Comparing Consumption of Green Leafy Vegetables to Cruciferous Vegetables in Relations to Incidence of 17 Different Cancers: A Meta-Analysis

By Richard Lee Pollock
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Introduction - Cancer is a group of over 100 different types of malignancies and there are several potential substances in green leafy vegetables (GLV) and cruciferous vegetables (CV) that may exhibit anticancer effects [1]. GLV are leaf vegetables, greens, vegetable greens, leafy greens or salad greens. They come from a very wide variety of plants all over the world, with nearly one thousand species of plants with edible leaves are known. Table 1 shows 11 of these GLV and some of the elements and phytochemicals that may reduce the incidence of cancer, and these same GLV are high in Vitamin C, Vitamin E, Vitamin K, and Vitamin A [2].

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Comparing Consumption of Green Leafy Vegetables to Cruciferous Vegetables in Relations to Incidence of 17 Different Cancers: A Meta-Analysis

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I. Implications and Contributions

This study will contribute to people’s knowledge of the importance of frequent daily intake of green leafy vegetables and cruciferous vegetables. Limited knowledge about the importance of these vegetables intake appears to be a serious worldwide health problem. The significant findings of this study will help provide some remedial measures to solve this problem of increased risk of cancers.

II. Introduction

Cancer is a group of over 100 different types of malignancies and there are several potential substances in green leafy vegetables (GLV) and cruciferous vegetables (CV) that exhibit anticancer effects [1]. GLV are leaf vegetables, greens, vegetable greens, leafy greens or salad greens. They come from a very wide variety of plants all over the world, with nearly one thousand species of plants with edible leaves are known. Table 1 shows 11 of these GLV and some of the elements and phytochemicals that may reduce the incidence of cancer, and these same GLV are high in Vitamin C, Vitamin E, Vitamin K, and Vitamin A [2].

CV are from the family Cruciferae which are widely cultivated, with many genera, species, and cultivars being raised for food production such as cauliflower, cabbage, cress, bok choy, broccoli, kale, collard greens and similar leafy vegetables and their roots such as turnips and radishes. Most researchers evaluating the association of fruit and vegetable intake with the risk of cancer place GLV and CV into two separate food categories even though most CV have edible green leaves. They are separated because only CV contain isothiocyanates which are plant phytochemicals that are known to be potent chemopreventives possessing the ability to prevent and inhibit tumorigenesis [3].

There is a need to research the worldwide scholarly journals to investigate case-control studies dealing with GLV and CV intake and the incidence of human cancers. After reading many previously published articles on this topic, there are apparent contradictions in research findings on whether GLV and CV intake does significantly lower incidence of cancer. The problem is that people worldwide are risking their health by not consuming enough GLV and CV on a daily basis. What could happen if we do not solve the problem? The World Health Organization (WHO) write on their website that cancer is a leading cause of death worldwide, accounting for 7.6 million deaths (around 13% of all deaths) in 2008 [4].

This meta-analysis research approach attempted to fill this knowledge gap by combining data from multiple studies to a common effect size (odds ratio) and statistically examine relations between study characteristics and findings. Findings between these different studies were compared by transforming the results into a single common effect size to better understand these apparent contradictions in prior research findings. The specific aims of this study were to attempt to answer the following: (1) assess the relationship between GLV intake and incidence of cancer; (2) assess the relationship between CV intake and incidence of cancer; and (3) determine which has a better genuine protective effect against cancer incidence, GLV or CV intake?

III. Materials and Methods

a) Experimental design

Searching for relevant studies was primarily performed by computer search engines seeking databases which included information about the subject. PubMed Central, Academic Search Complete, Medline, Proquest Central, Science Direct, Google, and Yahoo online were the most online periodical databases used. The criteria for including studies in this meta-analysis included: (1) a time period for collecting source studies which was from 1980 until 2015; (2) include only full text scholarly journal studies; (3) only studies showing no severe methodological flaws were included;

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(4) the collection of primary studies had to be case-control study design; (5) only include relations between similar independent variables (GLV and/or CV intake levels) and dependent variables (incidence of any cancer studied); (6) all studies had to measure GLV and/or CV consumption which was estimated by highest versus lowest quantiles (quintiles, or quartiles, or tertiles); (7) studies that reported an effect size of: odds ratio (OR), and their respective 95% confidence intervals (CI) data; and (8) source studies collected in this meta-analysis had to use logistic regression models to control for confounding or interaction variables and the results were expressed as adjusted OR if needed. IRB at Trident University International ethically approved the content of this meta-analysis (no human subjects used).

