development may be worthwhile, but studies are currently limited.

Keywords: Body mass index; Diagnostic factor; Kidney cancer; Obesity; Prognostic factor

1.0 BACKGROUND

Renal cell carcinoma (RCC) is the most lethal of all the common urologic cancers [1]. Numerous factors are thought to contribute to the growth and development of RCC such as cigarette smoking, consumption of alcohol, obesity, hypertension, reproductive and hormonal factors, lack of physical activity, diet, occupation, the environment, as well as genetics and pre-existing comorbidities [2]. There is a growing concern for the role of obesity in cancer development, with several studies finding that obesity increases the risk of malignancies, including RCC [3, 4].

Leptin (often abbreviated as Ob) is a 16kDa polypeptide. It is one of the adipokines that is secreted by adipocytes and is found at increased levels in the serum of obese people [5, 6]. The hormone leptin was initially discovered in 1994 through its association with the homeostatic regulation of body weight [7]. The primary function of leptin is to maintain the food intake and energy expenditure in the human body, thus leptin is thought of as a satiety hormone. The high levels of circulating leptin in obese people reflect higher amounts of adipose tissue in the body, with a deficiency in, or resistance to, leptin perhaps contributing to severe obesity [8]. Studies have shown that leptin also activates other molecular pathways such as Janus kinase2-signal transducer and activator of transcription 3 (JAK2-STAT3), phosphoinositol-3 kinase (PI3K) and extracellular signal regulating kinase (ERK)1/2 which may promote cancer cell survival, proliferation and migration [9-11].

In the past years, the relationship between obesity and RCC has been studied, in particular to correlate any mechanism that underlies a relationship between leptin and RCC development and progression. To date, most studies have not identified the mechanism by which leptin may contribute to the development of RCC [12, 13]. Moreover, some studies have found that leptin levels are inversely proportional to development and progression of RCC, [14] whereas other studies report a positive association linking leptin and the

development and progression RCC [15]. A hypothesis was formed based on the previous literature. In this paper, we hypothesised that leptin may be responsible for the progression of RCC and can be an early diagnostic and prognostic marker for RCC. Therefore, to support the mentioned hypothesis, a systematic review and a meta-analysis were carried out to analyze any association between leptin and the development and prognosis of RCC.

2.0 EVALUATING THE HYPOTHESIS BY USING SYSTEMATIC REVIEW AND META-ANALYSIS

The Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) flow diagram was used for selection of studies [16]. The Population, Intervention, Comparison and Outcome (PICO) method was used before initiating an electronic search [17].

2.1 Search strategy and study selection

Electronic databases used to identify relevant studies were PubMed, CINAHL, Web of Science, Science Direct and Google Scholar from the year 2000-2017. A comprehensive search strategy was developed using the following keywords: “leptin”, “Ob”, “adipokines” “adipose tissue”, “obesity”, “kidney cancer” and “renal cell carcinoma”.

Three authors (KP, HWK and YNY) autonomously retrieved and assessed potentially applicable articles according to the specified selection criteria. Each study was required to meet the following criteria: (a) published as an original article from 2000 - 2017; (b) having definite description of leptin in RCC patients; (c) having description of cohort design; (d) serum leptin concentrations had to be provided; (e) and having investigator provided data on the number of cases with controls, with 95% confidence intervals (95% CIs) for quantification of leptin in RCC. The studies were excluded if they were case reports, meeting

records, letters, review articles, or animal studies. Figure 1 demonstrates study selection by The PRISMA system that was used for this study.

The studies included in this meta-analysis were cohort studies. The quality of each included cohort study was evaluated by using the Newcastle-Ottawa Scale (NOS) by three independent authors [18]. The studies with 6 scores or more were classified as high quality studies. Table 2 shows Risk of Bias assessment for case control studies. Table 3 shows Risk of Bias assessment for cohort studies.

