Correlation between RBC changes and coagulation parameters in high altitude population

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Introduction

It was reported that over 140 million people worldwide permanently live at altitudes >2500 m above sea level. Low ambient temperature and hypobaric hypoxia are two challenges to life at high altitude (HA) [1,2], with an increased risk of thrombotic diseases following prolonged stay at HA [3–6]. The environmental conditions in HA affect the morphology and phenotype of blood cells [3]. Red blood cell (RBC) count, hemoglobin (HGB) concentrations and hematocrit (HCT) would reach a fairly high level in most of the individuals following long-term exposure to HA situations. Moreover, excessive erythropoiesis, higher HCT and overburdened HGB will lead to hyperviscosity [3,4,7–9].

Except the change of blood flow, thrombosis also involves platelet-vessel interactions and coagulation system [10]. Many studies suggested that HA-induced thromboembolic disorders are a complex or multifactorial trait. The relationship between HA and factors which may increase the incidence of thrombosis have well documented [11], such as HA and RBC, HA and main clotting factors, but whether there is any association among these factors has not been studied.

The objective of this study was to evaluate the correlation of main RBC index (HGB and HCT) with the basic coagulation parameters, through a large cross-sectional study within volunteers reside in Aba Tibetan and Qiang Autonomous Prefecture (>2500 m, located in northwestern Sichuan province of China) and provide data for further studies on the potential mechanism of high altitude-induced thrombotic disease.

Materials and methods

Ethics statement

In this study, the recruited volunteers provided anonymous informed consent, with the information of age, ABO type and gender, according to the requirements of the Ethics Committee of the Institute of Blood Transfusion. All procedures concerning the experiments with human plasma had been given prior approval by the Academic Board of Institute of Blood Transfusion. All clinical research was conducted according to the principles revealed in the Declaration of Helsinki. Participants received no remuneration for their role in the research.

Sample collection and preparation

All the 433 Tibetan volunteers who aged 18y–84y and with no organic disease were resident at high altitudes...
 (>2500 m) more than 1 year. Samples were taken at People’s Hospital of Aba Tibetan and Qiang Autonomous Prefecture, which located in Aba Tibetan and Qiang Autonomous Prefecture, Sichuan Province, China. Inclusion criteria were that all volunteers were ≥18 years and unrelated. Individuals who had prior history of thrombus or hemorrhage, usage of oral anti-coagulant therapy, hepatic disease, HIV infection, pregnancy, diabetes and others were excluded from this study. Plasma samples were drawn in 0.129 M sodium citrate in 9:1 volume ratio by plasmapheresis and then, aliquots were obtained and stored at −70°C until analysis. The samples were divided into the following categories as Table 1 showed and the basic information of volunteers were showed in Table 2.

### Laboratory assays

Hematological parameters (i.e. HGB concentration, HCT and PLT counts) were assessed using an automated hematology analyzer (Sysmex XE 2100, Kobe, Japan). Prothrombin time (PT), activated partial thromboplastin time (APPT), thrombin time (TT) and fibrinogen (Fbg) were measured by clotting assays using Siemens Healthcare Diagnostics Products GmbH (Marburg, Germany) on a CA-1500 automated coagulation analyzer (Sysmex Corporation, Kobe, Japan).

### Statistical analysis

Kolmogorov–Smirnov test was used for the normal distribution of all data, and values were expressed as means and standard deviation (SD) or as medians and quartiles. When appropriate, multi-group comparisons were conducted by one-way ANOVA followed by LSD posthoc test. Partial correlation analysis and multivariable linear regression analysis were used to check the association between basic coagulation parameters (APTT, PT, TT, Fbg and PLT) and RBCs indexes (HGB and HCT). A correlation coefficient (r) of 0.10–0.29 indicates a small correlation, 0.30–0.49 a moderate correlation and 0.50–1.0 a high correlation. Statistical analysis was performed using SPSS statistics software, version 17.0 (SPSS Inc., Chicago, IL, U.S.A.). A 95% CI [2.5%–97.5%] was used and a p-value <0.05 was considered statistically significant.

### Results

#### Effect of HGB and HCT on APTT and PT

As showed in Figure 1(A,B), APTT and PT in group 4 of HGB were significantly higher than group 1, group 2 and group 3 (42.3 ± 15.4 sec vs. 38.2 ± 12.8 sec vs. 38.1 ± 12.7 sec vs. 38.1 ± 13.2 sec for APTT, 14.2 ± 2.9 sec vs. 13.0 ± 1.6 sec vs. 12.6 ± 1.7 sec vs. 12.9 ± 2.2 sec for PT, all p < 0.05). Small negative correlations between APTT and HGB (r = −0.168, p < 0.001, Figure 2(A)), and PT and HGB (r = −0.165, p = 0.001, Figure 2(B)) were found by Partial correlation analysis. Further, in a multivariable regression model, APTT and PT were associated with a decrease in HGB (b = −0.478 and −0.072, both p < 0.01; Table 3).

