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The association between alcohol consumption and hemoglobin level among reproductive age non-pregnant women in Ethiopia: Further analysis of the 2016 Ethiopian Demographic Health Survey

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The association between alcohol consumption and hemoglobin level among reproductive age non-pregnant women in Ethiopia: Further analysis of the 2016 Ethiopian Demographic Health Survey

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Abstract

Objective: The aim of this study was to assess the effect of alcohol consumption on hemoglobin levels among non-pregnant reproductive age women using national representative data.

Method: A secondary data analysis was conducted using data from the 2016 Ethiopia Demographic Health Survey (EDHS). The main outcome of interest was anemia defined as a hemoglobin value <12g/which was measured using HemoCue, and adjusted for both altitude and smoking status. The main exposure variable was alcohol consumption. Both multivariable logistic regression and generalized linear model were employed to assess the association between alcohol consumption and anemia and to compare the mean of hemoglobin between the nondrinkers and three alcohol consumption frequency categories, respectively, after adjusting for the potential confounders.

Result: In the current analysis, a total of 13,436 non-pregnant women were included. The overall prevalence of anemia among non-pregnant women was 23.2 % of which 17.9% were mildly anemic, 4.6%, moderately anemic, and 0.7%, severely anemic. There was a significant negative association between anemia and respondents' history and frequency of alcohol consumption in the pooled sample. Among non-pregnant women, the odds of having anemia were decreased with a history of alcohol drinking (AOR=0.32(0.214-0.394)). Women who drunk alcohol less than once a week ((AOR=0.54(95% CI: 0.31-0.94), those who drunk alcohol at least once a week (AOR=0.50(0.28-0.88)) and who drunk alcohol almost every day in the last 12 months (AOR=0.42(0.21-0.81)) had significantly lower odds of having anemia than women who hadn't ever taken a drink that contain alcohol.

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Conclusion: This study showed that nearly a quarter of non-pregnant women in Ethiopia were found to be anemic. Alcohol consumption increase hemoglobin concentration. Further cohort or experimental studies are therefore needed to further assess the relationship between alcohol consumption and hemoglobin level among Ethiopian populations.

Keywords: Alcoholics; Anemia; Hematocrit; Substance use

Strengths and limitations of this study

- ✓ Used large population-based data with a large sample size, which is representative of all regions of the country.
- ✓ As cross-sectional data were used, we cannot assign causality.
- ✓ Estimation of alcohol consumption was entirely on self-reporting which might result in social desirability biases and may have been subject to underreporting.
- ✓ The EDHS survey did not incorporate important behavioral factors(dietary intake, physical activity)

Background

Anemia is a global health problem that affects one-quarter of the world's population(1). The prevalence of anemia has gradually decreased in the developed countries, but it still remains a major public health problem in most of the developing countries particularly in Africa(2). Ethiopia is a country with the highest prevalence of anemia ranges from 17% to 52.3%(2-5). Currently, reproductive-age women were disproportionally affected groups by anemia, making it a global public health problem(1).

Globally, the prevalence of anemia is 30% in non-pregnant women (15 to 49 years)(6). Previous studies reported that half of the anemic mothers during pregnancy start at the time of conception(7,

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8). Low hemoglobin level and anemia before pregnancy has been shown to increase risk perinatal death, low birth weight, preterm delivery, and maternal mortality(9, 10). Hence, reducing anemia in women of reproductive age is considered an essential part of improving the health of a woman, and WHO has set a global target of achieving a 50% reduction of anemia among women of reproductive age by 2025(11). The recommended policies targeted at prevention and reduction of anemia are mainly focused on the modifiable risk factors such as lifestyles, behavioral and nutritional factors(12, 13). Although there is no concrete evidence, excessive alcohol consumption might be one of the behavioral factors contributing to a higher prevalence of anemia(13). Nowadays, alcohol consumption is also one of the major public health problems in many countries(14). It is associated with various chronic medical conditions and responsible for causing about 2.5 million deaths per year and 5% of all women deaths globally (14-17).

An association between alcohol consumption and iron overload would be clinically important because alcohol and iron are believed to have synergistic hepatotoxic effects(18, 19). Besides, understanding the effect of alcohol drinking on anemia is crucial for the development of evidence-based interventions to reduce the anemia burden in Ethiopia. The association between excessive alcohol consumption and hemoglobin concentration among chronic alcoholism patients has been well described (20-23). In contrast, only few studies have investigated the effects of mild or moderate alcohol consumption among the general population (24-26). Most importantly, these few studies had inconsistent and conflicting results. Some studies in different parts of the world including Ethiopia identified alcohol drinking as risk factors for anemia (27-30). On the other hand, studies in Tanzania(26), United States(25), and Denmark(31) showed that alcoholic beverage consumption increases hemoglobin levels and suggested that moderate consumption of alcohol has a beneficial effect to reduce the prevalence of iron deficiency anemia.

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Furthermore, the majority of previous studies conducted in Ethiopia were restricted to pregnant or lactating women and women living with Human Immunodeficiency Virus infection (32-34). In addition, almost all of the previous studies were conducted in specific geographic areas that lacked national representation (35-38). Therefore, the objective of this study was to evaluate alcohol consumption on hemoglobin concentrations in non-pregnant women using nationally representative Ethiopia Demographic and Health Survey (EDHS) data to inform policymakers.

Methods and Materials

This analysis used secondary data from the 2016 EDHS. A detailed explanation of the methodology of 2016 EDHS is found somewhere else(39). Data were obtained from the DHS program website: https://www.dhsprogram.com. In 2016 EDHS, 15,683 reproductive-age women have participated in the survey. However, we restrict our analytical sample to 13,636 women reproductive age group after excluding pregnant women and women who had no hemoglobin measurement.

Study population and eligibility criteria

All non-pregnant women of reproductive age were the study population. In this study, we included women who meet the following criteria; (1). Non -pregnant women (2). Women who gave consent to draw a droplet of blood for hemoglobin measurement after reading a consent statement to woman/parent/responsible adult.

Study Variables

Outcome of interest

The main outcome of interest was anemia. Based on WHO cutoff levels, non-pregnant women with a hemoglobin value <12g/dL were considered anemic(40). This variable was used to determine categories of anemia status described as anemia (mild, moderate, severe) or normal. In

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the EDHS 2016, hemoglobin levels of the women were measured using HemoCue, and all hemoglobin values were adjusted for both altitude and smoking status.

Exposure variables

In this study, the main exposure variable is alcohol drinking (history and frequency of alcohol consumption). In 2016 EDHS, survey respondents were asked "have you ever taken a drink that contains alcohol?" For this question, two response categories were included: **Yes** and **No**.

Survey respondents who responded "yes" to the above question were also asked, "how often they drank alcohol in the last 12 months prior to the survey?" Regarding the frequency of alcohol consumption, four response categories were included: almost every day, at least once a week, less than once a week, and no in the last 12 months. However, in this study non-drinker group includes both women who hadn't ever taken a drink that contains alcohol in a lifetime and who have taken alcohol but no in the last 12 months.

Covariates

Age(15–24, 25–34, and 35–49 years), residence(urban and rural), educational status(no education, primary, secondary and higher), region (major, developing, and major city administrative), occupation (working or not working outside home at the time of the survey), marital status (married and not married), wealth index (poor, middle, and rich), media access, body mass index (BMI), number of birth in the last 5 years, access to care and source of drinking water (improved, non-improved and surface water). Previous studies have identified the above variables as determinants of anemia. Therefore, these variables were selected a priori for inclusion in regression models as potential confounders.