2.2 Data extraction for hypothesis evaluation

Reviewers (KP, HWK and YNY) extracted the data independently using a standardised data collection method. Extracted information included first author name, title of article, published year, country, number of patients, age range, stage and grade of tumour and subgroups of RCC with 95% CIs. Table 1 describes the characteristics of the selected studies (N = 6). Subgroups of RCC were defined as clear cell RCC (ccRCC) or non-ccRCC.

2.3 Statistical analysis of hypothesis

Prior to the statistical analysis, the results of included studies that are presented with median and inter-quarter range were changed to mean and standard deviation by the formula mentioned by Wan et al[18]. For prognostic value, stage and grade of RCC from I to IV were grouped as early (stage I & II) (grade I & II) and late (stage III & IV) (grade III & IV). The grouping was made by using the method as described in the Cochrane Handbook for Systematic Review of Interventions [19].

Review Manager Software version 5.3 was used to pool all the data of different outcomes in a meta-analysis. Diagnostic and prognostic value of leptin and concentration of leptin were pooled as mean difference. Statistical significance was determined at $p<0.05$ for

all outcomes. The random effect model was used to pool the results if there was high clinical heterogeneity.

3.0 EMPIRICAL DATA OF HYPOTHESIS

3.1 Description of studies

Characteristics of the included studies are described in Table 1. Four out of six included studies analysing the diagnostic value of serum leptin in RCC patients. A total of 1090 RCC patients versus 1056 controls were analysed [14, 15, 20, 21]. Three studies were evaluated for the prognostic value of serum leptin in RCC by early and late tumour stage. 190 early stage cases versus 193 late stage cases were analysed [14, 22, 23]. Four studies were evaluated for the prognostic value of serum leptin in RCC by early and late grade. 226 early grade RCC versus 300 late grade RCC were analysed [14, 21-23]. Three studies were included for the evaluation of serum leptin in ccRCC compared with non-ccRCC. A total of 247 ccRCC versus 78 non-ccRCC was analysed [14, 21, 22].

3.2 Evaluation of publication on leptin

Six included studies evaluated serum leptin solely or compared with other adipokines such as adiponectin, leptin receptor, insulin like growth factor 1 (IGF-1), retinoal-binding protein 4 (RBP4), and nicotinamide phosphoribosyltranferase (NAMPT) which are secreted from adipoctytes. In the works by Choi et al., Liao et al. and Wang et al., leptin was compared with adiponectin, to identify the link between obesity and RCC [15, 20, 21, 24]. In all those studies, serum leptin levels were found to be inversely associated with prognosis of RCC [15, 20, 21, 24]. A study carried out by Rasmuson et al. found that IGF-1 rather than leptin was not associated with prognosis of RCC [23]. Horiguchi et al. found serum leptin to be a good indicator of RCC prognostication [22]. Figure 2 demonstrates the diagnostic value of serum leptin for RCC. Figure 3 demonstrates prognostic value of leptin by stage of RCC.

Figure 4 demonstrates prognostic value of leptin by grade of RCC. Figure 5 demonstrates evaluation on leptin in ccRCC with non-ccRCC.

3.3 Association of leptin with RCC

The six studies of this meta-analysis (Table 1) were grouped into three specific objectives, with meta-analysis completed with RevMan version 5.3 for each specific objective. Forest plots were obtained.

3.3.1 Diagnostic value of leptin in RCC

The mean difference of leptin in RCC and control were analysed. Figure 2 shows serum leptin level was not associated with RCC, (mean difference= 1.39, 95% CI = -3.32 – 6.10, P=0.56).

3.3.2 Prognostic value of leptin in RCC

The mean differences of leptin in early and late stages of RCC were analysed. Figure 3 shows no association when the concentration of leptin was compared in early stage (Stage I and II), with late stage (Stage III and IV) (mean difference= 0.82, 95% CI = -1.68 – 3.32, P = 0.52). Hence, leptin level does not give any prognostic value in comparing early and late stage of RCC. When we stratified the studies based on grade of RCC (early and late), no association was found between the two groups. The mean difference was 0.07 (95% CI = -0.56 – 0.70, p = 0.82). This is demonstrated in Figure 4.