All meta-analysis calculations were performed by the software package Comprehensive Meta-Analysis Version 2 by Biostat (CMA v.2). These calculations include determining OR and their 95% CI, heterogeneity of the studies, relative weights for each study, significance (p) for each study, and for determining methods for detecting the presence of publication bias and assessing its impact on the meta-analysis. CMA v.2 was also used to create a high-resolution plot (Forest plot) which shows all the combined studies, their p-value, common OR, 95% CI for each study, relative weights for each study, and either a fixed effect model or random effect model. Borenstein, Hedges, and Higgins et al. [5] write that the selection of a model must be based solely on the question of which model fits the distribution of effect sizes, and when studies are collected from published literature, the random-effects model is a more plausible match for the meta-analysis. These same authors are experts on meta-analysis research, so only the random effect model was chosen for this meta-analysis.

The relative weights for each study were calculated by CMA v.2 software package. Small studies tend to have wide confidence intervals and large studies tend to have narrow confidence intervals with larger studies given greater percent relative weights [6]. An OR of 1.00 represents no treatment effect. Whereas when the OR falls below 1.00, this indicated participants that consumed GLV or CV in the highest quartile were less likely to develop incidence of cancer. If the effect size falls above 1.00, this indicated study subjects were more likely to develop incidence of cancer due to GLV or CV intake in the highest intake quantiles. The 95% CI bounding each study reflects the precision of the estimate, with small studies tending to have wide 95% CI and large studies tending to have narrow 95% CI [6].

The use of 95% CI in this meta-analysis was used, so each meta-analysis performed in this study was statistically significant (p< .05) if and only if the confidence interval excluded the null value of 1.0 for each effect model synthesized [6]. The conventional value of significance level for this meta-analysis was preset to an alpha of 0.05 [7].

CMA v.2 allows the meta-analyst to record data by subgroups within the study. Some studies collected in this meta-analysis used subgroups, e.g., male, female, GLV, CV, postmenopausal, premenopausal, colon, rectum, ever tobacco, never tobacco, colorectal, stomach, dark GLV, and light GLV. In this study, it emerged that the OR were comparable for each subgroup, so it was decided to use the study as the unit of analysis. This required calculating a “combined” effect size (utilizing the CMA v.2 software) for subgroups within each study, and imputes the values for the full group which recorded one treatment effect for each study.

CMA v.2 was also used to detect the possible presence of publication bias. All studies used in this meta-analysis were examined using a funnel plot of the natural logarithm of the OR versus its precision (1/standard error). Duval and Tweedie’s trim and fill method was also calculated by CMA v.2 software for detecting the presence of publication bias and assessing its impact on this meta-analysis study. Duval and Tweedie’s trim and fill builds on the key idea behind the precision funnel plot; that in the absence of publication bias the plot would be symmetric about the summary effect. If there are more small studies on the right than on the left of the mean effect size, the concern is that studies may be missing from the left. Duval and Tweedie’s method imputes these missing studies, adds them to the analysis, and then re-computes the summary effect size.

