Methods

The data used in this study is from an imputed version of NHANES II study. The exposure in this study is alcohol consumption (drinks/week) measured at the baseline. After exploring the distribution of alcohol intake, we decided to divide the continuous alcohol intake to categorical variables to reduce the influence of outliers. We found about 43.7% of people didn’t take alcohol per week. After excluding people with 0 alcohol intake, the minimum, median and maximum alcohol intake were 0.5, 2.0, and 77.0 per week. Then we divided people into 4 groups based on their alcohol intake: 0 per week, 0-0.5 drinks per week, 0.5-2 drinks per week and >2 drinks per week. The outcome is time to death of cancer during the follow-up, or time to censoring, or time to death from other causes, whichever comes first.

Descriptive statistics are utilized to measure the baseline characteristics in the study dataset. Number and percentages for categorical variables and mean and standard deviation for continuous variables are reported. Through these descriptive statistics, we measured the unbalanced distribution for the covariates between alcohol consumption groups, and thus, explore potential confounding in the association between alcohol assumption and cancer mortality.

Given the cross-sectional nature of the study, the alcohol intake can be perceived as prevalent intake, which could induce the “prevalent user” bias. To test the robustness of the findings given this potential bias, in the main analysis, we chose to conduct Cox proportional hazard models through two approaches. The first approach is setting the age 21 (which is the legal age to buy alcohol in the US) to be the time origin and choosing age as the time-scale. In this approach, we conducted crude model and fully-adjusted model. No model adjusting for baseline age was conducted since age was set as the time scale. The fully-adjusted model now only adjusted for sex, and other socio-economic status (SES) related covariates, because all the biomarkers, biometrics, and disease status at baseline might be the consequence of the alcohol intake before. The second approach is setting baseline time as the time origin, and time since entry is set to be the time scale. We conducted crude model, models adjusting for baseline age, and the fully-adjusted model. In the fully-adjusted model, we adjusted for confounders which are reasonable common causes of prevalent alcohol intake as well as future risk of death because of cancer. The confounders were chosen based on the descriptive statistics mentioned above. The conceptual framework as well as DAGs is shown in figure 1.

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Fig 1. Conceptual framework for Cox proportional hazard models in the primary analysis

To explore potential effect modification by sex, we conducted the fully-adjusted models using two method 1) including the product term in the fully-adjusted model, 2) conducting stratified Cox regression by sex and adding an interaction term by sex and alcohol intake, 3) conducting Cox regressions in males and female separately (called subgroup analysis). Wald-tests were utilized to test the significance of effect modification.

We checked the proportional hazard assumption using Schofield residuals and reported the corresponding chi-squared p-values for the exposure as well as for the whole model. In the fully-adjusted model non-linearity was also checked first by comparing the category-specific hazard ratio. Then, we fit a natural cubic spline replacing the linear term of alcohol consumption to explore potential non-linear relationship. Based on the results from the main analysis, we the non-linear relationship analysis was only conducted on the fully-adjusted model following the second approach.

In the sensitivity analysis, we adopted the first approach which using time since baseline as the time scale, and conducted logistic and Poisson regression to check the influence of model selection on our findings.