3.3.3 Evaluation of leptin in ccRCC compared with non-ccRCC

The mean differences of leptin in ccRCC and non-ccRCC were analysed. Figure 5 shows a significant difference on leptin level when ccRCC was compared with non-ccRCC. (mean difference = -6.41, 95% CI = -11.14 to -1.68, P=0.008).

4.0 THEORETICAL IMPLICATION

Leptin is an adipose-derived hormone that is manufactured in proportion to fat stores. Circulating leptin serves to relate the condition of body energy repletion to the central nervous system in order to suppress food intake and energy expenditure [25]. While leptin homeostasis takes place, it activates other molecular pathways, namely JAK2-STAT3, PI3K and ERK1/2 which promote cancer cell survival, proliferation and migration [26-28].

Multiples approaches were carried out for targeting leptin as a biomarker or factor in development and progression of RCC. However, there remain unanswered questions about the role or pathway of leptin in progression of the disease. This current meta-analysis included six different studies and demonstrated that the expression of leptin does not play a role in prognosis of RCC. This result appeared independent of the case control cohort, tumour-node-metastasis (TNM) staging, Fuhrman grading and subgroup of tumour RCC with leptin localization.

The meta-analysis of the diagnostic value of leptin shows leptin was not associated with the development and prognosis of RCC. Prognostic value analysis also shows leptin not significant in both early stages and grades compared to late stages and grades. In subgroup of RCC analysis, serum leptin was detected as significant in non-ccRCC cases. This suggests that leptin might play a role in non-ccRCC compared to ccRCC.

Various objectives were formulated to evaluate the role of serum leptin in prognosis of RCC. However, none of the objectives correlated serum leptin with development and prognosis of RCC. Therefore, our meta-analysis with six studies indicates that leptin could not be a key biomarker for the early detection of RCC.

Some limitations exist in this meta-analysis. First, a small number of studies was included (six studies only) for analysis of leptin. Specifically, fewer than four studies were analysed for each objective. This could lead to publication bias. Second, significant heterogeneity was found in each analysis. This might be due to the sample collection, and the method used for analysis. Heterogeneity across studies cannot be completely discounted despite the use of appropriate meta-analytic techniques with random effect models. In conclusion, our hypothesis that serum leptin is a useful biomarker for early detection and prognostication of RCC was not supported by results of this systematic review and meta-analysis. Since obesity is a known factor for RCC development and progression, future research should be carried out on a larger cohort or should study other adipokines that are secreted by adipocytes which might be biomarkers for the early detection of RCC.

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REFERENCES

1. Mohammadian, M., et al., *Incidence and mortality of kidney cancer and its relationship with HDI (Human Development Index) in the world in 2012.* Clujul Medical, 2017. **90**(3): p. 286-293.

2. Chow, W.-H., L.M. Dong, and S.S. Devesa, *Epidemiology and risk factors for kidney cancer.* Nature reviews. Urology, 2010. **7**(5): p. 245-257.

3. Drabkin, H.A. and R.M. Gemmill, *Obesity, cholesterol, and clear-cell renal cell carcinoma (RCC).* Adv Cancer Res, 2010. **107**: p. 39-56.

4. Klinghoffer, Z., et al., *Obesity and renal cell carcinoma: epidemiology, underlying mechanisms and management considerations.* Expert Rev Anticancer Ther, 2009. **9**(7): p. 975-87.

5. Ouchi, N., et al., *Adipokines in inflammation and metabolic disease.* Nature reviews. Immunology, 2011. **11**(2): p. 85-97.

6. Rabe, K., et al., *Adipokines and Insulin Resistance.* Molecular Medicine, 2008. **14**(11-12): p. 741-751.

7. Castracane, V.D. and M.C. Henson, *The Obese (ob/ob) Mouse and the Discovery of Leptin*, in *Leptin*, V.D. Castracane and M.C. Henson, Editors. 2007, Springer US: Boston, MA. p. 1-9.