IV. Theory

This study recognized the many theories of how GLV intake reduces incidence of disease. The intervening variable facilitates a better understanding of the relationship between GLV intake and reduction of disease. Some of these hypothesized intervening variables found in GLV are folic acid, the antioxidants beta-carotene and vitamin E, soluble fiber, calcium, and vitamin K. It has been theorized in numerous studies that these essential nutrients and phytochemicals found in GLV, if consumed in adequate amounts, reduces the incidences of some human diseases. The researchers in these studies theorize on the mechanisms of disease reduction caused by GLV intake. In the 2010 decade, researchers are conducting extensive research studies to discover phytochemicals connections to disease prevention, but so far, solid evidence is mostly lacking [19]. There are thousands of these phytochemicals in GLV and researchers are just beginning to understand and theorize how a handful of these phytochemicals work, and what is current in the 2010 decade may change tomorrow [19].
V. Data analysis and Results

Over a two year search period (2012-2015) thousands of scientific papers were reviewed for this meta-analysis. Table 2 shows the total number of collected case-control studies (N=45) that were relevant and reviewed in this meta-analysis. Twenty-nine case-control studies where combined in meta-analysis which included the relations between CV intake and incidence of cancer and used OR as the effect size. Thirty-four case-control studies where combined which included the relations between GLV intake and incidence cancer and used OR as the effect size. A total of 17 cancers were examined in the 45 case-control studies which included thyroid, renal cell carcinoma, non-Hodgkin lymphoma, lung, breast, gastric, endometrial, colorectal, ovarian, pancreatic, prostate, hypopharyngeal, nasopharyngeal, cervical, cutaneous melanoma, esophageal, and urothelial cancer.

a) Research Question 1

Does an increased intake of CV significantly reduce incidence of cancer?

Twenty-nine studies shown had a similar common effect size (OR) and a meta-analysis was used to combine results from the 29 different studies. Figure 1 shows a Forest plot of the 29 studies and meta-analysis. The random effect model was selected for combining the source studies. The model indicates an overall OR effect size of the ‘almost every day’ highest vs. lowest quantile intake category of CV on cancer as: OR = 0.753 (95% CI .695 to .816), p<.001.

b) Detecting the Presence of Publication Bias------CV

All the collected studies were evaluated for the likelihood of publication bias using a funnel plot of the log odds ratio versus its precision (1/standard error) and Duval and Tweedie’s trim and fill method. Note in Figure 2 that the large case-control cancer studies appear toward the top of the funnel plot graph, and tend to cluster near the mean of the log OR in the relationship between 29 cancer case-control studies. The smaller studies appear toward the bottom of the funnel plot, and since there is more random variation in smaller studies, they are dispersed across a wide range of log OR. Figure 2 shows a possible presence of publication bias in the 29 studies with the studies distributed asymmetrically about the mean effect size. By contrast, in the absence of publication bias, the bottom of the funnel plot would tend to show an even concentration of studies around the mean [5]. Duval and Tweedie’s method imputes nine missing studies to the right and adjusts new OR= 0.822, 95% CI = 0.753 to 0.894 from the observed values (0.753, 95% CI = 0.695 to 0.816).

c) Research Question 2

Does an increased intake of GLV significantly reduce incidence of cancer?

Thirty-four case-control studies shown had a similar common effect size (OR) and a meta-analysis was used to combine results from the 34 different studies. Figure 3 shows a Forest plot of the case-control studies and meta-analysis. The random effect model was selected for combining the source studies. The model indicates an overall OR effect size of the ‘almost every day’ highest vs. lowest quantile intake category of GLV on cancer as: OR = 0.659 (95% CI .590 to .736), p<.001.

d) Detecting the Presence of Publication Bias------GLV

Figure 4 shows a possible presence of publication bias in the 34 case-control studies with the studies distributed asymmetrically about the mean effect size. Duval and Tweedie’s method imputes nine missing studies to the right and adjusts new OR= 0.739, 95% CI = 0.659 to 0.828 from the observed values (0.659, 95% CI = 0.590 to 0.736).

e) Research Question 3

Which has a better genuine protective effect against cancer incidence, GLV or CV intake?

It was determined from final meta-analysis results, GLV’s OR was 0.659 which is a 34.1% reduced incidence of the researched cancers. CV’s meta-analysis results indicated an OR of 0.753 which is a 24.7% reduced incidence of the researched cancers. Results indicate that GLV have a 9.4% better genuine protective effect against cancer incidence than CV in the highest quantile intake as compared to the lowest intake.

