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Association between Alkaline Phosphatase and Anemia in Rural Japanese Men: The Nagasaki Islands study

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Although bone metabolism is reportedly associated with production and maturation of blood corpuscles, and serum alkaline phosphatase (ALP) levels have been associated with bone metabolism, no published study has investigated the association between ALP and anemia. Furthermore, although ALP is known as an enzyme affected by alcohol consumption, there are no reports in the literature on associations between ALP and the risk of anemia in relation to drinking status. We conducted a cross-sectional study of 907 men aged 30-89 years undergoing a general health check-up to investigate the associations between ALP and anemia in relation to drinking status. Of the 907 participants, 120 men were diagnosed with anemia. The association between ALP and anemia was J-shaped. With the second quartile of ALP (194-228 IU/L) (Q2) as the reference group, the multivariable adjusted OR and 95%CI for anemia were 1.91 (95%CI: 0.96-3.82) for <194 IU/L (Q1), 1.84 (95%CI: 0.93-3.62) for 229-277 IU/L (Q3) and 2.83 (95%CI:1.49-5.37) for >277 IU/L (Q4). When the analysis was limited to non-drinkers, the associations became stronger with corresponding values of 3.34 (95%CI: 1.28-8.74), 3.18 (95%CI: 1.28-7.88) and 3.22 (95%CI:1.37-7.59). Not only lower but also higher levels of serum ALP are associated with anemia for men, especially non-drinkers. For analyses of associations between ALP and anemia, alcohol consumption should thus be considered a potential confounder.

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1. Introduction

Alkaline phosphatase (ALP) is an enzyme that catalyzes the hydrolysis of inorganic pyrophosphate.[1] ALP is expressed in a variety of tissues, but its concentrations are notably high in bone, liver, and kidney.[1] Previous studies reported that osteoblastic cells regulate the hematopoietic stem cell niche and are also important for production of blood corpuscles including red blood cells.[2,3] Because activity of osteoblasts can be evaluated by bone-type ALP expression,[1,4] lower ALP might be associated with anemia. Furthermore, under anemic conditions, activity of osteoblasts might increase to compensate for hemoglobin insufficiency.[5] These findings led us to speculate that not only reduced, but also elevated ALP levels may be associated with anemia.

However, women might not be suitable subjects for such an investigation not only because ALP is confounded by menopausal status,[4,6] but also because hemoglobin levels are

confounded by menstruation.

On the other hand, serum ALP levels are influenced by alcohol consumption,[7,8] which has been found to be positively associated with hemoglobin levels.[9] Since the prevalence of drinkers is high among Japanese men,[10] a study to investigate the association between serum ALP and anemia among Japanese men should also take drinking status into account.

We therefore limited our investigation to community-dwelling Japanese men aged 30-89 who participated in a general health check-up between 2005 and 2010.

2. Material and Methods

2.1 Participants

This study was approved by the Ethics Committee for Human Use of Nagasaki University (project registration number: 0501120073).

The source population included 1,355 residents, 30 to 89 years old, of the western rural community of the Goto Islands, who participated in this study between 2005 and 2010. Data for blood pressure were missing for 3 men, for drinking status for 23 men, and for serum for 422 men so that a total of 448 subjects were excluded from this study. The remaining 907 men with a mean age of 65.1 years ($\pm$10.5 standard deviation (SD); range 30-89) were enrolled in this study.

2.2 Data Collection and Laboratory Measurements

Trained interviewers obtained information on smoking status, drinking status and medical history, as well as on the use of antihypertensive agents and medication for diabetes mellitus. Body weight and height were measured with an automatic body composition analyzer (BF-220; Tanita, Tokyo, Japan) when blood samples were obtained. Systolic and diastolic blood pressures were recorded at rest.

Fasting blood samples were collected in an EDTA-2K tube and a siliconized tube. Samples from the EDTA-2K tube were used for measuring hemoglobin with the sodium lauryl surfate (SLS)-hemoglobin method. Serum was separated and stored at $-20°C$ until assay. Following collection of blood samples after overnight fasting, serum and plasma were separated and stored at $-20°C$ and $-80°C$, respectively, until assay. Serum concentrations of triglyceride (TG), hemoglobin A1c (HbA1c), γ-glutamyltranspeptidase (GTP), alkaline phosphatase (ALP) and serum creatinine were measured with standard laboratory procedures.