8. Wang, X., G. Ye, and J. Sun, *[Leptin and the feedback regulation of body weight].* Wei Sheng Yan Jiu, 1999. **28**(5): p. 318-20.

9. Saxena, N.K., et al., *Concomitant activation of the JAK/STAT, PI3K/AKT, and ERK signaling is involved in leptin-mediated promotion of invasion and migration of hepatocellular carcinoma cells.* Cancer research, 2007. **67**(6): p. 2497-2507.

10. Li, L., et al., *Concomitant activation of the JAK/STAT3 and ERK1/2 signaling is involved in leptin-mediated proliferation of renal cell carcinoma Caki-2 cells.* Cancer biology & therapy, 2008. **7**(11): p. 1787-1792.

11. Chen, C., et al., *Leptin stimulates ovarian cancer cell growth and inhibits apoptosis by increasing cyclin D1 and Mcl-1 expression via the activation of the MEK/ERK1/2 and PI3K/Akt signaling pathways. Corrigendum in/10.3892/ijo. 2016.3564.* International journal of oncology, 2013. **42**(3): p. 1113-1119.

12. Albiges, L., et al., *Body Mass Index and Metastatic Renal Cell Carcinoma: Clinical and Biological Correlations.* Journal of Clinical Oncology, 2016. **34**(30): p. 3655-+.

13. Klinghoffer, Z., et al., *Obesity and renal cell carcinoma: epidemiology, underlying mechanisms and management considerations.* Expert Review of Anticancer Therapy, 2009. **9**(7): p. 975-987.

14. Spyridopoulos, T.N., et al., *Inverse association of leptin levels with renal cell carcinoma: Results from a case-control study.* Hormones-International Journal of Endocrinology and Metabolism, 2009. **8**(1): p. 39-46.

15. Liao, L.M., et al., *Serum leptin and adiponectin levels and risk of renal cell carcinoma.* Obesity (Silver Spring), 2013. **21**(7): p. 1478-85.

16. Liberati, A., et al., *The PRISMA Statement for Reporting Systematic Reviews and Meta-Analyses of Studies That Evaluate Health Care Interventions: Explanation and Elaboration.* PLOS Medicine, 2009. **6**(7): p. e1000100.

17. Methley, A.M., et al., *PICO, PICOS and SPIDER: a comparison study of specificity and sensitivity in three search tools for qualitative systematic reviews.* BMC Health Services Research, 2014. **14**: p. 579.

18. Wan, X., et al., *Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range.* BMC Medical Research Methodology, 2014. **14**(1): p. 135.

19. *Analysing data and undertaking meta-analysis*, in *Cochrane Handbook for Systematic Reviews of Interventions (5.1.0 ed.).* J.J. Deeks, Higgins, J. P. T., Altman, D. G. , Editor. 2011.

20. Choi, S.H., et al., *Identifying the emerging role of adipokine as a diagnostic and prognostic biomarker of renal cell carcinoma.* Urol Oncol, 2016. **34**(6): p. 259.e15-9.

21. Wang, H., et al., *Serum Adiponectin Level May be an Independent Predictor of Clear Cell Renal Cell Carcinoma.* J Cancer, 2016. **7**(10): p. 1340-6.

22. Horiguchi, A., et al., *Increased serum leptin levels and over expression of leptin receptors are associated with the invasion and progression of renal cell carcinoma.* J Urol, 2006. **176**(4 Pt 1): p. 1631-5.

23. Rasmuson, T., et al., *Serum insulin-like growth factor-1 is an independent predictor of prognosis in patients with renal cell carcinoma.* Acta Oncol, 2004. **43**(8): p. 744-8.

24. de Martino, M., et al., *Serum Adiponectin Predicts Cancer-specific Survival of Patients with Renal Cell Carcinoma.* Eur Urol Focus, 2016. **2**(2): p. 197-203.

25. Bates, S.H. and M.G. Myers, Jr., *The role of leptin receptor signaling in feeding and neuroendocrine function.* Trends Endocrinol Metab, 2003. **14**(10): p. 447-52.