VI. Discussion of Findings

A noteworthy finding of this meta-analysis study is the protective effect associated with high consumption of GLV and CV. These vegetables are a characteristic and traditional dietary habit of worldwide populations as shown in this study. It has been previously postulated that this could explain the very low cancer incidence rates observed in populations that consume these vegetables. This meta-analysis study has been able to provide some clues for further investigation into the role of diet of GLV and CV prevalent in regions where causation of many forms of cancer occurs.

The intent of this study was to investigate potential influences of GLV and CV intake on incidences cancers on worldwide human populations. An extensive search for relevant studies was initiated to learn more about these diet-cancer relationships. Forty-five studies were collected and used in two separate meta-analysis to investigate the effects GLV and CV intake have on incidences of 17 different cancers. A composite of the research questions in this meta-analysis study was; does an increased intake of GLV and CV significantly reduce the worldwide incidence of 17 aggregated...
cancers studied? Results shows both meta-analysis indicated a statistical significant reduction in incidence of cancer with an adequate intake of GLV including CV. Even after adjusting effect sizes for possible publication bias via Duval and Tweedie’s method, both meta-analysis results indicated GLV and CV consumption significantly reduced cancer incidences.

Forty five case-control studies were collected that investigated the relationship between the incidences of researched cancers with the consumption of GLV and CV which used OR as their effect size. These studies included 77,563 case participants and controls, with 28,543 case participants having 17 different type cancers. The first research question of this meta-analysis study was; does an increased intake of CV significantly reduce the incidence of these 17 cancers? The random effect model indicated an overall OR effect size of the ‘almost every day’ highest vs. lowest quantile intake category of CV on cancer as: OR = 0.753 (95% CI .695 to .816), p<.001, showing 24.7% lower odds that an intake of CV significantly reduces the incidence of these 17 cancers in the highest intake category as compared to the lowest. GLV showed even a better genuine protective effect against cancer incidence: OR = 0.659 (95% CI .590 to .736), p<.001, showing a significant 34.1% lower odds.

a) Phytochemicals and Minerals Fight Cancer

Why does the intake of GLV appear to reduce the incidences of forms of cancer? The phytochemicals in GLV and CV appear to provide much of the disease fighting power. GLV and CV provide adequate amounts of soluble fibers, retinol, carotenoids, vitamin C, riboflavin, folic acid and mineral salts like calcium, iron, and phosphorus [8]. Antioxidants, such as retinol, carotenoids, and vitamin C have been found to exert protective effects against cancer [9]. Individuals with high intakes of soluble fiber appear to be at significantly lower risk for developing coronary heart disease, stroke, hypertension, diabetes, obesity, and certain gastrointestinal cancers [10]. Antioxidants, especially flavonoids and vitamin C found in GLV, are a class of compounds thought to prevent certain types of chemical damage caused by an excess of free radicals. Flavonoids and vitamin C inhibits or quenches free radicals and reactive oxygen species in the body which helps fight cancer, heart disease, stroke and other immune compromising diseases [11]. These and other experts believe that over time free radicals contribute to the development of disease and if antioxidants can help neutralize harmful compounds, antioxidants found in GLV can reduce cell damage and prevent some forms of cancer. The primary dietary source of vitamin K is generally GLV and both in vitro in vivo studies have shown that vitamin K exhibits anticancer effects [12]. Carotenoids have antioxidant potential in the scavenging of harmful free radicals [13] and they appear to play an important role in the prevention of hepatitis virus-related liver carcinogenesis [9]. Rajalakshmi, and Agalyaa [1] found that watercress (Nasturtium officinale) has an anti-cancer effect in their study of oral cancer. Watercress is one of the richest sources of dietary phenethyl isothiocyanates and they found it inhibited a chemical in tobacco that may cause oral cancer. Also, in several epidemiological studies, high intake of calcium has been associated with reduced risk of colorectal and breast cancer [[14], [15]]. The risk of lymphoma cancer could be affected by reactive oxygen species, which might alter immune responses by damaging DNA and phospholipid membrane structures in lymphocyte cells, but especially the antioxidant properties of beta carotene and vitamin E found in GLV can hinder membrane damage [16]. The antioxidant beta carotene and vitamin A content of GLV and CV are 100-fold greater than in fruits and these two antioxidants have been said to possess the greatest protective effects against lung cancer [17]. These antioxidants may have the capability to prevent oxidative degradation of DNA, they also could act as an immunoenhancer, boosting the body’s immune system by helping identify and destroy anomalous cells recognized as foreign such as cancer cells in the lungs [[17], [18]].