**Results**

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Table 1 Baseline characteristics of study population by alcohol intake(N=9190).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Alcohol intake per week | | | |  |
|  | 0/week | 0-0.5/week | 0.5-2/week | >2/week | p-value |
|  | N=1052 | N=209 | N=319 | N=530 |
| Sex |  |  |  |  | <0.001 |
| Male | 515 (49.0%) | 110(52.6%) | 205(64.3%) | 407(76.8%) |  |
| Female | 537 (51.0%) | 537(51.0%) | 114(35.7%) | 123(23.2%) |  |
| Mean Age at entry (SD) | 65.2 (7.81) | 64.9 (8.32) | 62.2 (9.43) | 62.1 (9.67) | <0.001 |
| Race |  |  |  |  | <0.001 |
| white | 109 (10.4%) | 21 (10.0%) | 51 (16.0%) | 87 (16.4%) |  |
| black | 388 (36.9%) | 93 (44.5%) | 121(37.9%) | 234(44.2%) |  |
| other | 555 (52.8%) | 95 (45.5%) | 147(46.1%) | 209(39.4%) |  |
| Mean year of schooling (SD) | 9.24 (3.69) | 10.4 (3.42) | 10.3 (3.57) | 10.9 (3.35) | <0.001 |
| Marital status |  |  |  |  | . |
| Married | 699 (66.4%) | 143 (68.4%) | 225 (70.5%) | 379 (71.5%) |  |
| Widowed | 231 (22.0%) | 36 (17.2%) | 49 (15.4%) | 67 (12.6%) |  |
| Divorced | 44 (4.18%) | 12 (5.74%) | 13 (4.08%) | 12 (9.76%) |  |
| Separated | 24 (2.28%) | 7 (3.35%) | 10 (3.13%) | 11 (2.08%) |  |
| Never married | 53 (5.04%) | 9 (4.31%) | 21 (6.58%) | 32 (6.04%) |  |
| Blank | 1 (0.10%) | 2 (0.96%) | 1 (0.31%) | 5 (0.94%) |  |
| Mean BMI (SD) | 26.3 (5.58) | 25.8 (4.51) | 26.1 (5.21) | 25.1 (4.17) | <0.001 |
| Mean smoke per day (SD) | 5.60 (11.8) | 6.74 (11.9) | 9.78 (15.2) | 12.0 (15.0) | <0.001 |
| Size of place | 5.70 (2.57) | 5.07 (2.70) | 4.53 (2.69) | 4.44 (2.69) | <0.001 |
| Standard Metropolitan  Statistical Area |  |  |  |  | <0.001 |
| In central city | 265 (25.2%) | 59 (28.2%) | 105(32.9%) | 186(35.1%) |  |
| Not in central | 201 (19.1%) | 57 (27.3%) | 102(32.0%) | 183(34.5%) |  |
| Not in SMSA | 586 (55.7%) | 93 (44.5%) | 112(35.1%) | 161(30.4%) |  |
| Resides in urban area, Yes | 578 (54.9%) | 578(54.9%) | 235(73.7%) | 387(73.0%) | <0.001 |

We assessed the association between categorical alcohol consumption through two approaches using 5 models. Across all the 5 models, no significant associations are found for alcohol consumption 0-0.5 drinks per week, 0.5-2 drinks per week compared to 0 drinks per week. For the category >2 drinks per week, the first approach yields a HR of 1.57 (95% CI, 1.29, 1.91) in the crude model and 1.25 (95% CI, 1.00, 1.54) in the fully-adjusted model. Following the second approach, the estimated HR is 1.15 (95% CI, 0.94, 1.39) in the crude model and is 1.24 (95% CI, 1.00, 1.55) in the fully-adjusted model. When assessing the association between continuous alcohol consumption and hazard from cancer death, all the 5-model yield very similar results. The point estimates are around 1.03 and are of marginal significance. To test the sensitivity of the findings to the model specification, we performed logistic regressions and Poisson regressions. The findings are consistent with the findings from the Cox regressions. Only group with >2 drinks per week demonstrated statistically significant association with death from cancer. The odds ratio from the fully-adjusted logistic regression is 1.29 (95% CI, 1.02, 1.63) and the incidence rate ratio from the fully-adjusted Poisson regression is 1.24 (95% CI 1.00, 1.55). Like the beforementioned Cox regressions, The continuous alcohol consumption demonstrated weak associations with death from cancer in the fully-adjusted logistic regression (95% OR = 1.03, 95% CI, 1.01, 1.04) and fully-adjusted Poisson regression (IRR = 1.02, 95% CI, 1.01, 1.09).

Table 2. Association between alcohol intake and hazard of death from cancer in overall NHANES II (N=9190).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | 0/week | 0-0.5/week | 0.5-2/week | >2/week | Continuous | Ptrend |
| First approach, defining the exposure as alcohol intake age 21-entry, time scale as age | | | | | | |
| Crude model | Ref | 1.10 (0.82, 1.48) | 1.13 (0.88, 1.44) | 1.57 (1.29, 1.91) | 1.04 (1.03, 1.05) | <0.001 |
| Age-adjusted | Ref |  |  |  |  |  |
| MV-adjusteda | Ref | 1.06 (0.78, 1.43) | 0.98 (0.76, 1.27) | 1.25 (1.00, 1.55) | 1.02 (1.01, 1.04) | 0.084 |
| Second approach, defining the exposure as prevalent alcohol intake at baseline, time scale as years since entry | | | | | | |
| Crude model | Ref | 0.95 (0.70, 1.28) | 0.83 (0.65, 1.06) | 1.15 (0.94, 1.39) | 1.03 (1.02, 1.04) | 0.335 |
| Age-adjusted | Ref | 1.11 (0.82, 1.45) | 1.13 (0.88, 1.45) | 1.59 (1.30, 1.94) | 1.04 (1.03, 1.05) | <0.001 |
| MV-adjustedb | Ref | 1.06 (0.79, 1.44) | 0.98 (0.76, 1.26) | 1.24 (1.00, 1.55) | 1.03 (1.01, 1.04) | 0.092 |

a. In the MV-adjusted model with age as the time-scale, we adjusted for sex, race (categorical), education years, marital status (categorical), size of place (categorical), average cigarettes per day, Standard Metropolitan Statistical Area (categorical), and residence in urban region.

b. In the MV-adjusted model with time since entry as the time-scale, in addition to the covariates in a., we also adjusted for hypertension at baseline, RBC, diagnosed MI, and diabetes.