Region was categorized based on the Federal Democratic Republic of Ethiopia (FDRE) government administrative classification (Major, Developing, and City administrative). Media

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exposure was classified based on response to how often respondents read a newspaper, listened to the radio, or watched television. Those who responded at least once a week to any of these sources were considered to have access to media. Access to care is defined as having visited a healthcare facility within 12 months of the survey. The source drinking water is categorized into three groups according to definitions recommended by the WHO/UNICEF Joint Monitoring Programme (JMP) for Water Supply and Sanitation(41). **Improved water source** includes Piped water into dwelling, yard, or plot, Public tap or standpipe, Tube well or borehole, Protected spring, and Protected dug well. Unprotected dug well, unprotected spring, cart with small tank or drum, tanker truck, and bottled water were classified as **unimproved water sources. Surface water source** includes river, dam, lake, pond, stream, canal, irrigation channel and rainwater collection**.**

Statistical Analysis

Participant characteristics were summarized using frequency and weighted percentage. Multi-collinearity between independent variables was cheeked before fitting the final regression model. When two independent variables were found highly correlated, one was dropped.

The anemia level was first analyzed bivariably using logistic regression to estimate unadjusted odds ratios (ORs) and 95% confidence intervals (CIs). Thereafter, multivariable logistic regression models were used to calculate the ORs with 95% CIs for the presence of anemia by using women who hadn't ever taken a drink that contain alcohol as the reference group. In the multivariable model, the following variables were adjusted for: age, educational status, religion, marital status, occupation, wealth index, media exposure, chat chewing, BMI, contraceptive use, number of birth in the last 5 years, access to care and source of drinking water.

The goodness of fit of the final logistic model was tested using Hosmer–Lemeshow test at a p-value of >0.05. Adjusted odds ratios with 95% CI were used to measure the association

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between alcohol consumption and the outcome variable(anemia). All statistical techniques used a complex sampling design applied in the 2016 EDHS used a two-stage stratified sampling technique.

A Complex Samples General Linear Model (multiple linear regression and ANOVA) was also used to see if the frequency of alcohol drinking is related to the hemoglobin level, and to compare the mean difference in the levels of hemoglobin between the nondrinkers and the women in the 3 alcohol consumption frequency categories, respectively.

Patient and public involvement

There was no patient and public involvement in this study since we conducted a secondary data analysis based on already available DHS data. For the original project, data were obtained by engaging patients and the public, which was essential since biomarker data such as anemia, HIV testing, and anthropometric measurements were collected

Result

Sociodemographic and Household Characteristics of non-pregnant women

In the current analysis, a total of 13,436 non-pregnant women of reproductive age were included. Of the total women who participated in this analysis, 39.1% of the women were in the age group of 15-24 years and 78.1% lived in a rural area. Nearly half (47.9%) of women were non-educated and 72.9% got married. Half of the women had no occupation at the time of the survey. Almost a quarter of women were undernutrition (BMI<18.5 kg/m^2) and 7.4% were overweight (BMI≥25 kg/m^2). From the total, 64.4% of mothers have an improved source of drinking water (Table 1.).

Table 1. Characteristics of the study sample (N=13,436)

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Variables	Categories	Number	Weighted %
Age	15-24	5452	39.1
	25-34	4230	32.9
	35-49	3754	28.0
Residence	Urban	4500	21.9
	Rural	8936	78.1
Educational status	No education	6000	47.9
	Primary	4545	35.1
	Secondary	1911	11.5
	Higher	980	5.5
Current occupation	Not working	6817	49.4
	Working	6619	50.6
Marital status	Not married	3800	27.1
	Married	9636	72.9
Wealth index	Poor	5048	34.2
	Middle	1762	19.4
	Rich	6626	46.4
Region	Major	6385	47.5
	Developing	4579	34.1
	Major city administrative	2472	18.4
BMI status	<18.5 kg/m^2	3343	22.3
	18.5-24.99 kg/m^2	8565	70.4
	≥25.0 kg/m^2	1507	7.4
Current contraceptive use	No contraceptive use	10303	72.3
	Use hormonal contraceptive	2777	25.1

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	Use non-hormonal contraceptive	356	2.6
Number of birth in the last 5 years	No birth	7363	52.4
	One birth	3617	28.8
	Two birth	2038	15.9
	Three or more birth	418	2.8
Source of drinking water	Improved	9232	64.4
	Unimproved	868	4.4
	Surface water	3336	32.2

Weighted proportion of alcohol drinking and anemia

In this study, the weighted proportion of women who had ever taken a drink that contains alcohol was 36.3 %(95% CI: 33.4-39.2). Of the total women who had ever taken alcohol, 5.6% of women drink almost every day in the last 12 months, 32.0% of women drink at least once a week and 59.3 % of women drink less than once a week.

The mean hemoglobin level was 12.8g/dl in women who hadn't ever taken a drink that contains alcohol and 13.2 g/dl in women who had ever taken a drink that contains alcohol (P, 0.024). The overall prevalence of anemia among non-pregnant women was 23.2 %(95% CI: 21.6-24.9). The prevalence of anemia was 25.9% among women who hadn't ever taken a drink that contains alcohol and 18.5% among women who drink alcohol (Figure 1).

Figure 1: Proportion of anemia among non-pregnant women in Ethiopia

The prevalence of anemia among non-pregnant women were varied across different regions in Ethiopia. It was ranged from 15.8% in Addis Ababa to 59.1% in the Somali regional state (Figure 2).

Figure 2: Prevalence of anemia among non-pregnant women by Region, Ethiopia.

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Association between alcohol drinking and anemia

During bivariable logistic analysis, history of alcohol drinking was significantly associated with anemia (hemoglobin level). Similarly, at the multivariable-adjusted model, alcohol drinking is negatively and significantly associated with anemia after adjusted for other independent variables. The odds of having anemia was 68% less likely among women who had ever taken a drink that contains alcohol than women who hadn't ever taken a drink that contain alcohol (AOR=0.32(0.214-0.394)).

The frequency of alcohol drinking was also significantly associated with the presence of anemia in both unadjusted and adjusted analyses. The odds of having anemia is 46% lower among women who drunk alcohol less than once a week in the last 12 months as compared to women who hadn't ever taken a drink that contain alcohol (AOR=0.54(95% CI: 0.31-0.94; p=0.045)). The odds of having anemia was 50% less likely among women who drink alcohol at least once a week as compared to women who hadn't ever taken a drink that contain alcohol (AOR=0.50(0.28-0.88)). The odds of having anemia is 58% lower among women who drunk alcohol almost every day in the last 12 months as compared to not drinking alcohol (AOR=0.42(0.21-0.81)) (Table 2.).

Table 2. Association between alcohol consumption, frequency of alcohol consumption and anemia among non-pregnant reproductive women in Ethiopia, (N=13,436)

Variable		**Anemia**		
		OR (95%CI)		**P-value**
		Unadjusted analysis	**Adjusted analysis** [b]	
Alcohol drinking	Yes	0.65(0.559-0.759)	0.32(0.214-0.394)	0.014
	No	Ref.	Ref.	
Frequency of alcohol consumption in	No alcohol drink	Ref.	Ref.	
	Less than once a week[a]	0.64(0.538-0.771)	0.54(0.31-0.94)	0.045
	At least once a week	0.64(0.503-0.803)	0.50(0.28-0.88)	0.028

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the last 12 months	Almost every day	0.55(0.372-0.800)	0.42(0.21-0.81)	<0.001

Abbreviation:-AOR-adjusted odds ratio; COR-crude odds ratio; CI-confidence interval

Note:

[a] less than once per week includes women who didn't drink in the last 12 months

[b] Adjusted for age, educational status, religion, marital status, occupation, wealth index, media exposure, *chat* chewing, BMI, contraceptive, number of birth in the last 5 years, access to care, and source of drinking water.