26. McCubrey, J.A., et al., *Roles of the Raf/MEK/ERK pathway in cell growth, malignant transformation and drug resistance.* Biochim Biophys Acta, 2007. **1773**(8): p. 1263-84.

27. Liu, P., et al., *Targeting the phosphoinositide 3-kinase (PI3K) pathway in cancer.* Nature reviews. Drug discovery, 2009. **8**(8): p. 627-644.

28. Thomas, S.J., et al., *The role of JAK/STAT signalling in the pathogenesis, prognosis and treatment of solid tumours.* British Journal Of Cancer, 2015. **113**: p. 365.

FIGURE LEGENDS

Figure 1
Study selection by The PRISMA system
This demonstrates the study selection by the PRISMA system that was used for the current systematic review and meta-analysis

Figure 2
Diagnostic value of leptin in RCC
Analysis shows diagnostic value of leptin in RCC compared to control

Figure 3
Prognostic value of leptin by stage in RCC
Analysis shows prognostic value of leptin in early stage compared to late stage of RCC

Figure 4
Prognostic value of leptin by grade in RCC
Analysis shows prognostic value of leptin in low grade compared to high grade of RCC

Figure 5
Evaluation of leptin in ccRCC with non-ccRCC
Analysis shows evaluation of leptin in ccRCC compared with non-ccRCC

Table 1 Characteristics of studies

Author	Title	Publication year	Country	No of Participant	Age range	
						Diagn Val
Choi et al.,	Identifying the emerging role of adipokine as a diagnostic and prognostic biomarker of renal cell carcinoma	2016	Korea	54 RCC 25 control	49-66	Case 2.93±0.6 Control 2.85±0.3
Horiguchi et al.,	Increased serum leptin levels and over expression of leptin receptors are associated with the invasion and progression of renal cell carcinoma	2006	New York	57 RCC	36-78	
Liao et al.,	Serum leptin and adiponectin levels and risk of renal cell carcinoma	2013	USA	Caucasians (RCC 581, control 558) African American (RCC 187, control 359)	20-79	Case 29.76±6. (768) Control 21.26±4. (917)
Rasmuso et	serum insulin-like growth factor-1 is an	2009	Sweden	256 RCC	25-86	NIL

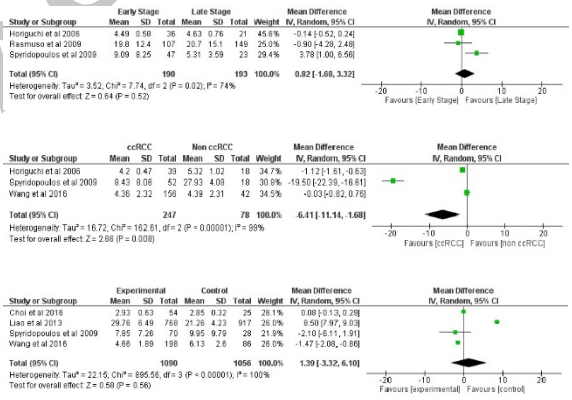
al.,	independent predictor of prognosis in patients with renal cell carcinoma					
Spyridopoulos et al.,	Inverse association of leptin levels with renal cell carcinoma: Results from a case-control study	2009	Greece	70 RCC 28 control	22-83	Case 4.66±1.8 (198) Control 6.13±2.6 (86)
Wang et al.,	Serum adiponectin level may be an independent predictor of clear cell renal cell carcinoma	2016	China	198 ccRCC 86 control	>18	Case 4.66±1.8 (198) Control 6.13±2.6 (86)

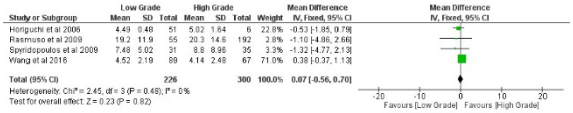
RCC = renal cell carcinoma; ccRCC = clear cell renal cell carcinoma