Further research in the twenty first century should be focused on conducting extensive research studies to discover phytochemicals connections to disease prevention because solid evidence is mostly lacking [19]. Researchers are just beginning to understand and theorize how a small percent of the different phytochemicals in GLV work. There are potentially thousands of phytochemical compounds from extracts of plant roots, leaves, and stems that have shown promising potential as anticancer drugs, or for serving as lead compounds in the synthesis of new drugs [19].

b) Study Limitations

This research meta-analysis study was restricted by the paucity of qualifying studies that evaluated the relationship between GLV intake and cancer. Thus the findings, although found to be statistically significant cannot be generalized with confidence. An experimental group vs. a control group was not determined as a requirement, so long as the source studies were case-control.

One major limitation in meta-analysis is that for any given research topic, the meta-analyst cannot know for sure how many studies had been conducted but never reported and the results filed away due to lack of significant findings. This “file drawer problem” results in the distribution of effect sizes that are biased and possibly skewed which creates a serious base rate fallacy, in which the significance of the published studies is overestimated [20]. Rosenthal [21] writes that
the heavy reliance on published studies may create exaggerated final results. Thus the decision on whether the fail safe number calculated and reported for each meta-analysis performed in this study, are “realistic”, needs to be determined by the researcher.

**References Références Referencias**


**Table 1**: Amounts of Chemical Elements and Phytochemicals in GLV.

<table>
<thead>
<tr>
<th>GLV</th>
<th>Ca (mg)</th>
<th>Mg (mg)</th>
<th>Folate (µg)</th>
<th>Lutein (µg)</th>
<th>B-carotene (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broccoli</td>
<td>62</td>
<td>33</td>
<td>168</td>
<td>1,685</td>
<td>1,449</td>
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<td>Brussels Sprouts</td>
<td>59</td>
<td>31</td>
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<td>2,012</td>
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<td>19</td>
<td>45</td>
<td>41</td>
<td>72</td>
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<td>38</td>
<td>129</td>
<td>14,619</td>
<td>9,147</td>
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<td>94</td>
<td>23</td>
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<td>41</td>
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<td>21</td>
<td>102</td>
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<td>245</td>
<td>157</td>
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<td>170</td>
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<td>RDA =</td>
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<td>400 mg</td>
<td>400 µg</td>
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### Table 2: GLV and CV on cancer qualifying studies showing location and number of subjects (N=case + controls).