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Multivariable linear regression

In addition to logistic regression, we also employed a multivariable linear regression model to assess the association between alcohol drinking and hemoglobin level. There is a positive association between the frequency of alcohol consumption and hemoglobin level. Women who drink alcohol less than once a week will have a hemoglobin level of 0.55g/dl higher than women who hadn't ever taken a drink that contains alcohol (β=0.55, 95% CI=0.431-0.617). Similarly, women who drink alcohol almost every day will have a hemoglobin level of 0.68g/dl higher than women who hadn't ever taken a drink that contains alcohol (β=0.68, 95% CI; 0.645-0.713) after adjusting for age, educational status, marital status, occupation, wealth index, media exposure, BMI, contraceptive, number of birth in the last 5 years, access to care and source of drinking water (Table 3 .).

Table 3. Multiple linear regression to assess the association between alcohol intake and Hemoglobin level

Variables	**Coefficient(β)[d]**	**95% CI**	**P-value**
Drink alcohol less than once a week	0.553	0.431-0.617	0.005
Drink alcohol at least once a week	0.660	0.641-0.690	0.001

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Drink alcohol almost every day	0.684	0.645-0.713	0.002
[d] adjusting for age, educational status, marital status, occupation, wealth index, media exposure, BMI, contraceptive, number of birth in the last 5 years, access to care, and source of drinking water.			

ANOVA

To compares the mean hemoglobin among different categories of alcohol drinking frequency, we used ANOVA (General Linear Model in the complex sample). Mean hemoglobin was 12.79 g/dL among non-drinkers. But, it started increasing among women who drink alcohol at least once a week and those who drink almost every day in the last 12 months. In this study, we compared each level of alcohol drinking against the non-drinkers (reference category). There was a significant difference in the mean of hemoglobin for women who drink alcohol almost every day, at least once, and less than once when compared with non-drinker women(P-value<0.001) (Table 4).

Table 4. Comparison of mean hemoglobin level between the frequencies of alcohol consumption with non-alcoholic subjects

	Frequency of alcohol consumption in the last 12 months N(%)				**P-value**
	Non-drinkers (n=8995(64.9%))	**Less than once a week** (n=2758(21.5%))	**At least once a week** (n=1406(11.6%))	**Almost every day** (n=277(2.0%))	
Mean hemoglobin with	12.79±0.049	13.15±0.049	13.23±0.068	13.25±0.102	<0.001

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standard error					
The comparison is based on simple contrasts(compare each level of alcohol drinking against the reference category(non-drinkers))					

Discussion

Both anemia and excessive alcohol consumption were a major public concern in Ethiopia. Therefore, evaluating the association between alcohol drinking and anemia is crucial for evidence-based public health intervention. The finding of this analysis revealed that the frequency of alcohol consumption has a positive significant effect on anemia (hemoglobin level). The finding is comparable with a study done in Tanzania reported alcoholic beverage consumption was a positive predictor of high hemoglobin concentration(26). A nationwide study in the United States also showed that the risks of iron deficiency and iron-deficiency anemia were approximately 40% lower among persons who consumed any amount of alcohol compared with nondrinkers (42). Similarly, a retrospective cohort study showed that blood hemoglobin concentrations are higher in heavy alcohol consumers than in abstainers. In that study, alcohol consumption of more than seven drinks/week increased the mean hemoglobin by 1.3% compared with those consuming less than or equal to seven drinks/week(43). The first possible justifications for the positive association between alcohol and hemoglobin level might be due to the fact that alcohol enhancing iron absorption (44, 45). Another hypothesis suggests that alcohol-related anemia reduction is caused at least in part by the iron present in certain alcoholic beverages such as red wine. Similarly, drinking alcohol, which contains folate, reduces the risk of anemia(46). We were unable to test this hypothesis because alcohol consumption was not subdivided according to the type of alcohol in 2016, EDHS.

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The third possible reason might be related to the type of alcohol consumed in Ethiopia. In the country, especially in rural communities, many peoples consumed homemade traditional alcoholic beverages like *tella, tej, areki, borde, and shamita* (47-49) on a daily basis. In addition, traditional alcoholic beverages are widely consumed as a prominent part of the local traditions of major social events including public holidays, weddings, funerals, and other forms of festivities. These traditional alcoholic beverages are brewed from maize, *Dagusa, Teff*, and sorghum. *Dagusa and Teff* are a good source of essential minerals especially calcium and iron(50). This might reduce the risk of anemia among alcohol drinkers. Besides, *borde* and *shamita* are alcoholic beverages with a thick consistency consumed as a meal replacement in some districts of Ethiopia(51, 52). Therefore, the protective effect of alcohol on anemia might be due to the iron content of the local alcohol beverage either from the raw material or from the fermentation.

However, the finding of this study is inconsistent with previous studies done in different parts of the world (53-56). The difference might be mainly due to the variation in the type of alcohol commonly used in different countries. A study done in India reported that moderate and chronic alcohol consumption decrease hemoglobin levels (54). The difference might be due to the difference in sample size and study setting. The previous study was done on very small sample size (n=75) and it was an institutional-based study. On the contrary, the current study used large nationally representative data.

The current study has several strengths that include a large nationally representative sample size, availability of detailed data on confounders, and standardized, high-quality data collection. However, there are several limitations to consider. First, as cross-sectional data were used, we cannot assign causality. Second, estimation of alcohol consumption in 2016 EDHS was entirely on self-reporting which might result in social desirability biases and may have been subject to

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underreporting. Some people who drink small amounts of alcohol may report their intake as none that result in the odds ratio to be systematically low.

Conclusion

The prevalence of anemia among non-pregnant women was relatively high. Consumption of any amount of alcohol was associated with a reduction in the risk of anemia among non-pregnant women. This association suggests that Ethiopian alcoholic beverages may contain iron important to erythropoiesis. Further cohort or experimental studies are therefore needed to further assess the relationship between alcohol consumption and hemoglobin level among Ethiopian populations.

List of Abbreviation

AOR-Adjusted Odds Ratio; **BMI**-Body Mass Index; **COR**-Crude Odds Ratio; **CI-**Confidence interval

Acknowledgment

Not applicable

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Ethical consideration

Not applicable

Consent for publication

Not applicable

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The data can be available from the corresponding author on a reasonable request.

Competing interest

The author declares that they have no competing interests

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Author contribution

GD: conceived of the presented idea, drafted the manuscript, and analyzed the data. **ML**: discussed the results and commented on the manuscript. All authors read and approved the final manuscript.

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Figures Legend

Figure 1: Proportion of anemia among non-pregnant women in Ethiopia

Figure 2: Prevalence of anemia among non-pregnant women by Region, Ethiopia.