With the findings from the main analysis, we explore the potential modification of sex. First, we compared the estimated HRs comparing >2 drinks per week to 0 drinks per week across males and females. The HR’s estimated in the females are greater than in the males in all the models, indicating the alcohol consumption’s harmful effects are more pronounced in the females than in the males. However, the differences are not of statistical significance across all the six models. Then we treated the alcohol consumption as a continuous variable. All the six models yield similar results for males and females, both are very close to the estimates in the overall sample analysis. The differences are of no statistical significance. Overall, no matter focusing on categorical or continuous alcohol consumption, we detected no statistically significant effect modification by sex.

Table 3. Associations between alcohol intake and hazard of death from cancer in males and females from NHANES II (N=9190).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | >2/week vs 0/week | | Continuous | |
| First approach, defining the exposure as alcohol intake age 21-entry, time scale as age | | | | |
| Adding interaction term | | | | |
|  | MV-adjusted | Heterogeneity | MV-adjusted | Heterogeneity |
| Female | 1.06 (0.82, 1.36) | 0.053 | 1.02 (1.01, 1.04) | 0.712 |
| Male | 0.69 (0.38, 1.27) |  | 1.02 (0.97, 1.06) |  |
| Stratified Cox | | | | |
|  | MV-adjusted | Heterogeneity | MV-adjusted | Heterogeneity |
| Female | 1.52 (1.06, 2.18) | 0.122 | 1.03 (0.99, 1.07) | 0.868 |
| Male | 1.08 (0.84, 1.40) |  | 1.02 (1.01, 1.04) |  |
| Subgroup analysis | | | | |
|  | MV-adjusted | Heterogeneity | MV-adjusted | Heterogeneity |
| Female | 1.35 (0.92-1.96) | 0.231 | 1.02 (0.97-1.06) | 0.293 |
| Male | 1.13 (0.87-1.48) |  | 1.03 (1.01-1.04) |  |
| Second approach, defining the exposure as prevalent alcohol intake at baseline, time scale as years since entry | | | | |
| Adding interaction term | | | | |
|  | MV-adjusted | Heterogeneity | MV-adjusted | Heterogeneity |
| Female | 1.59 (1.11-2.28) | 0.063 | 1.03 (0.99-1.07) | 0.758 |
| Male | 1.06 (0.82-1.37) |  | 1.02 (1.01-1.04) |  |
| Stratified Cox | | | | |
|  | MV-adjusted | Heterogeneity | MV-adjusted | Heterogeneity |
| Female | 1.58 (1.10-2.27) | 0.067 | 1.03 (0.99-1.07) | 0.772 |
| Male | 1.06 (0.82-1.38) |  | 1.02 (1.01-1.04) |  |
| Subgroup analysis | | | | |
|  | MV-adjusted | Heterogeneity | MV-adjusted | Heterogeneity |
| Female | 1.35 (0.93-1.98) | 0.240 | 1.01 (0.97-1.06) | 0.717 |
| Male | 1.15 (0.88-1.49) |  | 1.03 (1.01-1.04) |  |

We checked the proportional hazard assumption in all the fully-adjusted models using Scofield’s residuals. The assumption satisfied for the alcohol consumption in all the models. We also explored potential non-linear relationships by using a natural cubic spline with 3 degrees of freedom. The curve is linear, and we found no clear curvature pattern. The estimated 95% CI for hazard ratio covers 1 before alcohol consumption of 5 drinks per week. This finding can partially explain why we found non-significance for the findings from group 0-0.5, and 0.5-2 drinks per week but significant association for the group >2 drinks per week.

