The Percentage of Hemoglobin A_2 in the Participants with Hemoglobin E

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Abstract:

Objective: To quantify the percentage of hemoglobin A_2 in the participants with Hb E trait and Hb E disease and to compare them with that of the normal participants. **Participants and Methods:** This retrospective study was conducted at the Department of Medicine, Maharat Nakhon Ratchasima Hospital recruiting the consecutive healthy participants who were tested for CBC and Hb analysis using the Sebia capillary zone electrophoresis method and the participants would be classified into the normal, Hb E trait and Hb E disease groups. The Hb E traits with Hb concentration < 12 g% for females and < 13 g% for males and Hb E disease with Hb concentration < 10 g% were excluded. The mean Hb A_2 concentrations of Hb E disease, Hb E trait and the normal groups were compared and analyzed with the ANOVA and un-paired student-T tests and p value less than 0.05 would be considered statistically significant. **Results:** From 30 participants with Hb E disease, 30 Hb E traits and 30 normal, mean Hb A_2 concentrations were found to be 5.72$\pm$0.59% in Hb E diseasewhich was significantly higher than 4.07$\pm$0.43 % of Hb E trait ($p < 0.001$) and 2.63$\pm$0.27% of the normal group by the ANOVA method. Likewise, mean Hb A_2 concentration in Hb E traits was also sigficantly higher than that of the normal ($p < 0.001$) by the un-paired student-T test. **Conclusion:** The mean Hb A_2 concentration in Hb E disease is higher than that of Hb E trait whereas Hb A_2 of Hb E trait is higher than that of the normal participants with statistic significance.

Key words: Percentage of Hemoglonim A_2, Participants with Hemoglobin E

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บทคัดย่อ: **ร้อยละของฮีโมโกลบินเอ$_2$ ในผู้ที่มีฮีโมโกลบินอี**

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*หน่วยโลหิตวิทยากลุ่มงานอายุรกรรม โรงพยาบาลมหาราชนครราชสีมา จ.นครราชสีมา, 30000
**นักศึกษาแพทย์มหาวิทยาลัยแพทยศาสตร์เสิ่นหยางสาธารณรัฐประชาชนจีน
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วัตถุประสงค์: หาร้อยละของฮีโมโกลบินเอ$_2$ ในผู้ที่มีฮีโมโกลบินอีแฝงและผู้ที่มีโรค ฮีโมโกลบินอีและเปรียบเทียบกับฮีโมโกลบินเอ$_2$ ของกลุ่มคนปกติ **ผู้ป่วยและวิธีการ:** เป็นการศึกษาย้อนหลังที่กลุ่มงานอายุรกรรม โรงพยาบาลมหาราชนครราชสีมา โดยรวบรวมจากรายงานคนปกติที่มาตรวจร่างกายเป็นประจำตรวจ CBC และตรวจแยกชนิดของฮีโมโกลบินโดยวิธีของ Sebia capillary zone electrophoresis โดยแบ่งคนออกเป็น 3 กลุ่มได้แก่ กลุ่มคนปกติคนที่มีฮีโมโกลบินอีแฝงและกลุ่มคนที่มีโรคฮีโมโกลบินอีถ้ากลุ่มที่มีฮีโมโกลบินอีแฝง มีความเข้มข้นของฮีโมโกลบิน< 12 กรัม% สำหรับเพศหญิงและ< 13 กรัม% สำหรับเพศชายกับกลุ่มที่มีโรค ฮีโมโกลบิน อีที่มีความเข้มข้นฮีโมโกลบิน< 10 กรัม% จะถูกตัดออกค่าร้อยละของฮีโมโกลบินเอ$_2$ ในกลุ่มผู้ที่มีโรคฮีโมโกลบินอี, ฮีโมโกลบินอีแฝงและกลุ่มคนปกติจะได้รับการเปรียบเทียบ และวิเคราะห์ด้วยวิธีของ ANOVA และ un-paired student-T tests และค่า p ที่น้อยกว่า 0.05 จะถือว่ามีนัยสำคัญทางสถิติ **ผลการศึกษา:** จากผู้ที่มีโรคฮีโมโกลบินอี 30 ราย, ฮีโมโกลบินอีแฝง 30 ราย และคนปกติ 30 รายพบว่าความเข้มข้นของฮีโมโกลบินเอ$_2$ เฉลี่ยของกลุ่มที่มีโรคฮีโมโกลบินอีคือร้อยละ 5.72$\pm$0.59 ซึ่งมากกว่าร้อยละ4.07$\pm$0.43 ของกลุ่มที่เป็นฮีโมโกลบินอีแฝง ($p<0.001$) และร้อยละ 2.63$\pm$0.27 ของกลุ่มคนปกติอย่างมีนัยสำคัญทางสถิติ โดยวิธี ANOVA และค่าเฉลี่ยความเข้มข้นของฮีโมโกลบินเอ$_2$ ในผู้ที่มีฮีโมโกลบินอีแฝงก็มากกว่าของกลุ่มคนปกติโดยการตรวจด้วยวิธี un-paired student-T test อย่างมีนัยสำคัญทางสถิติเช่นกัน ($p<0.001$) **สรุป:** ค่าเฉลี่ยของความเข้มข้นฮีโมโกลบินเอ$_2$ ในกลุ่มผู้ที่มีโรคฮีโมโกลบินอีมากกว่าของกลุ่มที่มีฮีโมโกลบินอีแฝงและของกลุ่มที่มีฮีโมโกลบินอีแฝงมากกว่าของกลุ่มคนปกติอย่างมีนัยสำคัญทางสถิติ