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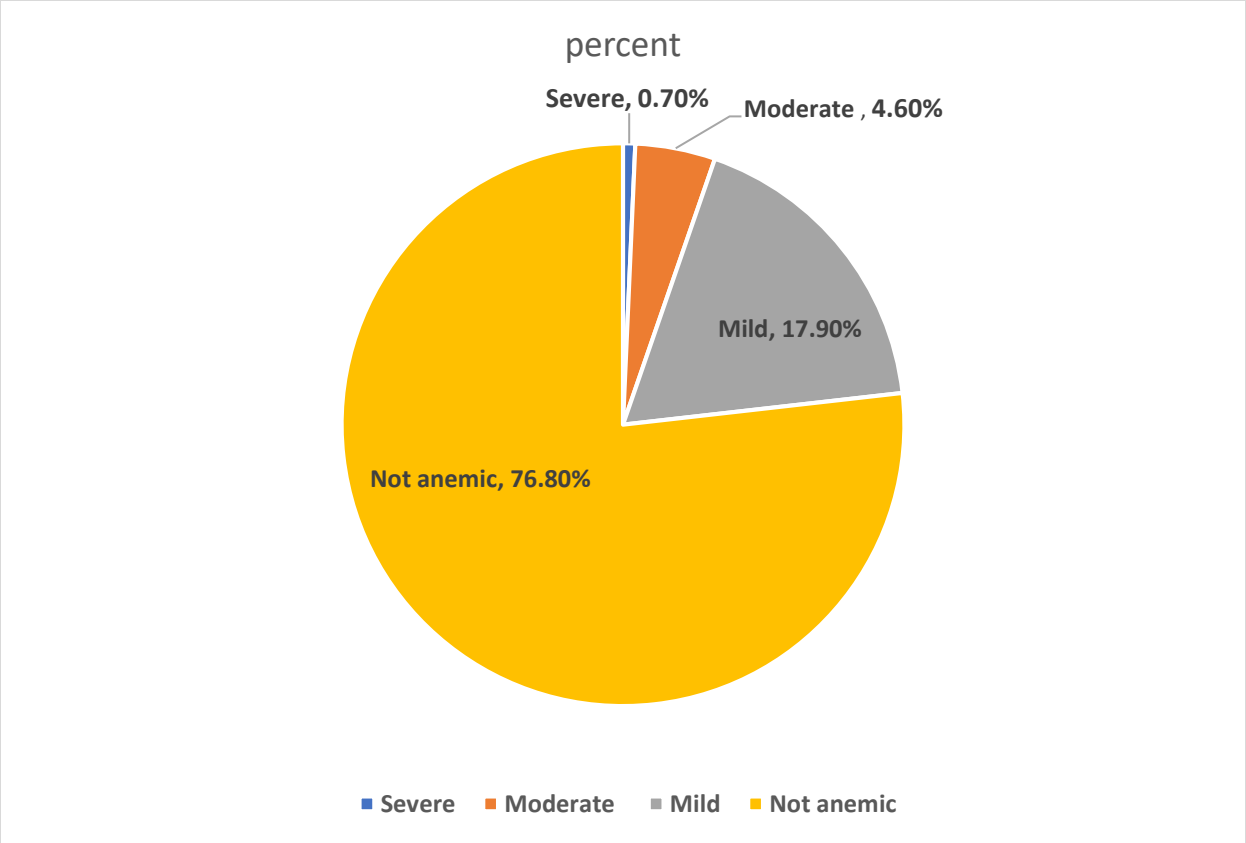

Figure 1: Proportion of anemia among non-pregnant women in Ethiopia

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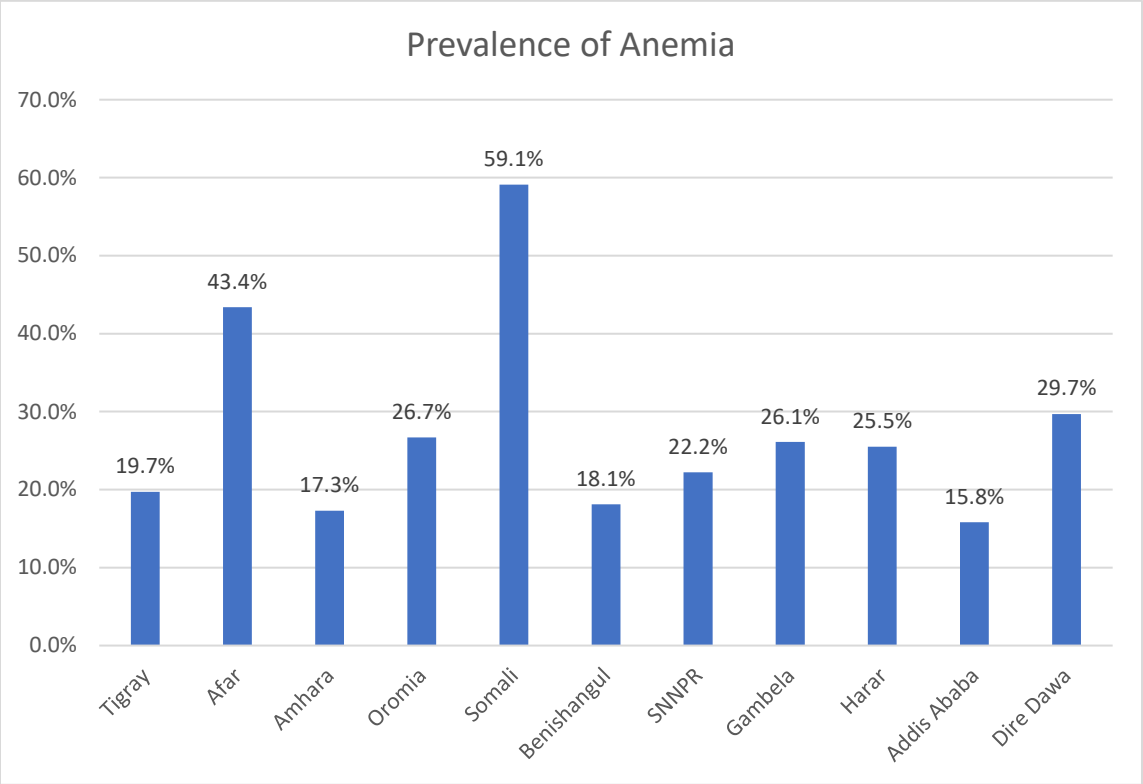

Figure 2: Prevalence of anemia among non-pregnant women by Region, Ethiopia.

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The effect of alcohol consumption on hemoglobin level among non-pregnant reproductive age women in Ethiopia: a cross sectional secondary data analysis of the 2016 Ethiopian Demographic Health Survey

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Abstract

Objective: The aim of this study was to assess the effect of alcohol consumption on hemoglobin levels among non-pregnant reproductive age women using national representative data.

Method: A secondary data analysis was conducted using data from the 2016 Ethiopia Demographic Health Survey (EDHS). The main outcome of interest was anemia defined as a hemoglobin value <12g/which was measured using HemoCue, and adjusted for both altitude and smoking status. The main exposure variable was alcohol consumption. Both multivariable logistic regression and generalized linear model were employed to assess the association between alcohol consumption and anemia and to compare the mean of hemoglobin between the nondrinkers and three alcohol consumption frequency categories, respectively, after adjusting for the potential confounders.

Result: In the current analysis, a total of 13,436 non-pregnant women were included. The overall prevalence of anemia among non-pregnant women was 23.2 % of which 17.9% were mildly anemic, 4.6%, moderately anemic, and 0.7%, severely anemic. There was a significant negative association between anemia and respondents' history and frequency of alcohol consumption in the pooled sample. Among non-pregnant women, the odds of having anemia were decreased with a history of alcohol drinking (AOR=0.32(0.214-0.394)). Women who drunk alcohol less than once a week ((AOR=0.54(95% CI: 0.31-0.94), those who drunk alcohol at least once a week (AOR=0.50(0.28-0.88)) and who drunk alcohol almost every day in the last 12 months (AOR=0.42(0.21-0.81)) had significantly lower odds of having anemia than women who hadn't ever taken a drink that contain alcohol.

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Conclusion: This study showed that nearly a quarter of non-pregnant women in Ethiopia were found to be anemic. Alcohol consumption increase hemoglobin concentration. Further cohort or experimental studies are therefore needed to further assess the relationship between alcohol consumption and hemoglobin level among Ethiopian populations.

Keywords: Alcoholics; Anemia; Hematocrit; Substance use

Strengths and limitations of this study

- ✓ Used large population-based data with a large sample size, which is representative of all regions of the country.
- ✓ As cross-sectional data were used, we cannot assign causality.
- ✓ Estimation of alcohol consumption was entirely on self-reporting which might result in social desirability biases and may have been subject to underreporting.
- ✓ The EDHS survey did not incorporate important behavioral factors(dietary intake, physical activity)

Background

Anemia is a global health problem that affects one-quarter of the world's population(1). The prevalence of anemia has gradually decreased in the developed countries, but it still remains a major public health problem in most of the developing countries particularly in Africa(2). Ethiopia is a country with the highest prevalence of anemia ranges from 17% to 52.3%(2-5). Currently, reproductive-age women were disproportionally affected groups by anemia, making it a global public health problem(1).