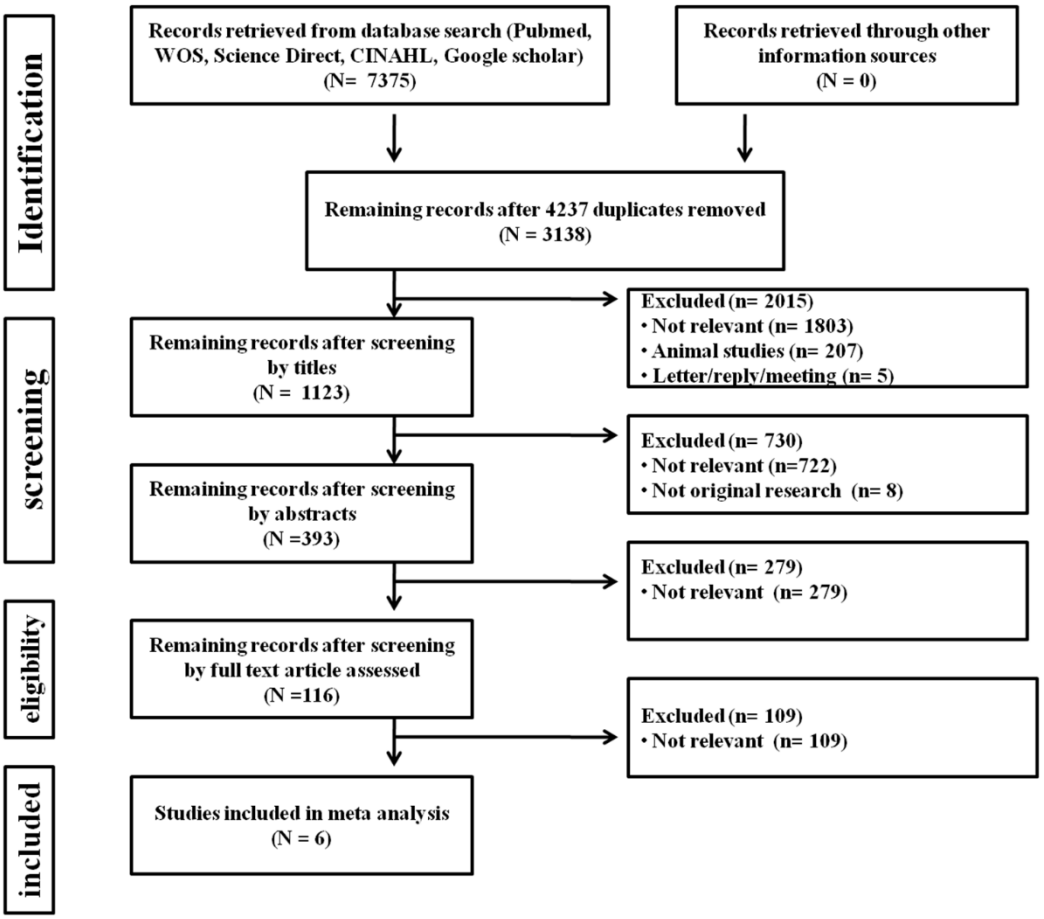
Table 2 Risk of bias assessment for case control studies

Included Studies	Year	Selection				Comparability		Exposure	
Included Studies	Year	Selection				Comparability		Outcome	
		Is case definition adequate	Representativeness of the exposed cohort	Representativeness of the cases	Selection of the non exposed cohort	Ascertainment of exposure	Definition of control	Demonstration that outcome of interest was not present at start of study	Comparability of cases and control on the basis of the design or analysis	Comparability of cohorts on the basis of the design or analysis	Ascertainment of exposure	Assessment of outcome
Spyridopoulos et al	2009	★		★		★	★		★			
Horiguchi et al	2006		★		★	★		★		★		★
Liao et al	2013	★		★		★	★		★			
Wang et al	2016	★	★	★★		★	★	★	★	★		★
Rasmuso et al	2009											
Choi et al	2016	★		★		★	★		★			

Table 3 Risk of bias assessment for cohort studies







Competing interests

The authors declare that they have no competing interests