คำสำคัญ: ร้อยละของฮีโมโกลบินเอ$_2$, ผู้ที่มีฮีโมโกลบินอี

Introduction

Hemoglobin (Hb) A_2 consists of 2 alpha globin and 2 delta globin chains. In normal adults, the range of Hb A_2 percentage is 2.4%$\pm$0.9%[1]. It is synthesized under the control of beta-globin gene complex on the chromosome 11. In the case of beta thalassemia trait, the genetically partial decrease of beta globin chain production, the delta and gamma globin chain production is increased for compensation, leading to the slightly increased production of Hb A_2($\alpha_2\delta_2$), 4.3-7.5% and of Hb F ($\alpha_2\gamma_2$), respectively.

Hb E is the combination of 2 alpha globin chains and 2 abnormal beta globin chains of which glumatine at the 26th position of amino acid polypeptide is substituted by lysine ($\alpha^{A}_{2}\beta^{56gly\rightarrow asp}_{2}$) . With the high performance liquid chromatography (HPLC) method, the Hb A_2 and Hb E are simultaneously found on the same band. The percentage of such band is used to classify the patients to be beta thalassemia trait, Hb E trait or Hb E disease. If it is less than 10 %, it is presumed to be Hb A_2, the surrogate marker of beta

thalassemia trait, 30 % more or less to be Hb E trait and 80 % to be Hb E disease. In conctrast, with the capillary zone electrophoresis (CZE) method, the migration of Hb A_2 and Hb E can be completely separated. And in normal individuals, Hb A_2 levels by CZE method are found to be 2.8±0.8% which is always slightly higher than 2.3±0.8% by HPLC method[2].

Hb A_2 should have been quantified in participants with Hb E in Nakhon Ratchasima after the Hb analysis by CZE method had been used instead of HPLC method because Hb E is highly prevalent in Nakhon Ratchasima, 37.5 %[3] as in other parts of the country that ranges from 17.9 % in the central part[4] to 41.7 % in the northeastern part[5]. The aim of this paper is to quantify and to compare the percentages of Hb A_2 among the normal, the Hb E trait and in Hb E disease participants using CZE method.

Participants and Methods

This retrospective study recruited the consecutive healthy participants who sought for the recommendation for the premarital screening of thalassemia and/or hemoglobinopathy in the Department of Medicine, Saint Marry Hospital, NakhonRatchasima, in 2016. They would be screened with CBC using the automated hematology analyzer and the hemoglobin type analysis using the Sebia® capillary zone electrophoresis method. They would be categorized into three groups, the normal if only Hb A_2 less than 3.5 %, the Hb E trait if Hb E less than 30 %, and Hb E disease if Hb E > 80 %. The participants who were diagnosed as normal, and as hemoglobin E trait would be excluded if the Hb concentration was less than 12.0 g% for females and less than 13.0 g% for males.And the participants who were diagnosed as Hb E disease must have Hb levels more than 10 g%[6]. The iron status and alpha thalassemia genotype were not explored in all participants. The percentage of Hb A_2 and Hb F of Hb E traits, Hb E disease and the nomal groups would be compared and analyzed using the student-T tests and the ANOVA method as appropriate. The p-value less than 0.05 would be considered statistically significant.

The participants who were found to have any chronic disease or got pregnant would be excluded.

Results

Among the normal people, Hb E trait and Hb E disease groups, 30 patients were consecutively selected from each group.Therefore 90 participants were recruited, consisting of 40 males and 50 females. Their ages ranged from 16 to 52 years, mean 33.8±11.2 years. The means and standard deviations of the percentage of Hb A_2 and Hb F as well as Hb concentration belonging to each group were shown and analyzed using the ANOVA method in the table.