Globally, the prevalence of anemia is 30% in non-pregnant women (15 to 49 years)(6). Previous studies reported that half of the anemic mothers during pregnancy start at the time of conception(7,

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8). Low hemoglobin level and anemia before pregnancy has been shown to increase risk perinatal death, low birth weight, preterm delivery, and maternal mortality(9). Hence, reducing anemia in women of reproductive age is considered an essential part of improving the health of a woman, and WHO has set a global target of achieving a 50% reduction of anemia among women of reproductive age by 2025(10). The recommended policies targeted at prevention and reduction of anemia are mainly focused on the modifiable risk factors such as lifestyles, behavioral and nutritional factors(11, 12). Although there is no concrete evidence, excessive alcohol consumption might be one of the behavioral factors contributing to a higher prevalence of anemia(12). Nowadays, alcohol consumption is also one of the major public health problems in many countries(13). It is associated with various chronic medical conditions and responsible for causing about 2.5 million deaths per year and 5% of all women deaths globally (13-16).

An association between alcohol consumption and iron overload would be clinically important because alcohol and iron are believed to have synergistic hepatotoxic effects(17, 18). Besides, understanding the effect of alcohol drinking on anemia is crucial for the development of evidence-based interventions to reduce the anemia burden in Ethiopia. The association between excessive alcohol consumption and hemoglobin concentration among chronic alcoholism patients has been well described (19, 20). In contrast, only few studies have investigated the effects of mild or moderate alcohol consumption among the general population (21-23). Most importantly, these few studies had inconsistent and conflicting results. Some studies in different parts of the world including Ethiopia identified alcohol drinking as risk factors for anemia (24-27). On the other hand, studies in Tanzania(23), United States(22), and Denmark(28) showed that alcoholic beverage consumption increases hemoglobin levels and suggested that moderate consumption of alcohol has a beneficial effect to reduce the prevalence of iron deficiency anemia.

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Furthermore, the majority of previous studies conducted in Ethiopia were restricted to pregnant or lactating women and women living with Human Immunodeficiency Virus infection (29-31). In addition, almost all of the previous studies were conducted in specific geographic areas that lacked national representation (32-35). Therefore, the objective of this study was to evaluate alcohol consumption on hemoglobin concentrations in non-pregnant women using nationally representative Ethiopia Demographic and Health Survey (EDHS) data to inform policymakers.

Methods and Materials

This analysis used secondary data from the 2016 EDHS. The sampling frame used for the 2016 EDHS is the Ethiopia Population and Housing Census (PHC), which was conducted in 2007 by the Ethiopia Central Statistical Agency. The census frame is a complete list of 84,915 enumeration areas (EAs) created for the 2007 PHC. An EA is a geographic area covering on average 181 households. Administratively, Ethiopia is divided into nine geographical regions and two administrative cities. The 2016 EDHS sample was stratified and selected in two stages. Each region was stratified into urban and rural areas, yielding 21 sampling strata. Samples of EAs were selected independently in each stratum in two stages. In the first stage, a total of 645 EAs (202 in urban areas and 443 in rural areas) were selected with probability proportional to EA size (based on the 2007 PHC) and with independent selection in each sampling stratum. In the second stage of selection, a fixed number of 28 households per cluster were selected with an equal probability systematic selection from the newly created household listing. All women age 15-49 who were either permanent residents of the selected households or visitors who stayed in the household the night before the survey were eligible to be interviewed(36). Data were obtained from the DHS program website: https://www.dhsprogram.com. In 2016 EDHS, a total of 15,683 reproductive-age women have participated in the survey. However, we restrict our analytical sample to 13,636

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women reproductive age group after excluding pregnant women and women who had no hemoglobin measurement.

Study population and eligibility criteria

All non-pregnant women of reproductive age were the study population. In this study, we included women who meet the following criteria; (1). Non -pregnant women (2). Women who gave consent to draw a droplet of blood for hemoglobin measurement after reading a consent statement to woman/parent/responsible adult.

Study Variables

Outcome of interest

The main outcome of interest was anemia. Based on WHO cutoff levels, non-pregnant women with a hemoglobin value <12g/dL were considered anemic(37). This variable was used to determine categories of anemia status described as anemia (mild, moderate, severe) or normal. In the EDHS 2016, hemoglobin levels of the women were measured using HemoCue, and all hemoglobin values were adjusted for both altitude and smoking status.

Exposure variables

In this study, the main exposure variable is alcohol drinking (history and frequency of alcohol consumption). In 2016 EDHS, survey respondents were asked "have you ever taken a drink that contains alcohol?" For this question, two response categories were included: **Yes** and **No**.

Survey respondents who responded "yes" to the above question were also asked, "how often they drank alcohol in the last 12 months prior to the survey?" Regarding the frequency of alcohol consumption, four response categories were included: almost every day, at least once a week, less than once a week, and no in the last 12 months. However, in this study non-drinker group includes

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both women who hadn't ever taken a drink that contains alcohol in a lifetime and who have taken alcohol but no in the last 12 months.

Covariates

Age(15–24, 25–34, and 35–49 years), residence(urban and rural), educational status(no education, primary, secondary and higher), region (major, developing, and major city administrative), occupation (working or not working outside home at the time of the survey)(38, 39), marital status (married and not married), wealth index (poor, middle, and rich)(38, 39), media access, body mass index (BMI), number of birth in the last 5 years, access to care and source of drinking water (improved, non-improved and surface water). Previous studies have identified the above variables as determinants of anemia. Therefore, these variables were selected a priori for inclusion in regression models as potential confounders.

Region was categorized based on the Federal Democratic Republic of Ethiopia (FDRE) government administrative classification (Major, Developing, and City administrative). Ethiopia is administratively divided into 9 regional states and 2 city administration. However, the Ethiopian government classified 9 regional states in to two categories based on economic growth and availability of infrastructure. Oromia, Amhara Tigray and Southern Nation, Nationality and people were classified as developed regional states. Whereas, Afar, Somali, Gambella, Harari and Benishangul-Gumuz were considered as developing regional state. Addis Abeba and Dire Dewa were categorized as city administration. Media exposure was classified based on response to how often respondents read a newspaper, listened to the radio, or watched television. Those who responded at least once a week to any of these sources were considered to have access to media. Access to care is defined as having visited a healthcare facility within 12 months of the survey. The source drinking water is categorized into three groups according to definitions recommended

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by the WHO/UNICEF Joint Monitoring Programme (JMP) for Water Supply and Sanitation(40). **Improved water source** includes Piped water into dwelling, yard, or plot, Public tap or standpipe, Tube well or borehole, Protected spring, and Protected dug well. Unprotected dug well, unprotected spring, cart with small tank or drum, tanker truck, and bottled water were classified as **unimproved water sources. Surface water source** includes river, dam, lake, pond, stream, canal, irrigation channel and rainwater collection**.**

Statistical Analysis

Participant characteristics were summarized using frequency and weighted percentage. Multi-collinearity between independent variables was cheeked before fitting the final regression model. When two independent variables were found highly correlated, one was dropped.

The anemia level was first analyzed bivariably using logistic regression to estimate unadjusted odds ratios (ORs) and 95% confidence intervals (CIs). Thereafter, multivariable logistic regression models were used to calculate the ORs with 95% CIs for the presence of anemia by using women who hadn't ever taken a drink that contains alcohol as the reference group. In the multivariable model, the following variables were adjusted for: age, educational status, religion, marital status, occupation, wealth index, media exposure, chat chewing, BMI, contraceptive use, number of birth in the last 5 years, access to care and source of drinking water.