Similarly, APTT and PT in group 4 of HCT were significantly higher than other three groups (42.4 ± 15.3 sec vs. 37.6 ± 12.8 sec vs. 38.1 ± 12.5 sec vs. 38.6 ± 13.4 sec for APTT, 14.3 ± 2.9 sec vs. 12.9 ± 1.6 sec vs. 12.6 ± 1.4 sec vs. 12.9 ± 2.3 sec for PT, all p < 0.05; Figure 1(F,G)). And, there was a small positive association between APTT and HCT (r = 0.225, p < 0.001; Figure 2(C)), and PT and HCT (r = 0.258, p < 0.001; Figure 2(D)). Moreover, using multivariable regression analysis, APTT and PT retained to have a significant relationship with HCT (b = 2.042 and 0.363, both p < 0.001; Table 3).

#### Effect of HGB and HCT on PLT

Contrary to the results of APTT and PT we assessed in this study, PLT in group 4 of HGB and HCT were
significantly lower than group 1, group 2 and group 3 ((182.0 ± 56.7)×10⁹/L vs. (231.6 ± 77.1)×10⁹/L vs. (220.3 ± 56.1)×10⁹/L vs. (215.0 ± 55.3)×10⁹/L for HGB groups and (181.5 ± 56.6)×10⁹/L vs. (226.4 ± 76.5)×10⁹/L vs. (225.0 ± 57.4)×10⁹/L vs. (217.3 ± 56.0)×10⁹/L for HCT groups; all p < 0.01; Figure 1(E,J)). No correlation was found between PLT and HGB, and PLT and HCT.

**Effect of HGB and HCT on TT and Fbg**

TT and Fbg showed no significant distinction among different HGB groups (all p > 0.05; Figure 1(C,D)), and so did HCT groups (all p > 0.05; Figure 1(H,I)). Furthermore, no correlation was found between HGB and TT, HGB and Fbg, HCT and TT, and HCT and Fbg (all p > 0.05).

**Discussion**

With increasing altitude, a progressive reduction in barometric pressure can be observed and one of HA (2500 m above sea level) features is low oxygen pressure [12,13]. It was reported that staying at HA may increase the risk of thrombotic diseases. Hypoxia may induce significant changes of the factors which contribute to thrombosis, such as blood pressure elevation, increase of RBC, HGB and HCT levels, prolongation of PT and APTT, decrease of PLT count and et al. [3,4,13–15]. However, it is still unclear that whether these factors are interrelated. The present study was designed to gain insights into relationship among major factors which play a critical role in thrombosis in hypoxic environment through the study of influences of main RBC indexes (HGB concentration and HCT) on main clotting parameters (PT, APTT, TT, Fbg and PLT count) in HA population.

As RBCs are key players in systemic oxygen transport, in line with their vital role in oxygen transport and delivery, they play a clear role in adaptations to hypoxia [16]. In addition, the function of RBC can reflect the amount of RBC and HGB. It was reported

![Figure 1](image_url). Basic coagulation parameters in different groups. The box plots encompass the 25th–75th quartiles, with the centre lines (—) representing the median values and the solid boxes (▪) showing the mean values. The whisker plots represent 95% CI. * p < 0.05; ** p < 0.01. The differences between main RBC indexes (HGB and HCT) and basic coagulation parameters (APTT, PT, TT, and Fbg), and main RBC indexes and platelet were calculated using one-way ANOVA followed by LSD post hoc test. (A)–(E) Basic coagulation parameters in different HGB groups; (F)–(J) Basic coagulation parameters in different HCT groups.
that in response to the insufficient amount of oxygen, HGB and HCT levels significantly increased at HA. The increased HGB is potentially important for improving the oxygen-carrying capacity of RBCs, meanwhile significantly increased HCT values will lead to hyperviscosity and lower blood flow velocity which may augment clinical risks of thrombosis [2-4,12-14]. Thus, the effects of HGB and HCT on coagulation parameters may be different.