Table 4. Tests for the proportional hazard assumption throughout the models (N=9190).

|  |  |  |  |
| --- | --- | --- | --- |
|  | Chi-squared statistic | p-value for alcohol consumption | Global p-value |
| First approach, defining the exposure as alcohol intake age 21-entry, time scale as age | | | |
| MV-adjusted categorical model | 6.93 | 0.074 | 0.178 |
| MV-adjusted continuous model | 0.15 | 0.697 | 0.2326 |
| Second approach, defining the exposure as prevalent alcohol intake at baseline, time scale as years since entry | | | |
| MV-adjusted categorical model | 2.92 | 0.404 | 0.653 |
| MV-adjusted continuous model | 0.53 | 0.465 | 0.672 |

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Fig 2.

**Discussion**

In this study with a cross-sectional dataset from NHANES II, we found a weak harmful but significant effect for alcohol consumption and on the risk of death from cancer. Taking alcohol less than 2 drinks per week is not found to significantly increase the risk. The exploratory non-linear analysis demonstrates that such relationship is close to linear with a significant harmful effect only after 5 drinks per week. No significant sex difference is found in the relationship.

In the main analysis, we employed two different approaches to assess the alcohol consumption’s effects on hazard from cancer-specific death. One approach is taking the age as the time scale and takes the temptation into account, which has a more reasonable causal structure and explicit causal assumption. However, another approach takes the whole dataset singly as a cross-sectional study and utilizes the time from entry as the time scale. The two approaches yield different results in the crude model but get similar results in the fully-adjusted model. Given the data at hand, there are some explanations for the very similar findings. The first explanation is the estimated association for the cancer-specific death (about 1.02 HR per 1 drink/week increment) is very weak which can be explained by the residual confounding or other unknown confounders. It suggests both approaches even including the logistic and Poisson regression doesn’t adjust for enough confounding. The Second explanation, in this study, the diseases’ distribution are pretty balanced across alcohol consumption groups. Thus, additional adjusting for these diseases and biomarkers such as baseline hypertension will not substantially influence the estimates. Last, the true effect is weak and both models get the correct estimand.

In this study, we didn’t detect significant effect modification by sex though the point estimates for the >2 drinks per week group shows a more harmful effect among women than in the men. However, we cannot totally exclude the presence of potential effect modification since the model is of low power to detect such difference. We also explore potential non-linear dose-response relationship, and we find the curve is pretty linear. This analysis also tells that with low dose of alcohol consumption, no significant harmful effects manifest. The harmful effect becomes significant when taking alcohol greater than 5 drinks per week.

There are several limitations. First, as we mentioned before, the estimated association is very weak, which is very sensitive to unmeasured confounders. Although the findings from different model are consistent, without additional confounder information, we must admit that the observed association is very likely to attributed to an unmeasured confounder. Second, the original data is in the cross-sectional form, which limits the survival analysis. It is hard to tell which exposure or confounder happens first, making the model adjustment difficult and obscure. Last, we must notify that the interested event in this study is death from cancer. Other deaths are competing risks but are treated as censoring in this study. Although the Cox model is still valid, the logistic and Poisson regression are not. Their estimations are biased.

Appendix 1. Association between alcohol intake and hazard of death from cancer in overall NHANES II (N=9190).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | 0/week | 0-0.5/week | 0.5-2/week | >2/week | Continuous | Ptrend |
| Logistics regressiona | | | | | | |
| Crude model | Ref | 0.96 (0.71, 1.30) | 0.86 (0.67, 1.11) | 1.22 (1.00, 1.49) | 1.03 (1.02, 1.05) | 0.144 |
| Age-adjusted | Ref | 1.11 (0.81, 1.51) | 1.15 (0.89, 1.49) | 1.66 (1.35, 2.04) | 1.04 (1.03, 1.06) | <0.001 |
| MV-adjusted | Ref | 1.05 (0.76, 1.43) | 0.99 (0.76, 1.30) | 1.29 (1.02, 1.63) | 1.03 (1.01, 1.04) | 0.054 |
| Poisson regressiona | | | | | | |
| Crude model | Ref | 0.95 (0.70,1.27) | 0.84 (0.66, 1.07) | 1.16 (0.95, 1.41) | 1.03 (1.02, 1.04) | 0.309 |
| Age-adjusted | Ref | 1.11 (0.83, 1.50) | 1.14 (0.89, 1.45) | 1.60 (1.31, 1.95) | 1.04 (1.03, 1.05) | <0.001 |
| MV-adjusted | Ref | 1.06 (0.79, 1.44) | 0.98 (0.76, 1.27) | 1.24 (1.00, 1.55) | 1.02 (1.01, 1.09) | 0.088 |

a. Odds ratios are reported for logistic regressions and incidence rate ratios are reported for Poisson regressions. Log of follow-up years are set as the offset in Poisson regressions.