The goodness of fit of the final logistic model was tested using Hosmer–Lemeshow test at a p-value of >0.05. Adjusted odds ratios with 95% CI were used to measure the association between alcohol consumption and the outcome variable (anemia). All statistical techniques used a complex sampling design applied in the 2016 EDHS used a two-stage stratified sampling technique. In complex sample design, when standard errors, confidence intervals or significance testing is required, it is necessary to consider three pieces of information: the primary sampling

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unit or cluster variable, the stratification variable, and the weight variable. If we assume simple random sampling and independence of observations, the standard errors will likely be underestimated and the resulting significance tests invalid.

A Complex Samples General Linear Model (multiple linear regression and Analysis of Variance (ANOVA)) was also used. The linear regression was to quantify the strength of association between alcohol drinking frequency and hemoglobin level. . The regression coefficient describes the average change in the hemoglobin level for the expected difference in alcohol drinking frequency versus a non-drinker group. The coefficient of determination (R^2), describes the proportion of the variability in the hemoglobin that can be explained by the independent variables. All variables with p-value less than 0.2 at bivariate analysis were entered into final regression model. Multicollinearity was checked using variance inflation factor (VIF).

ANOVA is used to compare the mean difference in the levels of hemoglobin between the nondrinkers and the three alcohol consumption frequency categories (almost every day, at least once a week, less than once a week), respectively. ANOVA test showed that there is an overall mean difference between three groups. Therefore, we performed post hoc tests to confirm where the differences occurred between groups. The data met the assumption of homogeneity of variances. Hence we used Tukey's honestly significant difference (HSD) post hoc test.

Patient and public involvement

There was no patient and public involvement in this study since we conducted a secondary data analysis based on already available DHS data. For the original project, data were obtained by engaging patients and the public, which was essential since biomarker data such as anemia, HIV testing, and anthropometric measurements were collected

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Result

Sociodemographic and Household Characteristics of non-pregnant women

In the current analysis, a total of 13,436 non-pregnant women of reproductive age were included. Of the total women who participated in this analysis, 39.1% of the women were in the age group of 15-24 years and 78.1% lived in a rural area. Nearly half (47.9%) of women were non-educated and 72.9% got married. Half of the women had no occupation at the time of the survey. Almost a quarter of women were undernutrition (BMI<18.5 kg/m^2) and 7.4% were overweight (BMI≥25 kg/m^2). From the total, 64.4% of mothers have an improved source of drinking water (Table 1.).

Table 1. Characteristics of the study sample (N=13,436)

Variables	Categories	Number	Weighted %
Age	15-24	5452	39.1
	25-34	4230	32.9
	35-49	3754	28.0
Residence	Urban	4500	21.9
	Rural	8936	78.1
Educational status	No education	6000	47.9
	Primary	4545	35.1
	Secondary	1911	11.5
	Higher	980	5.5
Current occupation	Not working	6817	49.4
	Working	6619	50.6
Marital status	Not married	3800	27.1
	Married	9636	72.9
Wealth index	Poor	5048	34.2

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	Middle	1762	19.4
	Rich	6626	46.4
Region	Major	6385	47.5
	Developing	4579	34.1
	Major city administrative	2472	18.4
BMI status	<18.5 kg/m^2	3343	22.3
	18.5-24.99 kg/m^2	8565	70.4
	≥25.0 kg/m^2	1507	7.4
Current contraceptive use	No contraceptive use	10303	72.3
	Use hormonal contraceptive	2777	25.1
	Use non-hormonal contraceptive	356	2.6
Number of birth in the last 5 years	No birth	7363	52.4
	One birth	3617	28.8
	Two birth	2038	15.9
	Three or more birth	418	2.8
Source of drinking water	Improved	9232	64.4
	Unimproved	868	4.4
	Surface water	3336	32.2

Weighted proportion of alcohol drinking and anemia

In this study, the weighted proportion of women who had ever taken a drink that contains alcohol was 36.3 %(95% CI: 33.4-39.2). Of the total women who had ever taken alcohol, 5.6% of women

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drink almost every day in the last 12 months, 32.0% of women drink at least once a week and 59.3 % of women drink less than once a week.

The mean hemoglobin level was 12.8g/dl in women who hadn't ever taken a drink that contains alcohol and 13.2 g/dl in women who had ever taken a drink that contains alcohol (P, 0.024). The overall prevalence of anemia among non-pregnant women was 23.2 %(95% CI: 21.6-24.9). The prevalence of anemia was 25.9% among women who hadn't ever taken a drink that contains alcohol and 18.5% among women who drink alcohol (Figure 1).

Figure 1: Proportion of anemia among non-pregnant women in Ethiopia

The prevalence of anemia among non-pregnant women were varied across different regions in Ethiopia. It was ranged from 15.8% in Addis Ababa to 59.1% in the Somali regional state (Figure 2).

Figure 2: Prevalence of anemia among non-pregnant women by Region, Ethiopia.

Association between alcohol drinking and anemia

During bivariable logistic analysis, history of alcohol drinking was significantly associated with anemia (hemoglobin level). Similarly, at the multivariable-adjusted model, alcohol drinking is negatively and significantly associated with anemia after adjusted for other independent variables. The odds of having anemia was 68% less likely among women who had ever taken a drink that contains alcohol than women who hadn't ever taken a drink that contain alcohol (AOR=0.32(0.214-0.394)).

The frequency of alcohol drinking was also significantly associated with the presence of anemia in both unadjusted and adjusted analyses. The odds of having anemia is 46% lower among women who drunk alcohol less than once a week in the last 12 months as compared to women who hadn't ever taken a drink that contain alcohol (AOR=0.54(95% CI: 0.31-0.94; p=0.045)). The odds of having anemia was 50% less likely among women who drink alcohol at least once a week as compared to women who hadn't ever taken a drink that contain alcohol (AOR=0.50(0.28-0.88)). The odds of having anemia is 58% lower among

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women who drank alcohol almost every day in the last 12 months as compared to not drinking alcohol (AOR=0.42(0.21-0.81)) (Table 2.).

Table 2. Association between alcohol consumption, frequency of alcohol consumption and anemia among non-pregnant reproductive women in Ethiopia, (N=13,436)

Variable		Anemia		
		OR (95%CI)		P-value
		Unadjusted analysis	Adjusted analysis [b]	
Alcohol drinking	Yes	0.65(0.559-0.759)	0.32(0.214-0.394)	0.014
	No	Ref.	Ref.	
Frequency of alcohol consumption in the last 12 months	No alcohol drink	Ref.	Ref.	
	Less than once a week[a]	0.64(0.538-0.771)	0.54(0.31-0.94)	0.045
	At least once a week	0.64(0.503-0.803)	0.50(0.28-0.88)	0.028
	Almost every day	0.55(0.372-0.800)	0.42(0.21-0.81)	<0.001

Abbreviation:-AOR-adjusted odds ratio; COR-crude odds ratio; CI-confidence interval

Note:

[a] less than once per week includes women who didn't drink in the last 12 months

[b] Adjusted for age, educational status, religion, marital status, occupation, wealth index, media exposure, *chat* chewing, BMI, contraceptive, number of birth in the last 5 years, access to care, and source of drinking water.

Multivariable linear regression

In addition to logistic regression, we also employed a multivariable linear regression model to assess the association between alcohol drinking and hemoglobin level. During simple linear regression, frequency of alcohol consumption, age, educational status, marital status, occupation, wealth index, media exposure,

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BMI, contraceptive, number of birth in the last 5 years, access to care and source of drinking water had p-value less than 0.2. Therefore, all these variables were entered into final regression model. The result of final regression model showed a positive association between the frequency of alcohol consumption and hemoglobin level. Women who drink alcohol less than once a week will have a hemoglobin level of 0.55g/dl higher than women who hadn't ever taken a drink that contains alcohol (β=0.55, 95% CI=0.431-0.617). Similarly, women who drink alcohol almost every day will have a hemoglobin level of 0.68g/dl higher than women who hadn't ever taken a drink that contains alcohol (β=0.68, 95% CI; 0.645-0.713) after adjusting for age, educational status, marital status, occupation, wealth index, media exposure, BMI, contraceptive, number of birth in the last 5 years, access to care and source of drinking water (Table 3). Multicollinearity was assessed using VIF values. The result showed that all VIF values were below 10, shows absence of multicollinearity between predictor variables.