The percentages of Hb A_2, Hb E, Hb F and Hb concentrations among the normal, Hb AE and Hb EE groups

	Normal	Hb AE	Hb EE	p
Hb (g%)	13.6±1.4	13.2±1.1	11.0±0.8	00
Hb A2(%)	2.6±0.3	4.1±0.4	5.7±0.6	00
Hb F(%)	0.1±0.3	0.6±0.6	2.1±1.6	00
Hb E(%)	-	24.4±1.2	92.1±1.5	

Note: Hb AE-Hb E trait, Hb EE-Hb E disease

The mean percentage of Hb A_2 among Hb E trait, Hb E disease and the normal control groups using the CZE method were different with the statistic significance, p 0.000. With an un-paired student-T test, the mean percentage of Hb A_2 of Hb E disease group was siginificantly higher than that of Hb E trait, $p < 0.00001$ and Hb A_2 of Hb E trait was also significantly higher than the control, $p < 0.00001$.

Likewise, the mean percentage of Hb F was significantly different among three groups, p 0.000. With an un-paired student-T test, the mean percentage of Hb F of the Hb E disease group was significantly higher than that of Hb E trait group, $p < 0.00001$ and that of Hb E trait group was also significantly higher than that of the normal control, $p = 0.00002$.

For the mean Hb concentration, it was significantly different among 3 groups with the ANOVA test.And with an un-paired student-T test, the mean Hb concentration of Hb E trait was significantly higher than that of Hb E disease, $p < 0.00001$ but not different from that of the control group, p 0.32.

Discussion

The means of Hb A_2 concentration in Hb E traits and Hb E disease in our study are significantly higher than that of the normal people. Our findings are consistent with the study of Mais et al. Although the ranges of Hb A_2 concentration of normal people in these two studies appeared quite similar, 2.63±0.27 vs. 2.6±0.4 %[7], it was found higher in Hb E trait and Hb E disease groups in our study as compared with that of the latter (4.07±0.43 vs 3.4±0.4 %) and 5.72±0.59 vs 4.4±0.4 %), respectively. Probably our study recruited only the Hb E traits that did not had anemia and Hb E disease patients who had Hb concentration of 10 g% or more because both Hb E trait and Hb E disease groups can be unexpectedly complicated by the iron deficiency anemia[6]. And it was well known that the percentage of Hb A_2 could be decreased in cases of iron deficiency anemia alone[8] or iron deficiency anemia with beta thalassemia trait[9]. The percentage of Hb A_2 of Hb E traits in our study appeared closed to 3.8±0.3 g% of Hb A_2 of Hb E traits who seemed to be free from anemia, Hb concentration between 12.9 and 13.5 g%[10].

The mechanisms why Hb A_2 is increased in Hb E trait and Hb E disease were not explored in our study. In the study of the percentage of Hb A_2 in blood samples containing Hb sickle cell, it was found increased in sickle cell trait, 4.09±0.42 %, range 2.20 to 5.20 %, in sickle cell disease, 3.90±1.08 %, range 0.60 to 5.90 %, and beta thalassemia trait, 4 to 9 %, compared to 2.57±0.25, range 2.1 to 3.0 % of the normal control by the HPLC method. Hb A_2 levels in Hb S-containing samples partially overlap with those expected from beta-thalassemia carriers[11]. This seems to suggest Hb A_2 will be slightly increased for compensation within the same range in the beta thalassemia trait or beta hemoglobinopathy trait. Hb E is resulted from one point of amino acid substitution at the 26^{th} position of beta chain from glumatine by lysine whereas Hb sickle is resulted from the similar abnormality, the glumatine is substituted by valine at the 6^{th} position of beta globin chains[12], so the pathogenesis of increased Hb A_2 in Hb E trait or even Hb E disease might be similar to those of Hb sickle.

The percentage of Hb E in Hb E trait using CZE method is 24.45±1.18 % or 25.6±0.9 %[10] that appears obviously lower than that found in the

HPLC method, 27.24 %[13] probably because of the complete separation between Hb A_2 and Hb E in the former. When Hb E trait was complicated with alpha thalassemia-1 trait, the percentage of Hb E was 16.6±1.6 % by CZE method[10] and 20.7±1.2 % by HPLC method[14]. So, the cut point of the percentage of Hb E for the recommendation of further investi-gation for the hidden alpha thalassemia-1 gene in any Hb E trait might be changed if using CZE method.

Conclusion

The Hemoglobin analysis using the capillary zone electrophoresis method can separate Hb E and Hb A_2. In this study, the mean percentage of Hb A_2 were found to be 5.72±0.59% in Hb E disease which was significantly higher than 4.07±0.43% of Hb E trait ($p < 0.001$) and 2.63±0.27% of the normal group. Likewise, the mean percentage of Hb A_2 in Hb E trait was also sigficantly higher than that of the normal ($p < 0.001$).

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