<table>
<thead>
<tr>
<th>Study</th>
<th>Location and (N)</th>
<th>Study</th>
<th>Location and (N)</th>
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</thead>
<tbody>
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<td>Chiu et al. (2011)</td>
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<td>Glynn et al. (1996)</td>
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<td>Fortes et al. (2008)</td>
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<td>USA (2,233)</td>
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<td>Hardin et al. (2011)</td>
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<td>Heck et al. (2008)</td>
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<td>Phukan et al. (2001)</td>
<td>India (1,506)</td>
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<td>Tao et al. (2005)</td>
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### Figure 1: Forest plot showing a significant 24.7% lower odds of incidence of cancer by consuming a high quantile intake of CV as compared to the lowest intake.
Figure 2: Funnel plot showing 29 case-control studies with 20 studies on the left of mean log odds ratio and 9 on the right signifying possible presence of publication bias.
### Study name | Subgroup within study | Statistics for each study | Odds ratio and 95% CI | Relative weight
--- | --- | --- | --- | ---
Annema et al. (2011) | GLV | 0.770 | 0.570, 1.040 | 0.083 | 4.02
Bae et al. (2012) | GLV | 0.930 | 0.792, 1.092 | 0.377 | 6.12
Chen et al. (2006) | GLV | 0.600 | 0.474, 0.837 | 0.001 | 4.15
Cheng et al. (2005) | Combined | 0.798 | 0.493, 1.321 | 0.065 | 3.42
Cheng et al. (1992) | GLV | 0.430 | 0.250, 0.711 | 0.001 | 2.61
Chi et al. (2011) | GLV | 0.600 | 0.385, 0.986 | 0.044 | 2.03
Duell et al. (2000) | GLV | 0.520 | 0.301, 0.832 | 0.003 | 2.53
Fortes et al. (2008) | GLV | 0.400 | 0.248, 0.648 | 0.000 | 2.75
Gaudet et al. (2004) | Combined | 0.797 | 0.638, 0.994 | 0.044 | 4.67
Glenn et al. (1999) | Combined | 0.735 | 0.527, 1.055 | 0.457 | 1.90
Grip et al. (2009) | GLV | 0.650 | 0.286, 1.656 | 0.073 | 1.93
Hardin et al. (2011) | GLV | 0.650 | 0.457, 0.953 | 0.027 | 3.50
Heck et al. (2008) | Combined | 0.221 | 0.119, 0.400 | 0.000 | 2.05
Holman et al. (2010) | GLV | 0.620 | 0.391, 0.932 | 0.000 | 4.15
Hosono et al. (2010) | GLV | 0.400 | 0.261, 2.608 | 0.333 | 0.32
Hsu et al. (2007) | GLV | 1.180 | 0.699, 2.049 | 0.233 | 4.26
Hsu et al. (2003) | Combined | 0.637 | 0.506, 0.833 | 0.000 | 4.63
Hsu et al. (2007) | Combined | 0.839 | 0.665, 1.074 | 0.164 | 4.46
Jain et al. (1999) | GLV | 0.940 | 0.778, 1.136 | 0.523 | 4.91
Janson et al. (2011) | GLV | 0.430 | 0.282, 0.685 | 0.000 | 3.12
Kelémen et al. (2006) | GLV | 0.590 | 0.381, 0.963 | 0.035 | 2.68
Kelémen et al. (2008) | GLV | 0.630 | 0.410, 0.959 | 0.035 | 3.05
Liu et al. (2012) | Combined | 0.516 | 0.303, 0.874 | 0.000 | 4.27
Marchand et al. (2002) | GLV | 0.600 | 0.204, 1.225 | 0.129 | 1.19
Memon et al. (2002) | GLV | 0.600 | 0.328, 1.949 | 0.623 | 1.20
Mozaffarian et al. (2012) | GLV | 0.232 | 0.151, 0.494 | 0.000 | 2.30
Norman et al. (2000) | GLV | 0.910 | 0.602, 1.375 | 0.664 | 3.17
Phukan et al. (2001) | GLV | 0.250 | 0.021, 3.212 | 0.294 | 0.18
Romans-Baxma et al. (2002) | GLV | 0.630 | 0.242, 1.642 | 0.344 | 1.07
Shirley et al. (2010) | GLV | 0.830 | 0.740, 0.930 | 0.001 | 5.42
Takaku-Kato et al. (2014) | GLV | 0.920 | 0.317, 2.657 | 0.673 | 0.93
Watari et al. (2004) | GLV | 0.570 | 0.270, 1.202 | 0.140 | 1.63
Wu et al. (2009) | GLV | 0.740 | 0.575, 0.982 | 0.019 | 4.42
Zheng et al. (2002) | GLV | 1.000 | 0.513, 1.949 | 1.000 | 1.64

**Random effect model**: 0.559

**Relative weight**: 0.590, 0.736, 0.000

**Figure 3**: Forest plot showing a significant 34.1% lower odds of incidence of cancer by consuming a high quantile intake of GLV as compared to the lowest intake.
Figure 4: Funnel plot showing 34 case-control studies with 23 studies on the left of mean log odds ratio and 11 on the right signifying possible presence of publication bias.