Table 3. Multiple linear regression to assess the association between alcohol intake and Hemoglobin level

Variables	Coefficient(β)[d]	95% CI	P-value
Drink alcohol less than once a week	0.553	0.431-0.617	0.005
Drink alcohol at least once a week	0.660	0.641-0.690	0.001
Drink alcohol almost every day	0.684	0.645-0.713	0.002

[d] adjusting for age, educational status, marital status, occupation, wealth index, media exposure, BMI, contraceptive, number of birth in the last 5 years, access to care, and source of drinking water.

ANOVA

To compares the mean hemoglobin among different categories of alcohol drinking frequency, we used ANOVA (General Linear Model in the complex sample). Mean hemoglobin was 12.79 g/dL among non-drinkers. But, it started increasing among women who drink alcohol at least once a

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week and those who drink almost every day in the last 12 months. In this study, we compared each level of alcohol drinking against the non-drinkers (reference category). There was a significant difference in the mean of hemoglobin for women who drink alcohol almost every day, at least once, and less than once when compared with non-drinker women(P-value<0.001) (Table 4).

Table 4. Comparison of mean hemoglobin level between the frequencies of alcohol consumption with non-alcoholic subjects

	Frequency of alcohol consumption in the last 12 months N (%)				**P-value**
	Non-drinkers (n=8995(64.9%))	**Less than once a week** (n=2758(21.5%))	**At least once a week** (n=1406(11.6%))	**Almost every day** (n=277(2.0%))	
Mean hemoglobin with standard error	12.79±0.049	13.15±0.049	13.23±0.068	13.25±0.102	<0.001
The comparison is based on simple contrasts(compare each level of alcohol drinking against the reference category(non-drinkers))					

The post hoc test result showed that there is significant difference between nondrinkers and all the remaining three groups(less than once a week, at least once a week, and almost every day)(p-value<0.001). However, there is no significant difference between a group who drink less than once a week and drink at least once a week (p-value=0.912) and there is significant difference between participants who drink alcohol

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less than a week and who drink almost every day (p-value=0.959). Similarly, there is no significant difference between the last two groups (at least once a week, and almost every day) (p-value=0.999).

Discussion

Both anemia and excessive alcohol consumption were a major public concern in Ethiopia. Therefore, evaluating the association between alcohol drinking and anemia is crucial for evidence-based public health intervention. The finding of this analysis revealed that the frequency of alcohol consumption has a positive significant effect on anemia (hemoglobin level). The finding is comparable with a study done in Tanzania reported alcoholic beverage consumption was a positive predictor of high hemoglobin concentration(23). A nationwide study in the United States also showed that the risks of iron deficiency and iron-deficiency anemia were approximately 40% lower among persons who consumed any amount of alcohol compared with nondrinkers (41). Similarly, a retrospective cohort study showed that blood hemoglobin concentrations are higher in heavy alcohol consumers than in abstainers. In that study, alcohol consumption of more than seven drinks/week increased the mean hemoglobin by 1.3% compared with those consuming less than or equal to seven drinks/week(42). The first possible justifications for the positive association between alcohol and hemoglobin level might be due to the fact that alcohol enhancing iron absorption (43, 44). Another hypothesis suggests that alcohol-related anemia reduction is caused at least in part by the iron present in certain alcoholic beverages such as red wine. Similarly, drinking alcohol, which contains folate, reduces the risk of anemia(45). We were unable to test this hypothesis because alcohol consumption was not subdivided according to the type of alcohol in 2016, EDHS. The third possible reason might be related to the type of alcohol consumed in Ethiopia. In the country, especially in rural communities, many peoples consumed homemade traditional alcoholic beverages like *tella, tej, areki, borde, and shamita* (46-48) on a daily basis. In addition, traditional

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alcoholic beverages are widely consumed as a prominent part of the local traditions of major social events including public holidays, weddings, funerals, and other forms of festivities. These traditional alcoholic beverages are brewed from maize, *Dagusa, Teff*, and sorghum. *Dagusa and Teff* are a good source of essential minerals especially calcium and iron(49). This might reduce the risk of anemia among alcohol drinkers. Besides, *borde* and *shamita* are alcoholic beverages with a thick consistency consumed as a meal replacement in some districts of Ethiopia(50, 51). The final possible justification for this positive association between alcohol consumption and hemoglobin level might be related to the effect of fermentation in traditional alcoholic beverages. Minirals like iron, calcium, and zinc are increased fermented foods (52, 53). This is due to the fact that fermentation increases the bioavailability of these minirals due to degradation of oxalates and phytates that complex with minerals thereby reducing their bioavailability (54). Therefore, the protective effect of alcohol on anemia might be due to the iron content of the local alcohol beverage either from the raw material or from the fermentation.

However, the finding of this study is inconsistent with previous studies done in different parts of the world (55-58). The difference might be mainly due to the variation in the type of alcohol commonly used in different countries. A study done in India reported that moderate and chronic alcohol consumption decreases hemoglobin levels (56). The difference might be due to the difference in sample size and study setting. The previous study was done on a very small sample size (n=75) and it was an institutional-based study. On the contrary, the current study used large nationally representative data.

The current study has several strengths that include a large nationally representative sample size, availability of detailed data on confounders, and standardized, high-quality data collection. However, there are several limitations to consider. First, as cross-sectional data were used, we

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cannot assign causality. Second, estimation of alcohol consumption in 2016 EDHS was entirely on self-reporting which might result in social desirability biases and may have been subject to underreporting. Some people who drink small amounts of alcohol may report their intake as none that results in the odds ratio to be systematically low.

Conclusion

Nearly one-fourths of non-pregnant women had anemia. Consumption of any amount of alcohol was associated with a reduction in the risk of anemia among non-pregnant women. This association suggests that Ethiopian alcoholic beverages may contain iron important to erythropoiesis. Further cohort studies are therefore needed to further assess the relationship between alcohol consumption and hemoglobin level among Ethiopian populations.

List of Abbreviation

AOR-Adjusted Odds Ratio; **BMI**-Body Mass Index; **COR**-Crude Odds Ratio; **CI**-Confidence interval

Acknowledgment

Not applicable

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Declaration

Ethical consideration

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Not applicable

Consent for publication

Not applicable

Availability of data

The data can be available from the corresponding author on a reasonable request.

Competing interest

The author declares that they have no competing interests

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Author contribution

GD: conceived of the presented idea, drafted the manuscript, and analyzed the data. **ML**: discussed the results and commented on the manuscript. All authors read and approved the final manuscript.

Figures Legend

Figure 1: Proportion of anemia among non-pregnant women in Ethiopia

Figure 2: Prevalence of anemia among non-pregnant women by Region, Ethiopia.