PT and APTT tests are commonly ordered in the evaluation of bleeding symptoms and they are measures of the procoagulant cascade. Some studies suggested that changes in the coagulation profile at HA can activate the coagulation cascade and lead to increased thrombosis [15]. However, it seems to be contradictory to that HA is a risk factor for thrombosis, since some studies demonstrated that PT and APTT notably prolonged at HA [2,11,16-19]. And as expected, the results of our study showed HGB and HCT had different influence on APTT and PT: HGB was weakly and negatively associated with APTT and PT ($r = -0.168$ and $r = -0.165$ resp., both $p < 0.001$), otherwise, there was a small positive association among HCT with APTT and PT ($r = 0.225$ and $r = 0.258$ resp.; both $p < 0.001$). These results may be explained by the fact that hypoxia-induced compensatory hyperplasia of RBCs and the increasing of blood viscosity may accelerate the consumption of coagulation factors and ultimately prolonged PT and APTT. It should be noted that correlations between the included variables were weak, they were probably an epiphenomenon, some statistical findings of changes produced by high-altitude itself, and these weak correlations may have little clinical or physiological significance. And it is difficult to make firm conclusions between the erythrocytes and the basic parameters of coagulation with these results. Furthermore, it was reported that some factors such as hypoxia-inducible factor-1 (HIF-1) can influence erythropoiesis and endothelial activation, which finally led to the changes of RBCs and coagulation cascade [20]. Therefore, further studies on these factors are needed to determine the potential mechanism of high altitude-induced thrombotic disease.

**Table 3.** Associations between coagulation parameters and RBCs indexes by multivariable analysis.

| APTT | PT | TT | Fbg | PLT |
|------|----|----|-----|-----|
| HGB  | HCT| HGB| HCT | HGB | HCT | HGB| HCT | HGB| HCT |
| $b$  | -0.478 | 2.042 | -0.072 | 0.363 | -0.029 | 0.086 | -0.019 | 0.067 | -0.690 | -1.036 |
| $\rho$ | <0.001 | <0.001 | 0.001 | <0.001 | NS | NS | NS | NS | NS | NS |
| $R^2$ | 0.095 | 0.187 | 0.004 | 0.003 | NS | NS | NS | NS | NS | NS |
PLT is another key factor which plays an indispensable role in thrombogenesis. Our study showed PLT count had no correlation with HGB and HCT in HA volunteers. In addition, there were studies showed different effect of HA conditions on PLT count [11]. Wang et al. showed that PLT counts were lower ($p = 0.017$) in HA than in low altitude [14]. Vij, Lehmann et al., Gray et al. and Chatterji et al. consistent with this result [19,21–23]. Conversely, Sharma, Simon-Schnass et al., Hartmann et al., Hudson et al. and Kotwal et al. demonstrated an elevation in PLT count at HA [24–28]. Furthermore, Sharma found there was no significant difference in PLT counts when 50 subjects were moved from low altitude to 3650 m [29].

These results may be explained by the fact that PLT function may be influenced by physiological changes that occur during adaptation to HA, such as increase of aggregation and adhesion of PLT, but not PLT function may be in HA [24]. Kotwal et al. demonstrated an elevation in PLT count at HA [24–28]. Furthermore, Sharma found there was no significant difference in PLT counts when 50 subjects were moved from low altitude to 3650 m [29]. These results may be explained by the fact that PLT function may be influenced by physiological changes that occur during adaptation to HA, such as increase of aggregation and adhesion of PLT, but not PLT function may be in HA [24].

Another blood viscosity parameter is Fbg, and TT is used to diagnose blood coagulation disorders and to assess the effectiveness of fibrinolytic therapy. Many studies showed, plasma Fbg level increased at HA and it was positively associated with PAI-1 level [5,11,14,19,28]. PAI-1 is an inhibitor of plasminogen activator and can decrease levels of plasmin, resulting in a decrease of fibrinolytic activity, and thus causes a tendency to thrombosis [28]. The results of our study showed that HGB and HCT had no significant effect on Fbg and TT, which suggest that the effect of Fbg on thrombotic diseases is relatively independent of RBCs at HA, and its mechanism of action remains to be further studied.

Limitations

Naturally, some limitations of the study should be considered. The first limitation of this study is its cross-sectional nature. Second, volunteers are often not fully comparable because they are at different altitude levels and are confounded by a number of different factors, such as climate and latitude. Finally, in this study, we provide evidence that HGB concentration and HCT were showed different effects on factors contributing to clot strength such as PLT count, Fbg, PT, APTT and TT, but how these parameters might relate to the risk of subsequent thrombotic events is not clear.

Conclusion

In summary, this study initiated the evaluation of the influences of HGB concentration and HCT on APTT, PT, TT, Fbg and PLT count in a HA population and provided evidence that HGB and HCT may not correlate with APTT and PT, and no associations were observed among TT, Fbg, PLT and HGB, TT, Fbg, PLT and HCT. These new data showed the changes of RBC, Fbg and PLT at HA may have independent implications to the risk of thrombotic events. Our findings might have important implications for further studies of the formation mechanism of altitude-related thromboses. And we hope that our study would encourage further research to explore the multifactorial induced thromboembolic disorders at HA.

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Disclosure statement

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