The glomerular filtration rate (GFR) was estimated by means of an established method with three variations recently proposed by a working group of the Japanese Chronic Kidney Disease Initiative.[11] According to this adapted version, the formula for determining GFR was: GFR(mL/min/1.73 m^2) = 194 $\times$ (serum creatinine (enzyme method))$^{-1.094}$ $\times$ (age)$^{-0.287}$ $\times$ (0.739 for women). HbA$_{1c}$ was calculated with the following equation based on standardization by the National Glycohemoglobin Standardization Program (NGSP) and recently proposed by a working group of the Japanese Diabetes Society (JDS): HbA$_{1c}$(NGSP) = HbA$_{1c}$(JDS) + 0.4%.[12] Diabetes was defined as HbA$_{1c}$(NGSP) $\geq$ 6.5%, and/or initiation of glucose-lowering medication or insulin therapy.[13] Anemia for men was defined in accordance with the recommendation by the World Health Organization (WHO) Study Group: hemoglobin <13 g/dL.[14]

2.3 Statistical Analysis

Differences in age-adjusted mean values or prevalence of potential confounding factors were calculated by ALP quartile using covariance or general linear models, and logistic regression models were used for calculating odds ratios (OR) and 95% confidence intervals (CI) to determine associations of anemia with ALP values.

Two different approaches were used to adjust for confounding factors. The first adjustment was only for age, and the second consisted of other possible confounding factors, that is, smoking status (never smoker, former smoker, current smoker), alcohol consumption (non-drinker, current light to moderate drinker (1-6 times/week) and current heavy drinker (every day)), diabetes (no, yes), history of cardiovascular disease (no, yes), history of liver disease (no, yes), thyroid disease (no, yes), TG (mg/dL), γ-GTP (IU/L) and GFR (mL/min/1.73m^2). In addition, subjects were stratified by drinking status because alcohol consumption has been associated with ALP levels,[7,8] and several studies reported that hemoglobin levels of drinkers were higher than those of non-drinkers.[8,15]

All statistical analyses were performed with the SAS system for Windows (version 9.3; SAS Inc., Cary, NC). All p-values for statistical tests were two-tailed, and values of <0.05 were regarded as statistically significant.

3. Results

Of the 907 men, 120 were diagnosed with anemia. The prevalence of non-drinkers was 49.1%.

Table 1 shows age-adjusted baseline characteristics by

ALP level. Serum γ-GTP was positively associated, and current drinker status inversely associated with ALP levels. No significant association was observed for hemoglobin.

Table 2 shows the odds ratios (OR) and 95% confidence intervals (CI) for anemia according to ALP levels. The association between ALP levels and anemia was J-shaped. With second quartile (Q2) of ALP (194-228 IU/L) as the reference group, the multivariable adjusted OR and 95%CI for anemia were 1.91 (95%CI: 0.96-3.82) for <194 IU/L (Q1), 1.84 (95%CI: 0.93-3.62) for 229-277 IU/L (Q3) and 2.83 (95%CI:1.49-5.37) for >277 IU/L (Q4).

We also investigated the influence of drinking status [non-drinker, current light-to-moderate drinker (1-6 times/week), and current heavy-drinker (every day)] on ALP and hemo-

globin levels. The association for ALP was significantly inverse while that for hemoglobin was significantly positive. The age-adjusted mean values of ALP were 253 for non-drinkers, 238 for current light-to-moderate drinkers, and 234 for current heavy drinkers (P=0.006) and the corresponding values of hemoglobin were 14.2 g/dL, 14.4g/dL and 14.6g/dL (P<0.001).

Table 3 shows OR and 95% CI for anemia by ALP level in relation to drinking status. The J-shaped association between ALP levels and anemia was observed only for non-drinkers. With the second quartile (Q2) as the reference group, the multivariable adjusted OR and 95%CI for anemia were 3.34 (95%CI: 1.28-8.74) for Q1, 3.18 (95%CI: 1.28-7.88) for Q3 and 3.22 (95%CI:1.37-7.59) for Q4.

Table 1. Age-adjusted mean values and proportions in terms of alkaline phosphatase (ALP) levels.

	Alkaline phosphatase (ALP) quartiles				p
	Q1 (low)	Q2	Q3	Q4 (high)	
Median values of Serum alkaline phosphatase (ALP), IU/L	173	209	251	318	
No. at risk	222	234	222	229	
Age, year	64.5 ± 10.1	63.9 ± 11.5	65.6 ± 10.4	66.6 ± 9.9	
Systolic blood pressure, mmHg	140	142	143	141	0.434
Antihypertensive medication use, %	26.5	25.3	27.0	28.8	0.849
Body mass index, kg/m^2	24.0	23.8	23.6	23.4	0.543
Current drinker, %	61	52	47	44	0.003
Current smoker, %	18	23	26	30	0.543
Diabetes, %	8.2	7.1	9.8	12.8	0.174
History of cardiovascular disease, %	7.6	7.9	5.2	9.3	0.405
History of liver disease, %	1.8	3.1	1.8	1.2	0.552
Thyroid disease, %	0.0	0.4	0.9	0.0	0.903
Hemoglobin, g/dL	14.3	14.4	14.4	14.3	0.903
Serum triglycerides (TG), mg/dL	128	138	150	141	0.074
Serum γ-glutamyltranspeptidase (γ-GTP), IU/L	36	41	50	61	<0.001
Serum creatinine, mg/dL	0.91	0.95	0.93	0.92	0.472
Glomerular Filtration Rate (GFR), mL/min/1.73m^2	69.2	67.6	68.1	69.7	0.523

Age: mean ± standard deviation. p: p factor. Serum alkaline phosphatase (ALP) levels for quartiles were <194IU/L, 194-228IU/L, 229-277IU/L, and >277IU/L.