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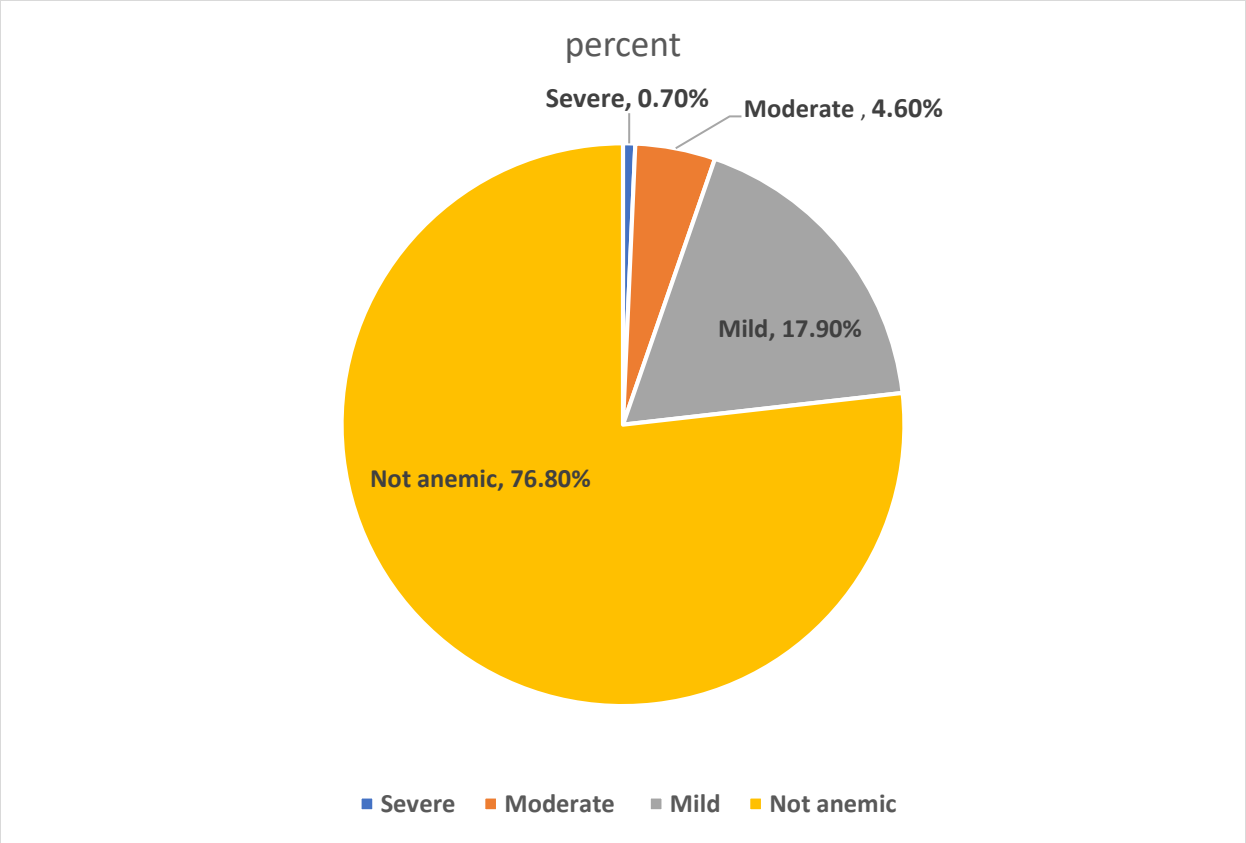

Figure 1: Proportion of anemia among non-pregnant women in Ethiopia

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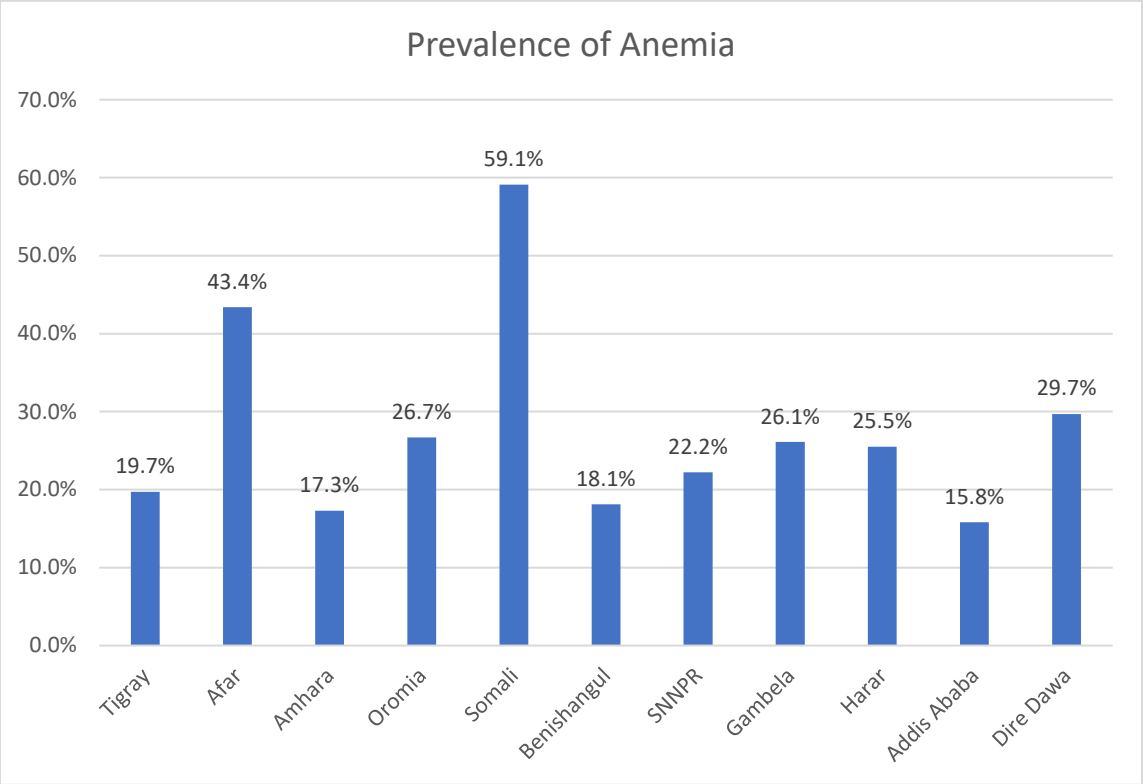

Figure 2: Prevalence of anemia among non-pregnant women by Region, Ethiopia.

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STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Items	Stated
Title and abstract	Indicate the study's design with a commonly used term in the title or the abstract	On page 1
	Provide in the abstract an informative and balanced summary of what was done and what was found	On page 2
Introduction		
Background/rationale	Explain the scientific background and rationale for the investigation being reported	On page 3 and 4
Objectives	State specific objectives, including any prespecified hypotheses	On page 5, paragraph 1
Methods		
Study design	Present key elements of study design early in the paper	On page 5, method section, paragraph 1
Setting	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	On page 5, method section, paragraph 1
Participants	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	On page 6
Variables	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	On page 6, second and third paragraph
Data sources/ measurement	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	On page 7
Bias	Describe any efforts to address potential sources of bias	Not appilicable
Study size	Explain how the study size was arrived at	On page 5 at last paragraph.
Quantitative variables	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	On page 7
Statistical methods	(*a*) Describe all statistical methods, including those used to control for confounding	On page 8 and 9, stastical analysis section
	(*b*) Describe any methods used to examine subgroups and interactions	NA
	(*c*) Explain how missing data were addressed	NA
	(*d*) If applicable, describe analytical methods taking account of sampling strategy	NA
	(*e*) Describe any sensitivity analyses	NA
Results		
Participants	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analyzed	On result section, on page 10, paragraph 1.
	(b) Give reasons for non-participation at each stage	NA
	(c) Consider use of a flow diagram	NA
Descriptive data	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	On result section, on page 10 and 11, paragraph 1 and on tabel1
	(b) Indicate number of participants with missing data for each variable of interest	No missing data
Outcome data	Report numbers of outcome events or summary measures	On result section, on

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		page 11 and 12
Main results	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Result section on page 12 and page 13 at first paragraph.in addition on table 2
	(*b*) Report category boundaries when continuous variables were categorized	NA
	(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Not done
Discussion		
Key results	Summarize From page 11-page 14 in discussion section key results with reference to study objectives	From page 16-page 17 in discussion section
Limitations	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	On page 17 and 18
Interpretation	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	page 16 and 17 in discussion section
Generalizability	Discuss the generalizability (external validity) of the study results	On page 18
Other information		
Funding	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	On page 22

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