Table 2. Odds ratios (OR) and 95% confidence intervals (CI) for anemia by ALP level quartile for all subjects

	ALP quartiles					1 SD increments in ALP
	Q1 (low)	Q2	Q3	Q4 (high)	P for trend	
No. at risk	222	234	222	229		
No. of cases (percentages)	28 (12.6)	19 (8.1)	28 (12.6)	45 (19.7)		
Age-adjusted OR	1.74 (0.92-3.29)	1.00	1.55 (0.82-2.94)	2.56 (1.41-4.66)	0.002*	1.32 (1.09-1.60)*
Multivariable OR	1.91 (0.96-3.82)	1.00	1.84 (0.93-3.62)	2.83 (1.49-5.37)	0.001*	1.45 (1.15-1.81)*

Multivariable OR: adjusted further for age, systolic blood pressure, antihypertensive medication use, body mass index, smoking, alcohol intake, diabetes, thyroid disease, history of cardiovascular disease, history of liver disease, serum triglycerides, serum γ-glutamyltranspeptidase (γ-GTP) and glomerular filtration rate (GFR). Anemia : defined as hemoglobin <13.0g/dL. ALP levels for quartiles were <194IU/L, 194-228IU/L, 229-277IU/L, and >277IU/L. * : analyses were performed for ALP ≥ 194IU/L.

Table 3. Odds ratios (OR) and 95% confidence intervals (CI) for anemia by ALP level quartile in relation to drinking status

	ALP quartiles					1 SD increments in ALP
	Q1 (low)	Q2	Q3	Q4 (high)	P for trend	
Non-drinkers						
No. at risk	86	112	118	129		
No. of cases (percentages)	18 (20.9)	10 (8.9)	22 (18.6)	31 (24.0)		
Age-adjusted OR	2.84 (1.21-6.67)	1.00	2.61 (1.14-5.99)	3.12 (1.41-6.89)	0.006*	1.41 (1.08-1.85)*
Multivariable OR	3.34 (1.28-8.74)	1.00	3.18 (1.28-7.88)	3.22 (1.37-7.59)	0.011*	1.46 (1.06-2.00)*
Drinkers						
No. at risk	136	122	104	100		
No. of cases (percentages)	10 (7.4)	9 (7.4)	6 (5.8)	14 (14.0)		
Age-adjusted OR	0.94 (0.35-2.53)	1.00	0.50 (0.16-1.58)	1.78 (0.69-4.62)	0.200*	1.24 (0.92-1.66)*
Multivariable OR	0.71 (0.21-2.45)	1.00	0.82 (0.23-2.89)	2.56 (0.80-8.16)	0.098*	1.48 (0.97-2.25)*

Multivariable OR: adjusted further for age, systolic blood pressure, antihypertensive medication use, body mass index, smoking, alcohol intake, diabetes, thyroid disease, history of cardiovascular disease, history of liver disease, serum triglycerides, serum γ-glutamyltranspeptidase (γ-GTP) and glomerular filtration rate (GFR). Anemia : defined as hemoglobin <13.0g/dL. ALP levels for quartiles were <194IU/L, 194-228IU/L, 229-277IU/L, and >277IU/L. * : analyses were performed for ALP ≥ 194IU/L.

4. Discussion

Our findings demonstrate that the association between serum ALP and anemia is J-shaped and that this association is confined to non-drinkers. For analyses of associations between ALP and anemia, alcohol consumption should thus be considered a potential confounder.

A previous study using 69,864 ALP measurements reported that 130 (0.19%) of them showed low ALP (<30U/L; reference range 30-115 U/L) and that 88 (67.7%) of these were comparable in age and sex and that only one case (1.2%) showed severe anemia.[16]

In our study, subjects whose ALP level was in the lowest quartile showed associations with anemia especially for non-drinkers. We found further evidence that not only lower but also higher levels of ALP were associated with anemia, again especially for non-drinkers.

Side population hematopoietic stem cells in bone marrow decrease as individuals age,[17,18] and this decline may be associated with an increase in the frequency of anemia and other hematopoietic disorders that are seen in the elderly.[19] Because osteoblasts, whose activity can be evaluated by bone-type ALP expression,[4, 20] regulate the production of hematopoietic stem cells in bone marrow,[2,3,20] serum ALP levels may correlate with production of hematopoietic stem cells which also constitute an important determinant factor for hemoglobin level. Lower ALP levels may therefore be associated with anemia by signifying lower production of red blood cells. Lack of hyperfunction of bone marrow to compensate for anemia may also constitute an important mechanism for those associations. Under anemic conditions, activity of bone-marrow should increase to compensate for hemoglobin insufficiency.[5] However, if the osteoblasts cannot be activated by anemia, lower ALP may be associated with anemia.

A few previous reports have dealt with associations between ischemic stroke and anemia.[21,22] And one of our studies, The Circulatory Risk in Community Study (CIRCS), found that not only higher but also lower ALP levels constitute a risk for stroke might partly support the mechanism between lower ALP and anemia.[7]

On the other hand, the mechanisms that account for higher ALP also being associated with anemia have not been elucidated. Since ALP may indicate productivity of hematopoietic stem cells,[2-4,20] higher ALP should be reflected in higher hemoglobin levels. In our present study, however, hemoglobin was not associated with ALP levels, whereas anemia showed a significantly association. This may partly be explained by a different cause of anemia that is not only lower productivity of hemoglobin but also a drastic reduction of hemoglobin such as bleeding. To compensate for anemia caused by hemoglobin reduction, activity of hematopoietic stem cell production in bone marrow may increase, which would also results in ALP elevation. As for the reduced hemoglobin productivity, the increased activity of hematopoietic stem cell production in bone marrow is comparatively lower than that of caused by bleeding, which would result in low ALP levels (Fig 1). Those mechanisms may act as a confounding factor on the association between hemoglobin and ALP, so that not only compensated anemia with hemoglobin levels above 13g/mL but also not yet compensated anemia (Hb<13g/mL) may be an indication of higher ALP.
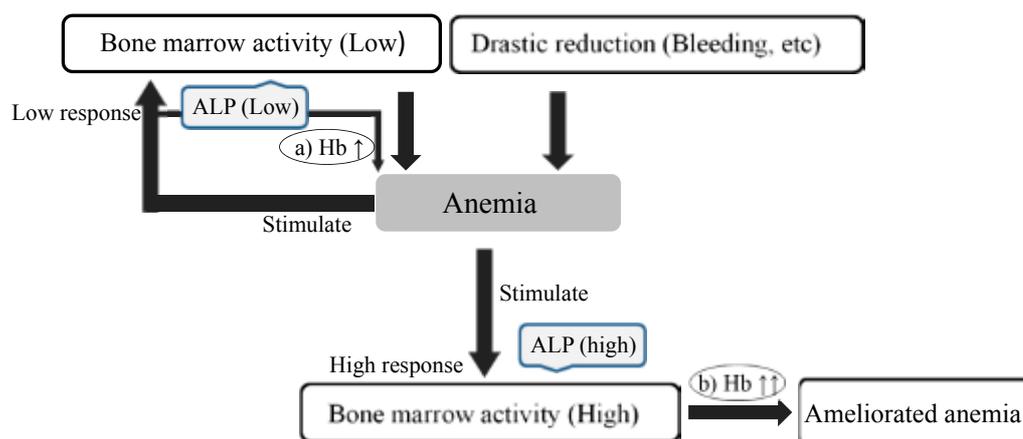
Figure 1. Association between serum ALP and anemia. Hb:hemoglobin. Ability of compensation for anemia is a) (Hb↑) < b) (Hb↑↑).

This study has certain potential limitations, which warrant consideration. First, because the ALP isozyme was not measured,[23] we could not assess which type of ALP was associated with the risk of anemia. Second, only limited data for thyroid disease were available, which may have confounded the associations between ALP and risk of anemia.[9, 24] Further investigations using thyroid function data are therefore necessary. Third, since no reticulocyte data were available, we could not evaluate any direct association between ALP and production of red blood cells. However, although we were able to establish a significant association between ALP and anemia, the age-adjusted mean values of hemoglobin showed no significant association with ALP. This could be partly explained by the effects of compensation for anemia. Further, although the association between ALP levels and risk of anemia was shown to be independent of the traditional risk factors, we did not adjust for other potential confounders whose values were associated with ALP, such as calorie, protein, vitamin C, magnesium, and zinc deficiencies,[16] nor the volume of alcohol consumption.

In conclusion, not only lower but also higher levels of serum ALP are associated with anemia among men, especially for non-drinkers. For analyses of associations between ALP and anemia, alcohol consumption should thus be considered a potential confounder.

Acknowledgment

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